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Control of citrus postharvest decay by ammonia gas fumigation and its influence on the efficacy of the fungicide imazalil

Clara Montesinos-Herrero[a], Joseph L. Smilanick[b,*], J. Steven Tebbets[b], Spencer Walse[b], Lluís Palou[a]

[a] Centre de Tecnologia Postcollita, Institut Valencià d'Investigacions Agràries (IVIA), Apartat Oficial, 46113 Montcada, València, Spain
[b] United States Department of Agriculture – Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, 9611 South Riverbend Avenue, Parlier, CA 93648, USA

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ABSTRACT

Postharvest green mold and blue mold, caused by *Penicillium digitatum* and *Penicillium italicum*, respectively, were effectively controlled by fumigation of lemons and oranges for 6 h at 22 °C with two applied dosages of 3000 μL L^{-1} of ammonia that was injected initially and again 2 h later. This treatment did not injure oranges, however, it caused the tissue within previously injured areas on the rind of lemons to become darker in color. Fumigation of lemons with 6000 μL L^{-1} of ammonia slightly accelerated the natural transition of rind color from green to yellow. The germination of conidia of *P. italicum* was more sensitive to ammonia than those of *P. digitatum*, although many survived fumigation. About 30% of the conidia of *P. digitatum* and 10% of those of *P. italicum* could germinate after a 6 h fumigation where two injections of 6000 μL L^{-1} of ammonia were applied, one initially and a second 2 h later. Ammonia fumigation controlled an isolate of *P. digitatum* with a high level of resistance to imazalil (IMZ). The influence of ammonia fumigation on the effectiveness of this common postharvest fungicide was examined. When fruit were first immersed in 10 or 30 mg L^{-1} IMZ (about 10% of typical commercial rates) before ammonia fumigation, a single fumigation with 1500 μL L^{-1} of ammonia was adequate to control both diseases and the increase in effectiveness was usually additive and sometimes synergistic. This effect was probably due in part to the influence of pH on IMZ activity, because the neutral form of IMZ increases with increasing pH and it has markedly higher antifungal activity than the ionized molecule. Fumigation with 1500, 3000, or 6000 μL of ammonia per liter increased the pH (±SD) of albedo tissue of pre-existing wounds on oranges and lemons from 5.9 (±0.2) before fumigation by 0.6 (±0.3), 0.9 (±0.4), or 1.3 (±0.3) units, respectively. IMZ can be applied immediately after harvest by drenching fruit within harvest bins with aqueous IMZ solutions. Subsequent ammonia fumigation on their arrival to packinghouses may be a feasible practice, since it could employ the existent ethylene degreening chambers present at all packinghouses, if these were modified to be gas tight. Ammonia could replace synthetic fungicides or augment IMZ performance in citrus postharvest decay management. Its capacity to control IMZ resistant isolates of *P. digitatum*, common in citrus packinghouses, is particularly valuable.

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1. Introduction

The most significant decay loses in arid production areas, such as Spain and California, during storage and marketing of citrus fruit, are caused by green and blue molds of citrus, caused by *Penicillium digitatum* (Pers.:Fr) Sacc., and *Penicillium italicum* Wehmer, respectively. Initially, these diseases were controlled by immersing fruit in heated solutions of borax, sodium bicarbonate, or sodium carbonate, later, synthetic fungicides such as sodium *ortho*-phenylphenate, imazalil, or thiabendazole (Eckert and Eaks, 1989) were used, and the last two remain in common use. Their repeated use has given rise to resistant biotypes of the pathogens that has diminished their effectiveness (Bus et al., 1991; Holmes and Eckert, 1999; Kinay et al., 2007). More recently the 'reduced-risk' fungicides azoxystrobin, pyrimethanil and fludioxonil were introduced (Adaskaveg and Förster, 2010). To control fungicide resistant isolates, treatment of fruit in heated tanks has again become popular, often containing potassium sorbate (Montesinos-Herrero et al., 2009), sodium carbonate, or sodium bicarbonate (Montesinos-Herrero and Palou, 2010), sometimes alone or in mixtures with conventional fungicides (Smilanick et al., 2005, 2008; Palou et al., 2008). Significant issues remain regarding the use of fungicides to manage these diseases, including concerns about the impact of the fungicide residues on human health and the environment (Dezman et al., 1986; Bates, 2002), the lack of residue tolerances in some markets, disposal of the used tank solutions, chemical costs, and the need to produce residue-free

* Corresponding author.
E-mail addresses: Joe.smilanick@ars.usda.gov, jsmilanick@fresno.ars.usda.gov (J.L. Smilanick).

