Use of Bicarbonates to Inhibit in vitro Colony Growth of *Botrytis cinerea*

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ABSTRACT

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Fungicide resistance in *Botrytis cinerea* has caused increased concerns about losses due to gray mold on many important agricultural and horticultural crops. Since bicarbonates have been reported to be an effective control of powdery mildew on greenhouse roses, the purpose of this research was to determine the effectiveness of bicarbonates against *B. cinerea*. Assessments were made of in vitro fungal colony growth in response to ammonium, potassium, and sodium bicarbonates. Bicarbonates inhibited colony growth at concentrations as low as 20 mM. In addition, comparisons of several ammonium, potassium, and sodium salts were conducted to determine whether cation or anion is the active moiety. Although the bicarbonate anion primarily affected growth, the ammonium cation also contributed greatly to fungal growth inhibition. With the exception of dibasic phosphate, only salts with high pK_a values or that are reducing agents, or both, decreased colony growth. The effect of pH on *B. cinerea* was then examined. Since bicarbonate anion concentration is related to pH, this parameter was examined in combination with several salts to separate pH effects from bicarbonate effects. As pH increased from 7.0 to 8.5, colony growth decreased with bicarbonates and phosphates, but not with ammonium sulfate. Since bicarbonates and phosphates decreased colony growth more than could be accounted for from pH alone, and since both have buffering characteristics, buffering capacity was examined and found to decrease colony growth but not as much as bicarbonate. Therefore, bicarbonates control *B. cinerea* colony growth in vitro, and both pH and buffering capacity contribute to, but are not solely responsible for, growth inhibition.

Botrytis cinerea Pers.:Fr., anamorph of *Botryotinia fuckeliana* (deBary) Whetzel and a member of the family Sclerotiniaceae, causes gray mold on many important agricultural and horticultural crops, including begonia, geranium, grape, lettuce, rose, and tomato (9). Gray mold can be problematic during all phases of crop production, resulting in heavy losses. For example, in the early stages of growth, damping-off can affect seedling geraniums. *B. cinerea* also affects mature plants by colonizing weak tissues such as flower petals and leaves within the lower canopy as well as attacking compromised stems from mother plants during cutting production. Moreover, postharvest problems can occur during the storage of cut flowers and of fruit.

Incidence of gray mold can be reduced through manipulation of crop environment and through usage of traditional chemical fungicides. Control for *B. cinerea* can be obtained in greenhouses by raising temperatures and by venting to lower humidity. However, many crops are grown in situations where environmental controls

are limited or nonexistent. Traditional chemical fungicides have had measurable success, but *B. cinerea* has developed resistance to common fungicides used to control gray mold. For example, benomyl resistance has been documented in the United States and in Israel (8,16). Since pesticide registration is a costly and lengthy process and many fungicides may be withdrawn from the turf and ornamental markets due to the Food Quality Protection Act, alternative means of safe control must be developed. Neem oil, horticultural oils, sulfur dioxide, silicates, phosphonates, phosphates, and bicarbonates have exhibited potential for disease control in other disease systems (1-5,12,18,26,28). Bicarbonates are generally regarded as safe by the Environmental Protection Agency and therefore will cause fewer problems with registration.

Bicarbonates have been demonstrated to effectively control a wide range of fungi, including food spoilage organisms and plant pathogens. Sodium bicarbonate reduces slightly the CFU of *Aspergillus parasiticus*, *Fusarium graminearum*, and *Penicillium griseofulvum* obtained from treated cracked-corn kernels, whereas ammonium bicarbonate greatly reduces CFUs of those same food spoilage fungi (15). Bicarbonates have been demonstrated to control powdery mildew diseases of cucumber (11), euonymus (30), and rose (12). Ziv and Zitter (31) found pronounced detrimental effects of bicarbonates on disease

incidence and in vitro growth of several cucurbit foliar pathogens: *Alternaria cucumerina*, *Colletotrichum orbiculare*, *Didymella bryoniae*, and *Ulocladium cucurbitae*. Bicarbonates and carbonate salts amended to water agar inhibit *Sclerotium rolfsii* sclerotial germination (24). In an initial report, Ziv and Hagiladi (30) claim bicarbonates control *B. cinerea*, but no host was mentioned; however, Ricker and Punja (27) tested *B. cinerea* on V8 agar containing 10 and 100 mM NaHCO_3 and found 28 and 100% inhibition, respectively.

This study was initiated to further elucidate the potential for bicarbonates to control *B. cinerea*. Research objectives to achieve this goal include (i) a determination of efficacies of bicarbonates against *B. cinerea* in vitro colony growth; (ii) an examination of cations and anions of various salts for active moieties; and (iii) an examination of the roles pH and buffering capacity play in bicarbonate activity. Preliminary results of this work have been presented (20-22).

MATERIALS AND METHODS

B. cinerea isolated from geranium (*Pelargonium × hortorum* L.H. Bailey) was used to evaluate bicarbonate inhibition of in vitro colony growth. Potato dextrose agar (PDA) at 39.0 g/liter was autoclaved for 17 min and then incubated at 55°C until it was poured into sterile plastic petri plates. Prior to cooling and solidification, supplements to PDA were added and, for appropriate experiments, pH was adjusted with HCl and NaOH or KOH. *B. cinerea* conidia were transferred to solidified plates with a heat-sterilized glass rod; plates were then sealed with Parafilm. Five plates per treatment were inoculated and then incubated at room temperature with natural cycles of light and dark. Colony diameters at the widest point were measured at 144 h. Colonies that grew appeared opaque within 48 h; colonies that did not grow had clear spore residues at 144 h. Spore residues were included in diameter measurements even if no colony formation occurred. *B. cinerea* cultures for inoculation were maintained on unsupplemented PDA.

Ammonium, potassium, and sodium bicarbonate (Church and Dwight Co., Inc., Princeton, NJ) concentrations from 0 to 100 mM were examined. To determine whether bicarbonates have fungistatic or fungitoxic effects on *B. cinerea*, plugs from treatments with no growth were transferred to unsupplemented PDA; colony diameters were measured 172 h later.

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To determine possible active moieties, the following salts were amended to PDA at 25 and 50 mM with no adjustment to pH: NaHCO_3 , NaCl , NaF , NaOH , NaNO_2 , NaH_2PO_4 , Na_2HPO_4 , Na_2SO_4 , $\text{Na}_2\text{S}_2\text{O}_3$, $\text{Na}_2\text{S}_2\text{O}_5$, KHCO_3 , K_2CO_3 , KCl , KOH , KNO_3 , KH_2PO_4 , K_2HPO_4 , K_3PO_4 , NH_4HCO_3 , $(\text{NH}_4)_2\text{CO}_3$, NH_4Cl , NH_4NO_3 , and $(\text{NH}_4)_2\text{SO}_4$.

Several experiments were conducted to determine effects of pH and buffering capacity on *B. cinerea* colony growth. The effect of pH on colony growth was examined on adjusted PDA at pH 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0. In addition, media supplemented with 25 mM and 50 mM NH_4HCO_3 or NaHCO_3 were evaluated at pH 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5. Monobasic and dibasic sodium phosphates at 50 mM were added to PDA, and pH was adjusted to 7.0, 7.5, 8.0, and 8.5. Media supplemented with 10 mM $(\text{NH}_4)_2\text{SO}_4$ or 20 mM NH_4HCO_3 , and unsupplemented PDA were tested at pH 7.0, 7.5, 8.0, and 8.5. To determine whether buffering capacity contributes to bicarbonate efficacy, *N*-[2-acetamido]-2-aminoethane sulfonic acid (ACES) buffer (Sigma, St. Louis, MO), pK_a 6.8, and KHCO_3 were compared at 50 mM.

Data were analyzed using statistical program SuperANOVA (Abacus Concepts, Inc., Berkeley, CA). Analyses performed included one-way and two-way ANOVA to determine significance of treatments in altering colony growth and Duncan new multiple range using $\alpha = 0.05$ to determine differences among treatments.