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fruit that comply with 'chemical free' or 'organic' marketing programs.

Ammonia fumigation was developed and rigorously evaluated more than 50 years ago but was not adopted because of the inconvenience and training needed to implement it (Eckert, 1967) and the fact that the first generation of the synthetic fungicides, sodium *ortho*-phenylphenate and diphenyl, had been introduced. Since that time developments within the citrus industry make revisiting ammonia fumigation timely. It could be a tool in the management of fungicide resistant pathogens. It is likely ammonia does not deposit residues of dietary concern and it could conceivably be part of 'residue-free' or 'organic' programs. Large capacity fumigation chambers are present in citrus packinghouses where ethylene gas is applied to eliminate the green rind color for marketing purposes (Wardowski et al., 2006); these could be made gas-tight and adapted for ammonia fumigation. The use of a gas for decay control purposes itself offers significant advantages over fungicides applied on packinglines; fumigation can be applied in mass to fruit in cartons or bins, and the need for mechanical handling and the rind injuries it can cause is avoided.

The objectives of this work were to assess the effectiveness of brief ammonia fumigations to control citrus green and blue molds and its influence on fruit quality. Several aspects of ammonia fumigation had not been assessed in prior work. To determine the interaction of ammonia fumigation with imazalil, fruit were treated with imazalil before ammonia fumigation to evaluate their interaction. The control of a *P. digitatum* isolate with a high degree of imazalil resistance by ammonia fumigation, the toxicity of ammonia gas to conidia of *P. digitatum* and *P. italicum*, and its effect on the pH of exposed albedo tissue and the rate of transition of green to yellow color of lemon fruit were determined. Ammonia concentration was measured during fumigation to determine losses of the gas during fumigation by sorption to chamber walls and fruit.

2. Materials and methods

2.1. Fruit

'Eureka' lemons (*Citrus limon* (L.) Burm) and 'Valencia' oranges (*Citrus sinensis* (L.) Osbeck) were used in this study. Lemons were collected from field bins in a packinghouse in Tulare County (CA, USA) 1 d after harvest. The oranges were harvested by hand from a grove of the University of California, Lindcove Research and Extension Center (Exeter, CA). No fungicides had been applied before harvest and no postharvest treatments had been applied to any of these fruit. Before use, fruit were stored at 15 °C for less than 2 weeks. Fruit were washed and randomized before the experiments. To determine the influence of ammonia fumigation on rind quality, fresh wounds were made at 3 points in the equatorial zone by cutting the flavedo with the tip of a knife leaving an open albedo area of 5 mm in diameter and 2 mm in depth immediately before fumigation.

2.2. Inoculum

All pathogens were isolated from infected lemon fruit and cultured for 1–2 weeks on potato dextrose agar (PDA, Difco Laboratories, Detroit) at 25 °C. Isolates used were two *P. digitatum* biotypes (IMZ-sensitive D90 and IMZ-resistant D201) and an isolate of *P. italicum*. The virulence, growth, and other characteristics of these *P. digitatum* isolates were characterized in prior work (Kinay et al., 2007). Isolate PD90 is representative of the IMZ sensitive isolates (EC_{50} on PDA of 0.05 mg L^{-1} IMZ) commonly encountered in citrus groves, while isolate D201 is representative of the IMZ resistant isolates (EC_{50} on PDA of 1.75 mg L^{-1} IMZ) commonly encountered in packinghouses (Holmes and Eckert, 1999; Kinay et al., 2007). Suspensions containing 10^6 conidia mL^{-1} were prepared by measuring the concentration of an initial preparation of conidia with a spectrophotometer and diluting the suspension until the absorbance was 0.1 (*P. digitatum*) or 0.11 absorbance (*P. italicum*). Fruit were inoculated by briefly dipping a stainless steel rod with a 1-mm wide and 2-mm long tip into the inoculum solution, and then immediately making a puncture on the equator of the fruit (Eckert and Brown, 1986). Inoculated fruit were left at room temperature for 24 h before treatment. After treatment, they were incubated under humid conditions (>95%) relative humidity at 20 °C for 7–10 d.