RESULTS

Bicarbonate concentration. Bicarbonates decreased *B. cinerea* colony growth as bicarbonate concentration increased (Fig. 1). Optimal concentrations for complete inhibition of *B. cinerea* varied depending upon which cation associated with the bicarbonate was incorporated into PDA. No colony expansion was measurable with NaHCO_3 at 50 mM, while the molarity for complete radial growth inhibition with KHCO_3 was slightly higher, 60 mM. Ammonium bicarbonate inhibited colony formation at a lower concentration, 20 mM. In addition to inhibiting *B. cinerea* colony growth, NH_4HCO_3 was also fungitoxic (Table 1). Even at 10 mM, no growth was observed 172 h after colonies were transferred. Fungistatic versus fungitoxic activity was not determined with KHCO_3 , because some colony growth occurred with salt concentrations up to 150 mM (data not shown).

Salt studies. Salts differed dramatically from no alteration to moderate reduction to complete inhibition of *B. cinerea* colony growth (Table 2). Bicarbonate, carbonate, hydroxide, dibasic and tribasic phosphate, fluoride, nitrite, sulfite, and metabisulfite significantly decreased ($P < 0.0001$) colony growth; whereas chloride, monobasic

phosphate, nitrate, and sulfate salts had no discernible activity on colony diameters. Except for NH_4HCO_3 , cations did not significantly alter anion activity. All inhibiting salts except sodium metabisulfite and sodium fluoride have pK_a values greater than 7.8. In addition to being alkaline, sodium sulfite and sodium nitrite are reducing agents, as is the acidic sodium metabisulfite.

pH studies. Growth of *B. cinerea* varied with pH; as pH increased from 6.5 to 10.0, *B. cinerea* colony growth decreased (Fig. 2). No differences were seen in colony growth from pH 5.0 to pH 6.0. *B. cinerea* can grow on PDA at pH 9.0, albeit at a reduced rate, but at pH 10.0, no growth occurred. Effects of bicarbonate concentration and pH on *B. cinerea* colony growth were assessed on PDA supplemented with NH_4HCO_3 at 0, 5, 10, and 20 mM. From pH 6.5 to pH 8.5, as bicarbonate concentration and pH increased, *B. cinerea* colony growth decreased ($P < 0.0001$). No colonies were formed at 10 and 20 mM NH_4HCO_3 above pH 7.5 (Fig. 3). Below

pH 6.5, no significant differences in growth occurred among bicarbonate concentrations. Similar results were obtained with NaHCO_3 . When grown on PDA containing four concentrations of NaHCO_3 (0, 5, 25, and 50 mM), *B. cinerea* grew significantly less on bicarbonate-supplemented PDA than on unsupplemented PDA at pH 6.5 and greater ($P < 0.0001$) (Fig. 4). Generally, increased inhibition was seen with increasing bicarbonate concentration and with increasing pH. No growth occurred with 50 mM NaHCO_3 above pH 7.5. Below pH 6.5, no significant differences were observed among the four treatments.

Dibasic phosphates reduced growth of *B. cinerea* from pH 7.0 through pH 8.5 ($P < 0.0001$) in comparison to pH controls (data not shown); no growth occurred with either phosphate above 8.0. Monobasic and dibasic sodium phosphate differed only at pH 7.0, which may be due to anomalous readings for dibasic sodium phosphate, since concentrations of monobasic and dibasic phosphate depend directly on solution pH.

Fig. 1. Effects of bicarbonate concentration on in vitro colony diameters of *Botrytis cinerea* on potato dextrose agar at 144 h. Media pH was not adjusted after amending with bicarbonate. Spore residues from inoculation were included in measurements. Bars represent critical difference at $\alpha = 0.05$.

Table 1. Diameters in cm of *Botrytis cinerea* colonies 144 h after placement onto potato dextrose agar (PDA) amended with NH_4HCO_3 at increasing concentrations up to 50 mM

NH_4HCO_3 concentration	Mean colony diameter at 144 h (cm) ^y	Mean colony diameter at 172 h after transfer (cm) ^z
0 mM	8.36 a	...
5 mM	2.18 b	...
10 mM	0.40 c	0.0 c
20 mM	0.38 c	0.0 c
30 mM	0.36 c	0.0 c
40 mM	0.34 c	0.0 c
50 mM	0.34 c	0.0 c

^y Values followed by the same letters are not significantly different at $\alpha = 0.05$.

^z Treatments exhibiting no colony formation were transferred to unsupplemented PDA, and subsequent growth from plugs was measured at 172 h.

Effects of ammonium sulfate and ammonium bicarbonate on *B. cinerea* colony growth were compared from pH 7.0 to pH 8.5 at equal molarities for NH_4^+ . While $(\text{NH}_4)_2\text{SO}_4$ significantly decreased growth ($P = 0.012$), NH_4HCO_3 completely inhibited growth ($P < 0.0001$) (Table 3). Ammonium sulfate above pH 8.0 slightly re-

duced colony growth in comparison to unsupplemented controls.

To determine whether buffering capacity reduces *B. cinerea* colony growth, 50 mM KHCO_3 and 50 mM ACES buffer were amended to PDA, and the pH was adjusted to 6.5, 7.0, 7.5, 8.0, and 8.5. The ACES buffer and KHCO_3 exhibited similar ef-

fects on *B. cinerea*: as pH increased, colony diameters decreased (Fig. 5). However, with the exception of pH 8.5, the decrease with ACES was not as great as with KHCO_3 .

DISCUSSION

Bicarbonate concentration. Ammonium, potassium, and sodium bicarbonates inhibit *B. cinerea* in vitro colony growth. *B. cinerea* has differential sensitivity to these bicarbonates, as demonstrated by its varying rates for complete inhibition of colony growth. Potassium and sodium bicarbonate have similar effects in reducing radial growth, but some abnormal growth can occur with KHCO_3 at the highest concentration tested. *B. cinerea* is most sensitive to NH_4HCO_3 . Different growth rates may be related to the ability of *B. cinerea* to compensate for elevated concentrations of the cations. The potassium cation is a critical component to maintain membrane integrity, but sodium is not utilized by cells as readily as potassium; this difference in metabolic usage explains the reason for *B. cinerea* to be slightly more tolerant of KHCO_3 . The ammonium cation can readily gain an electron to become ammonia gas, which by itself can prevent growth, providing NH_4HCO_3 two active moieties instead of one.

Concentrations for control of gray mold in field situations may be similar to concentrations needed for complete inhibition of *B. cinerea* radial colony growth. Several other pathogens have been demonstrated to be inhibited by bicarbonate both in vitro and in vivo. At 50 mM, NH_4HCO_3 prevented sclerotial germination of *S. rolfsii* (24) as well as significantly reducing the percentage of dead carrot plants from root rot (25). At 100 mM, NaHCO_3 completely inhibited *Rhizoctonia carotae* growth on V8 agar and reduced the number of unacceptable carrots (27). In addition, ammonium, potassium, and sodium bicarbonate reduced *Alternaria cucumerina* colony growth on water agar and the number of lesions formed on cucumber leaves (31). Thus, similar concentrations of bicarbonates modify disease incidence and in vitro fungal development in several disease systems. Since foliar sprays will dry on plant surfaces, increasing bicarbonate concentrations, lower initial concentrations may be sufficient to control gray mold development provided adequate coverage is achieved.

Salt studies. Because chloride, nitrate, and sulfate salts had no activity against *B. cinerea*, the bicarbonate anion appears to be the active portion. Punja and Grogan (24) found that chloride, sulfate, nitrate, and monobasic phosphate had no activity on *Sclerotium rolfsii*, also a member of the Sclerotinaceae family. Cations may have some minor effects, as demonstrated by differential sensitivities to various bicarbonate salts.