2.3. Ammonia fumigation

Fruit were placed in one of four 0.242-m^3 steel chambers with air circulation fans for fumigation with anhydrous ammonia (Airgas Inc., Radnor, PA, USA) or stored in air at 20 °C at high relative humidity (>95%) (control). The chambers were housed inside a temperature controlled room (22 °C) and attached to an exhaust manifold so the fumigant was safely exhausted from the chamber at the end of the 6 h exposure period. The relative humidity inside the chambers was not controlled. There was no detectable loss of fumigant due to leakage. Fruit were pre-conditioned overnight to the desired treatment temperature and weighed before being fumigated. The load factor (percentage of the chamber volume occupied by the fruit ±SD) with 20 kg of fruit inside the chambers was approximately 20 ± 1%. Initial concentrations of 0, 1500, 3000 and 6000 μL L^{-1} ammonia were injected by extracting a calculated volume of gas from the ammonia cylinder using a syringe (models S-500 or S-1500, Hamilton Co., Reno, NV, USA) and injecting it into the chamber through a stainless steel LuerLok® valve to which the Teflon® LuerLok® tip of the syringe was connected. Ammonia gas concentrations were recorded at the beginning of the fumigation (±2 min), and after 2, 4, and 6 h (±2 min). Ammonia detector tubes (models 3HM and 3 M, Gastec Corporation, Ayase-City, Japan) and gas sampling pump (model GV-100, Sensidyme/Gastec, Clearwater, Florida, USA) were used to make these measurements.

Chambers were loaded with 0, 3, 10 or 20 kg of fruit and injected with ammonia to a calculated applied dosage of 6000 μL L^{-1} ammonia, then repeated measurements were taken as described previously in order to determine the influence of the amount of fruit within chamber on the ammonia concentration. The concentration of ammonia during fumigation within the chamber was also measured and a concentration–time product (*C*–*T*) was calculated by the method of Bond (1984), which is the area under the line depicting the concentration of ammonia during fumigation expressed in μL L^{-1} h^{-1}. After fumigation, fruit were incubated at 20 °C at high relative humidity (>95%) and the number of infected fruit and the sporulation from decay lesions were observed after 4 and 7 d, and again after 10 d when oranges were evaluated. Disease incidence and sporulation were calculated as percentages within each treatment. In a second set of experiments, the same procedure was employed except a second injection into the chamber of the calculated initial applied dosage of ammonia was done 2 h after the first injection. These double injections of 1500, 3000, or 6000 μL L^{-1} ammonia were depicted as 2 × 1500, 2 × 3000, or 2 × 6000 μL L^{-1}, respectively. All experiments were done twice.

2.4. Influence of ammonia fumigations on germination of conidia

Conidia of *P. digitatum* isolate D201, *P. digitatum* isolate D90, and *P. italicum* were deposited on microscope slide cover slips and placed in the fumigation chambers where they were exposed to ammonia gas at initial concentrations of 1500, 3000, or 6000 μL L^{-1}. Control samples were similarly prepared but not fumigated. After fumigation, the cover slips were placed in sterile centrifuge tubes

and the conidia were suspended by adding sterile water with 0.05% (w/v) Triton X-100 to the tubes. A volume of 20 μL of each conidial suspension was placed on the surface of PDA. After 12 h incubation at 25 °C, the number of germinated and non-germinated conidia in each Petri dish were counted by observation of 150–250 conidia from each isolate using a compound microscope (200×). The experiment was done twice.

2.5. Interactions between imazalil treatment and subsequent ammonia fumigation

In tests to determine the influence of subsequent ammonia fumigation on the efficacy of the fungicide imazalil (IMZ; 44.6% imazalil, Fungaflor 500EC; Janssen Pharmaceutica, Beerse, Belgium), lower than commercial rates of IMZ and all tested dosages of ammonia, that partially controlled green mold and blue mold, were employed in order to reveal the interaction between these treatments. The fruit were inoculated as previously described with *P. digitatum* PD90 or *P. italicum*. After 24 h, IMZ treatments were applied by dipping the fruit for 30 s in aqueous solutions of 0 (control), 10 mg L^{-1} (on oranges) or 30 mg L^{-1} (on lemons) of IMZ at room temperature. Fruit were not rinsed and were allowed to dry in air at approximately 20 °C. Once the fruit surface was dry, fruit were fumigated with ammonia and incubated as previously described. The experiment was done twice.

2.6. Influence of ammonia fumigations on pH of fruit albedo

To determine the influence of ammonia fumigation on the pH within rind wounds, two pieces of fruit were used for each concentration of ammonia tested (initial applied dosages of 0, 1500, 3000, or 6000 μL L^{-1}). Before ammonia fumigation, the fruit were wounded on 3 points in the equatorial zone by cutting the flavedo with the tip of a knife leaving an open albedo area of 5 mm in diameter and 2 mm in depth. A pH meter with a 2 mm diameter combination electrode with automatic temperature compensation ('3 in 1'; Corning Incorporated Science Products Division, Corning, NY, USA) was used. A volume of 30 μl of distilled water was added to each wound before measuring the pH. The pH was measured immediately before the fumigations, immediately afterwards, and 24 h later. The pH of fruit albedo in contact with ammonia gas was measured before and after fumigations in three experiments, two of them with oranges and one with lemons.