Table 2. Diameter in cm of *Botrytis cinerea* colonies 144 h after placement onto potato dextrose agar amended with various salts

Salt	Chemical formula	pK _a	Colony diameter at 25 mM (cm)	Colony diameter at 50 mM (cm)
Ammonium bicarbonate	NH_4HCO_3	7.8	0.28	0.23
Ammonium carbonate	$(\text{NH}_4)_2\text{CO}_3$	...	0.28	0.28
Ammonium chloride	NH_4Cl	5.0-5.1	7.42	7.02
Ammonium nitrate	NH_4NO_3	5.4	8.40	8.38
Ammonium sulfate	$(\text{NH}_4)_2\text{SO}_4$	5.5	8.40	8.18
Potassium bicarbonate	KHCO_3	8.2	1.54	1.01
Potassium carbonate	K_2CO_3	11.6	0.30	0.30
Potassium chloride	KCl	7.0	7.98	8.00
Potassium hydroxide	KOH	13.5	0.58	0.32
Potassium nitrate	KNO_3	7.0	7.80	7.82
Potassium phosphate, dibasic	K_2HPO_4	Alkaline	2.64	2.00
Potassium phosphate, monobasic	KH_2PO_4	4.4-4.7	8.40	8.30
Potassium phosphate, tribasic	K_3PO_4	Alkaline	0.22	0.32
Potassium sulfate	K_2SO_4	7.0	8.40	8.40
Sodium bicarbonate	NaHCO_3	8.3	1.35	0.38
Sodium carbonate	Na_2CO_3	11.6	0.30	0.28
Sodium chloride	NaCl	6.7-7.3	8.38	8.40
Sodium fluoride	NaF	7.4	2.90	0.50
Sodium hydroxide	NaOH	12.0-14.0	0.40	0.40
Sodium metabisulfite	$\text{Na}_2\text{S}_2\text{O}_5$	Acidic	0.22	0.20
Sodium nitrite	Na_2NO_2	9.0	4.28	0.28
Sodium phosphate, dibasic	Na_2HPO_4	9.1	1.84	1.02
Sodium phosphate, monobasic	NaH_2PO_4	4.5	8.40	8.12
Sodium sulfate	Na_2SO_4	6.0-7.5	7.86	8.26
Sodium sulfite	Na_2SO_3	9.0	5.88	2.16
Sodium thiosulfate	$\text{Na}_2\text{S}_2\text{O}_3$	6.5-8.0	7.00	8.22
No salt	...	...	8.25	8.25
LSD $\alpha = 0.05$			0.75	0.75

Fig. 2. Effects of pH on in vitro colony growth of *Botrytis cinerea* on potato dextrose agar at 144 h. Media were adjusted with HCl and KOH or NaOH. Spore residues from inoculation were included in measurements. Bars represent critical difference at $\alpha = 0.05$.

Since many common buffers include phosphate, it was unexpected that dibasic and tribasic phosphates would decrease colony diameters at rates similar to bicarbonates. However, Punja and Grogan (24) found similar results, in that carbonates and bicarbonates at 50 mM completely inhibited germination of sclerotia, while dibasic phosphates greatly reduced germination. Moreover, phosphates have been implicated in rose powdery mildew control as well as inducing resistance, as demonstrated by reduced infections even 23 days after spray (26). Mucharromah and Kuc (17) demonstrated that phosphates induce systemic resistance in cucumber for diseases caused by bacteria, fungi, and viruses.

Bicarbonates and chlorides were tested at the same concentrations; hence osmotic effects within these colony growth studies were negligible. However, in field situations where bicarbonate solutions will become more concentrated due to evaporation, osmotic effects may play a role in disease reduction.

pH studies. Many salts with basic pK_a values inhibited colony growth, indicating that pH may be a reason for the detrimental activity of bicarbonates. Most fungi, including *B. cinerea*, grow better in acidic to neutral conditions than in alkaline environments. For example, *S. rolfii* sclerotial germination is inhibited above pH 7.0 (24). Also, Webb (29) demonstrated that several fungi, including *B. cinerea*, germinate primarily in acidic conditions. However, *B. cinerea* can grow at reduced rates on media with high pH. Bicarbonates reduce growth beyond that of merely elevated pH; this inhibition is mediated by but not solely due to pH. Curran and Montville (6) compared apple juice with bicarbonates and juice adjusted to pH similar to that amended with bicarbonate and observed that *Hansenula wingei* and *Saccharomyces cerevisiae* growth were reduced at elevated pH but not to the same extent as with bicarbonates. Homma et al. (10) conducted similar studies with pH on *Penicillium citri*, also concluding that another mechanism is involved.