2.7. Influence of ammonia fumigation on the natural degreening rate of lemons

Dark green lemons were randomized and divided in two sets of 20 fruit each; one set was stored at 20 °C and the second one was fumigated with an initial applied dosage of 6000 μL L^{-1} ammonia for 6 h at 22 °C. A colorimeter (Chromameter CR-300 Minolta Co. Ltd., Osaka, Japan) was used to measure the rind surface color using CIE color parameters L^* (lightness), C^* (chroma or saturation) and h° (hue angle). The fruit were stored at 20 °C. Color was determined initially before ammonia fumigation and after 12, 20, and 22 d of storage. The experiment was done once.

2.8. Statistical analysis

Combinations of IMZ and ammonia fumigation were applied to three replicates of 20 oranges or lemons. Fruit were randomized before each experiment. Values recording disease incidence were arcsine transformed (arcsin of the square root of the proportion of infected fruit) before an analysis of variance was applied (Statgraphics Plus 4.1, Manugistics Inc., Rockville, MD, USA) and means were separated by Fisher's least significant difference test (LSD; $P = 0.05$). Values shown are non-transformed data. In order to determine if the effectiveness of the combination of IMZ and ammonia fumigation was synergistic, Limpel's formula was applied (Richter, 1987). Data shown are means of all of the experiments.

3. Results

3.1. Control of green and blue molds

The incidence of green and blue molds was significantly lower on oranges and lemons treated with a single injection of ammonia gas than on non-treated fruit. In the case of oranges, control of the molds was evident even at the lower concentrations tested (Fig. 1). While non-treated fruit were completely decayed at the end of the incubation period, after 10 d of incubation the incidence of green mold on oranges treated with ammonia at any of the tested concentrations was about 40%, and the incidence of blue mold was 26, 18, and 15% on oranges treated with 1500, 3000, and 6000 μL L^{-1} ammonia, respectively. On lemons, the effectiveness of ammonia fumigations on decay was lower and more dependent on the concentration used. After incubation for 7 d, the incidence of green mold on lemons treated with 1500, 3000, or 6000 μL L^{-1} ammonia was 82, 46, and 22%, and the incidence of blue mold was 74, 42, and 10%, respectively. The incidence of green and blue molds on lemons treated with 30 mg L^{-1} IMZ alone was relatively high and the combination of IMZ with ammonia was consistently synergistic and it greatly reduced the incidence of both molds compared to lemons treated with either of the treatments alone (Fig. 1). The incidence of green mold on non-treated oranges inoculated with the IMZ-resistant isolate D201 was 90% after 10-days incubation (Fig. 2). Green mold developed on 45% of the oranges inoculated with this isolate and treated with 10 mg L^{-1} IMZ, while treatments with ammonia or combinations of 10 mg L^{-1} of IMZ with ammonia fumigation reduced the incidence of green mold to less than 10%, and as low as 2% on oranges treated with 6000 μL L^{-1} ammonia. No synergy between IMZ and ammonia fumigation was observed at any of the concentrations used (Fig. 2). Sporulation of green and blue molds on decayed fruit was similarly reduced by ammonia treatments, although on oranges inoculated with *P. digitatum* D201 the reduction in sporulation caused by ammonia treatment was greater than that observed with the other isolates (data not shown). Ammonia fumigation did not visibly damage oranges; however, on lemons, albedo tissue within wounds made before fumigation was darker in color after treatments with 3000 or 6000 μL L^{-1} of ammonia. A second injection with the same concentration of ammonia 2 h after the first injection consistently improved the effectiveness of the fumigations, but it increased tissue darkening on lemons. The incidence of green and blue molds was very low (less than 5%) or absent on fruit treated with 30 mg L^{-1} of IMZ before two injections of ammonia at any of the concentrations applied (Fig. 3). Darkening within the albedo tissue of wounds on lemons after a double injection of ammonia was noticeable when 3000 μL L^{-1} of ammonia was used, and the damage was more severe after one or two injections of 6000 μL L^{-1} ammonia (Table 1). The albedo tissue in wounds on oranges treated with two injections of 6000 μL L^{-1} of ammonia was darkened slightly.