The bicarbonate concentration in solution is directly related to the pH of that solution (Fig. 6A). Bicarbonates are ineffective under acidic conditions because carbonic acid predominates in solutions below pH 6.5. H_2CO_3 is unstable and decomposes into carbon dioxide and water. As pH increases to pH 8.5, the concentration of bicarbonate increases. Above pH 8.5, bicarbonate concentration decreases and the level of carbonate rises.

Ammonium bicarbonate has two active components: the ammonium cation, which can convert to ammonia gas, and the bicarbonate anion. At low pH, ammonium bicarbonate becomes ammonium, water, and carbon dioxide: $pH < 7.0$, $NH_4HCO_3 + H^+ = NH_4^+ + H_2O + CO_2$. Since at low pH, no

inhibition was observed, it is highly unlikely that the ammonium ion contributes to decreased growth. At high pH, ammonium bicarbonate degrades to ammonia gas, water, and bicarbonate: $pH > 7.0$, $NH_4HCO_3 + OH^- = NH_3 + H_2O + HCO_3^-$. Release of ammonia at high pH provides an additional active component. Ammo-

nium bicarbonate passively diffuses into fungal cells (13).

The phosphate anion exhibits similar ionic changes through pH. Below pH 2, phosphoric acid is dominant in solution (Fig 6B). As pH increases, phosphoric acid gives way to monobasic phosphate and then to dibasic phosphate. Above pH 12.5,

Fig. 3. Effects of pH and NH_4HCO_3 concentration on in vitro colony growth of *Botrytis cinerea* on potato dextrose agar at 144 h. After amending with bicarbonate, media were adjusted with HCl and KOH or NaOH. Spore residues from inoculation were included in measurements. Bars represent critical difference at $\alpha = 0.05$.

Fig. 4. Effects of pH and $NaHCO_3$ concentration on in vitro colony growth of *Botrytis cinerea* on potato dextrose agar at 144 h. After amending with bicarbonate, media were adjusted with HCl and KOH or NaOH. Bars represent critical difference at $\alpha = 0.05$.

Table 3. Comparisons of colony diameters of *Botrytis cinerea* 144 h after placement onto potato dextrose agar amended with ammonium bicarbonate or ammonium sulfate^y

pH	Colony diameter with 0 mM (cm) ^z	Colony diameter with 10 mM (NH ₄) ₂ SO ₄ (cm)	Colony diameter with 20 mM NH ₄ HCO ₃ (cm)
7.0	2.60 b	4.42 e	0.28 a
7.5	5.10 g	4.74 f	0.24 a
8.0	4.48 e	2.92 c	0.30 a
8.5	3.70 d	2.60 b	0.22 a

^y Salt molarities were chosen to have equal concentrations of NH₄⁺ in solution.

^z Values followed by the same letters are not significantly different at $\alpha = 0.05$.

Fig 5. Effects of KHCO₃ and ACES buffer through pH on in vitro colony growth of *Botrytis cinerea* on potato dextrose agar at 144 h. After amending with bicarbonate or ACES, media were adjusted with HCl and KOH or NaOH. Bars represent critical difference at $\alpha = 0.05$.

tribasic phosphate predominates. Since dibasic phosphate has a similar structure to bicarbonate, the potential exists for interactions with fungal membranes.

Examination of the ammonium bicarbonate and sulfate data indicates that DePasquale and Montville's (7) theory regarding the primary mode of action for ammonium bicarbonate cannot generally be applied to fungi. Unlike *Botrytis*, *Fusarium* readily grows in alkaline conditions without any significant reduction in colony growth and will germinate readily (29). In studies by DePasquale and Montville, release of ammonia gas from ammonium sulfate at high pH (pH > 8.3) gave inhibition equivalent to that previously seen by ammonium bicarbonate for *Fusarium* and *Penicillium*. Since ammonia has very little negative impact on *B. cinerea* colony growth, while ammonia does, their conclusion that ammonia release in high pH solutions inhibits fungi is valid. Sulfate is a polyprotic salt similar to bicarbonate and phosphate. However, the first dissociation constant is very high, and all H₂SO₄ completely dissociates into H⁺ and HSO₄⁻ (Fig. 6C). Above pH 4.25, sulfate is completely dissociated into SO₄⁻²