3.2. Ammonia concentration within chambers

Ammonia concentration in the chambers decreased rapidly after the initial injection and the rate of decline slowed as the ammonia concentration diminished. Ammonia concentration in the chambers had declined to 10% of the initial concentration 2 h after the injection (Fig. 4). When a second ammonia injection was made 2 h later, the ammonia concentration reached a higher peak, due to the addition to the amount of ammonia still present in the chamber,

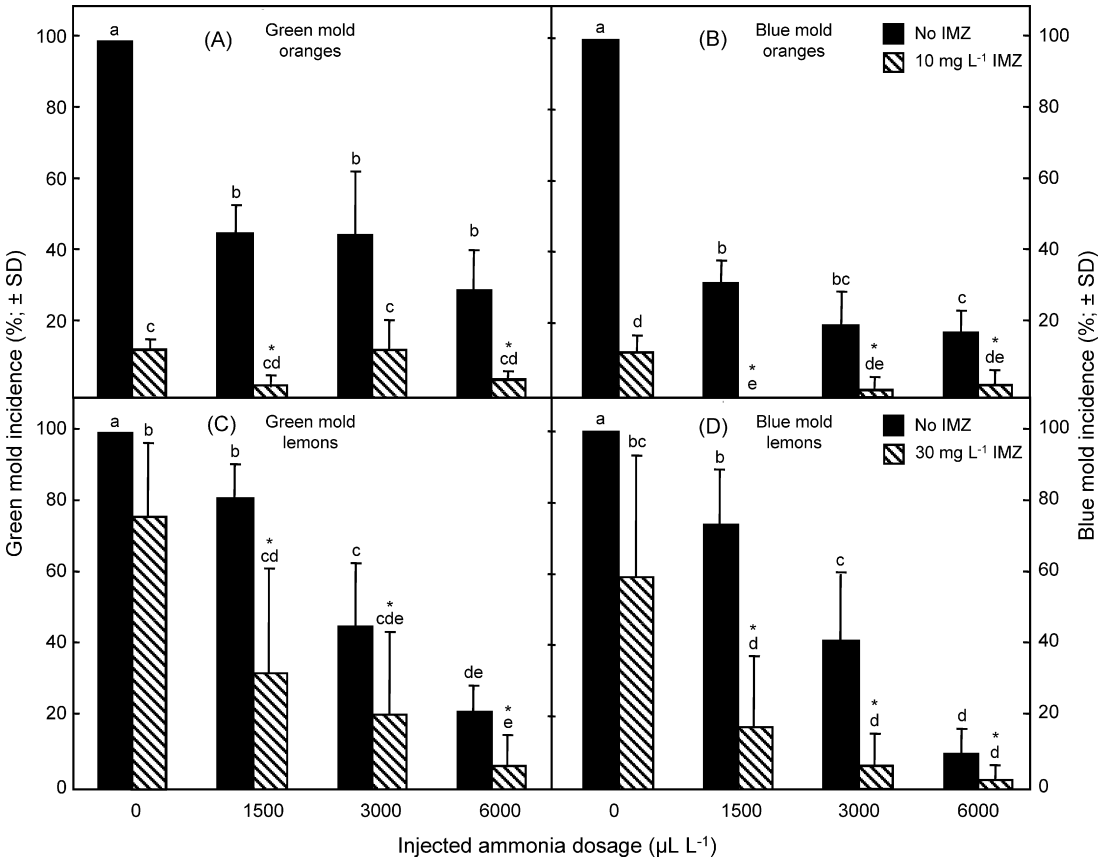

Fig. 1. Effectiveness of fumigation with one injection of ammonia on the incidence of green mold, caused by *Penicillium digitatum* isolate PD90, an imazalil (IMZ) sensitive isolate, and blue mold, caused by *Penicillium italicum*, respectively, on Valencia oranges (A and B) and Eureka lemons (C and D). The fruit were inoculated, half were treated 24 h later by immersion in 10 mg L^{-1} (oranges) or 30 mg L^{-1} (lemons) of IMZ for 30 s. When treated fruit were dry, all were fumigated with ammonia for 6 h at 22 °C with ammonia dosages of 0, 1500, 3000, or 6000 µL L^{-1} injected once at the beginning of the treatment. The incidence of infected fruit was determined after storage at 20 °C at high relative humidity (>95%) for 10 and 7 d, respectively, for green mold and blue mold. Values are the means of two experiments with three replicates of 20 fruit each. Error bars indicate standard deviations. Within each disease with unlike letters are significantly different by Fisher's Protected LSD test ($P \leq 0.05$). Asterisk indicates synergistic activity was present between ammonia and imazalil according to Limpel's formula (Richter, 1987).

and the following drop in the concentration was similarly rapid as after the first injection (Fig. 4). The weight of the lemons or oranges treated in each chamber was 20 ± 3 kg. The rate of ammonia decline in the chamber was influenced by the load of fruit within the chamber (Fig. 5). Initial concentrations of 6000 µL L^{-1} ammonia declined after 2 h to 4000, 3500, 2500, or 600 µL L^{-1} ammonia within chambers containing none, 3, 10, or 20 kg of non-wounded fruit, respectively. After 2 h the rate of decline in ammonia concentration was slower (Fig. 5). The *C–T* products calculated from the ammonia concentrations measured in this experiment and the observations of their effect on fruit appearance were summarized (Table 1).

Table 1
Applied ammonia dose, fruit load, measured concentration-time products (*C–T*), presence of fresh wounds before fumigation for 6 h, and rind appearance after the fumigation of lemons or Valencia oranges.

NH_3[a] (µL L^{-1})	Fruit load (kg)	*C–T* product[b] (µL L^{-1} h^{-1})	Wounded[c] (yes or no)	Rind appearance	
				Oranges	Lemons
2 × 1500	20	3303	Yes	Unaltered	Unaltered
2 × 3000	20	8564	Yes	Unaltered	Tissue slightly darker in wounds
2 × 6000	20	16,960	Yes	Tissue slightly darker in wounds	Tissue much darker in wounds
1 × 1500	20	1770	Yes	Unaltered	Unaltered
1 × 3000	20	3650	Yes	Unaltered	Unaltered
1 × 6000	20	7953	Yes	Unaltered	Tissue much darker in wounds
1 × 6000	20	7830	No	Unaltered	ND[d]
1 × 6000	10	14,320	No	Unaltered	ND
1 × 6000	3	19,100	No	ND	Unaltered
1 × 6000	0	23,800	No	NA[e]	NA

[a] Number of injections and calculated concentration of ammonia applied into the chamber. When a second injection was made, it followed the first injection by 2 h.
[b] Measured concentration times time product during fumigation.
[c] Fresh wounds were made at 3 points in the equatorial zone by cutting the flavedo to expose an albedo area of 5 mm in diameter and 2 mm in depth immediately before fumigation.
[d] NA = not applicable.
[e] ND = not done.

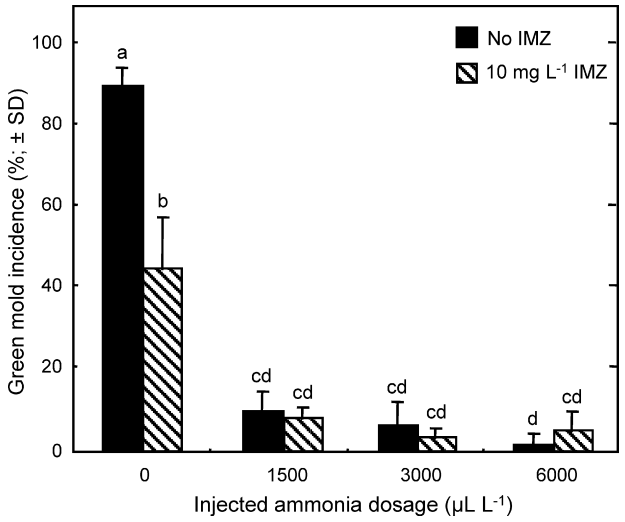

Fig. 2. Incidence of postharvest green mold, caused by *Penicillium digitatum* (isolate D201) with a high level of imazalil (IMZ) resistance, on Valencia oranges incubated at 20 °C at high relative humidity (>95%) for 10 d after fumigation for 6 h at 22 °C with ammonia dosages of 0, 1500, 3000, or 6000 μL L^{-1}, injected once at the beginning of treatment. The fruit were inoculated, treated 24 h later by immersion in 10 mg L^{-1} of IMZ for 30 s, and fumigated with ammonia once the fruit surface was dry. Values are the means of two experiments with three replicates of 20 fruit each. Error bars indicate standard deviations. Values with unlike letters are significantly different by Fisher's Protected LSD test ($P \leq 0.05$).