and its associated cations. Therefore, ammonia gas release would occur at any pH above 7.0, with NH₃ concentrations increasing as pH increased. However, DePasquale and Montville (7) concluded that "ammonium bicarbonate inhibits fungi because the bicarbonate anion supplies the alkalinity necessary to establish an antifungal concentration of free ammonia." If that were the case, ammonium sulfate and ammonium bicarbonate would affect *B. cinerea* similarly under alkaline conditions. The following conclusion can be reached based on the presented data: Ammonia gas contributes to the detrimental activity of NH₄HCO₃ on *B. cinerea* but is not the sole means for its activity.

Buffering capacity to maintain elevated pH may be one means for bicarbonate activity, since fungi secrete acid to reduce pH in their environment. If buffering capacity is high, fungi cannot release enough acid to lower pH sufficiently for optimal growth. Energy is allocated to acid secretion to obtain a more favorable environment instead of manufacturing components for hyphal extension. Bicarbonates raise internal pH in conidia of *Sphaerotheca pannosa* f. sp. *rosae* and *Alternaria brassi-*

cae (23), probably because H⁺ ions are being secreted to lower external pH. In addition, internal pH measurements of *Fusarium graminearum* and *Penicillium griseofulvum* after treatment with ammonium sulfate or ammonium bicarbonate were elevated (7). Although very important, the buffering capacity of bicarbonates and phosphates is not the main reason either salt inhibits *B. cinerea* colony growth.

Implications. This research achieved the objectives outlined in the introduction. Bicarbonates are effective against *B. cinerea* in vitro colony growth at concentrations as low as 20 mM. Moreover, cations contribute little to the activity of the anions, and anions primarily determine effect on *B. cinerea*. Buffering capacity and elevated pH contribute to bicarbonate efficacy but are not the sole reasons for it; data presented herein support evidence by Marloth (14) "that the bicarbonate ion in itself has the inhibitory power, and this must be ascribed either to its action on the enzymes or enzyme-secreting power of the protoplasm or to a direct toxic influence on the protoplasm itself."

Bicarbonates may have several modes of action against fungi, including buffering an elevated pH environment and increasing osmotic levels on leaf surfaces, both conditions detrimental to fungal spores. Even though elevated pH, such as that buffered by bicarbonates, can prevent germination, conidial adhesion is dramatically reduced only above pH 11.0 (14). Marloth (14) observed that *Penicillium italicum* spores treated with hydroxides and carbonates swell, an early step in germination, but the appearance of germ tubes is inhibited; elevated pH allows spores to remain viable but may interfere with extracellular enzymes required to solubilize and expand cell walls and membranes. Nobecourt (19) demonstrated that sodium bicarbonate inactivates extracellular enzymes from *Penicillium* species. In addition, bicarbonates may directly interact with membranes to alter normal membrane activities or may disrupt cellular physiology at concentrations well below saturation.

The impetus driving research on bicarbonates as agents to control fungal diseases is the search for chemicals that are safe for humans and compatible with the environment. There is a need for new methods to control *B. cinerea*, because many chemicals will no longer be available for growers of certain crops due to increasing registration costs and full or overflowing risk cups after evaluation under the new Food Quality Protection Act. Since bicarbonates have been proven to be efficacious against fungi in both in vitro and in vivo studies, it is not improbable that they would control gray mold under field conditions. The next paper to be presented by these authors will address bicarbonate control of gray mold

Fig 6. Concentrations of (A) carbonic acid, bicarbonate, and carbonate, (B) phosphoric acid, monobasic phosphate, dibasic phosphate, and tribasic phosphate, and (C) mono- and dibasic sulfate as modified by solution pH, based upon calculations from dissociation constants.

on geraniums. If bicarbonates are demonstrated by in vivo assessments to effectively control gray mold development, they may augment or supplant current fungicide regimens because of their safety and efficacy.

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