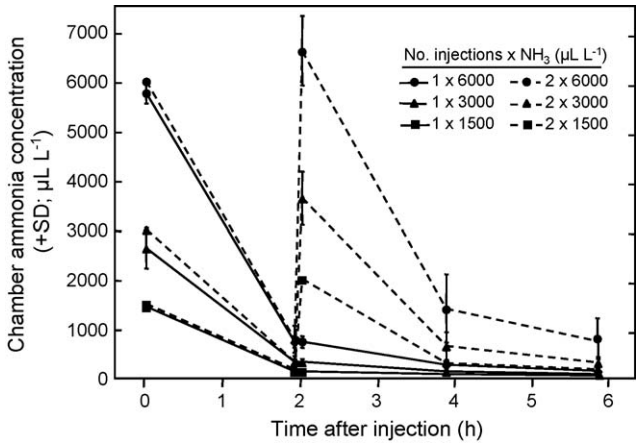

Fig. 4. Concentration of ammonia gas during fumigations in chambers with ammonia dosages of 1500, 3000, or 6000 μL L^{-1} injected at the beginning of the treatment (1500, 3000, 6000) or at the beginning of the treatment and again 2 h later (2 × 1500, 2 × 3000, 2 × 6000). The weight of the fruit treated in each chamber was 20 ± 3 kg.

3.3. Influence of ammonia fumigations on germination of conidia

Fumigations with injected concentrations of 1500, 3000, or 6000 μL L^{-1} of ammonia for 6 h inhibited the subsequent germination of conidia of *P. digitatum* isolate D90 from 97% to approximately 75–80% (Fig. 6). Two injections with the same dosage of ammonia gas inhibited the germination of conidia to between 45 and 70%.

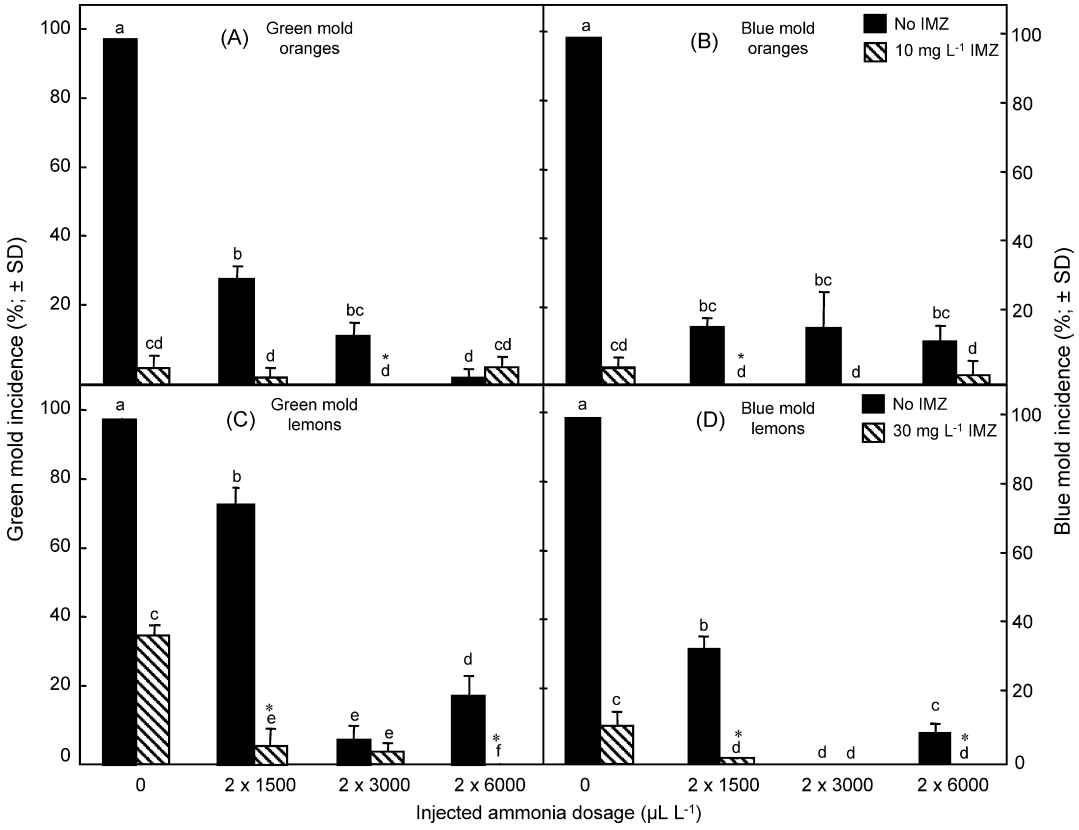

Fig. 3. Effectiveness of fumigation with two injections of ammonia on the incidence of green mold, caused by *Penicillium digitatum* (isolate D90, imazalil (IMZ) sensitive), and blue mold, caused by *Penicillium italicum*, respectively, on Valencia oranges (A and B) and Eureka lemons (C and D). The fruit were inoculated, half were treated 24 h later by immersion in 10 mg L^{-1} (oranges) or 30 mg L^{-1} (lemons) of IMZ for 30 s, and all were fumigated with ammonia once the fruit surface was dry. Values are the means of two experiments with three replicates of 20 fruit each. Error bars indicate standard deviations. Fumigation was for a period of 6 h at 22 °C with ammonia dosages of 0, 1500, 3000, or 6000 μL L^{-1} injected at the beginning of the treatment and again 2 h later. The incidence of infected fruit was determined after storage at 20 °C at high relative humidity (>95%) for 10 and 7 d, respectively, for green mold and blue mold. Values within panel with unlike letters are significantly different by Fisher's Protected LSD test ($P \leq 0.05$). Asterisk indicates synergistic activity was present between ammonia and imazalil according to Limpel's formula.

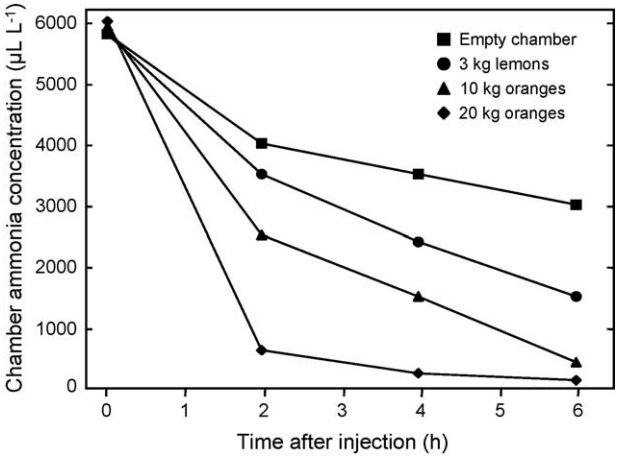

Fig. 5. Concentration of ammonia gas measured during fumigations with one injected dosage of ammonia at 6000 µL L^{-1} in chambers loaded with 0 (empty), 3, 10, or 20 kg of lemons or oranges. The test was repeated once with oranges and once with lemons and values are the means of both. The appearance of these fruit was recorded (Table 1).

Germination of the conidia of *P. digitatum* isolate D201 was reduced from 98 to 43% by one injection of 6000 µL L^{-1} of ammonia gas, and further reduced germination to 19% by a second injection (Fig. 6). *P. italicum* was the more sensitive to ammonia than *P. digitatum*; a single injection of 6000 µL L^{-1} of ammonia reduced the germination of its conidia from 99 to 22%, and further reduced germination to 11% by a second injection (Fig. 6).

3.4. Influence of ammonia fumigations on pH of fruit albedo

The pH of citrus albedo in contact with ammonia gas was significantly increased by the fumigations. Exposure of albedo to higher concentrations of ammonia significantly increased the difference between its pH before and after treatments. The pH (±SD) of the albedo of citrus fruit before fumigation was 5.9 (±0.3), while after fumigations with 1500, 3000, or 6000 µL L^{-1} ammonia it was 6.5 (±0.3), 6.8 (±0.4), or 7.2 (±0.3), respectively. When the pH was measured in the same wounds 24 h after treatment, it had declined and was not significantly different from those measured before the fumigations (data not shown).

3.5. The influence of ammonia fumigations on the natural degreening of lemons

A decrease in hue angle (h°), which indicates the transition from green to yellow color, was recorded for untreated and ammonia-fumigated lemons between days 0 and 22 of storage. On untreated lemons, hue angle values measured after 12, 20, and 22 d were 3.5°, 4.6°, and 8.8° lower than the initial value, respectively. On lemons fumigated with 6000 µL L^{-1} ammonia, hue values measured after 12, 20, and 22 d were 7.3, 9.1, and 13.2 lower, respectively (Table 2). At each time of measurement, the decrease of the hue values was significantly larger on lemons treated with ammonia than on untreated lemons. The parameters measuring lightness (L^*) and saturation (C^*) changed similarly on fumigated and untreated lemons. No injury was observed on the lemons used in this experiment.

4. Discussion

Fumigation of citrus fruit with ammonia effectively controlled postharvest green and blue molds. Generally, it controlled blue mold better than green mold, and it was more effective on oranges than on lemons. The inhibitory activity of ammonia increased as the concentration applied increased and a double injection of ammonia further improved its effectiveness. These results corroborate the findings in prior reports (Tomkins and Trout, 1931; Roistacher et al., 1955, 1957, 1958; Gunther et al., 1959a; Leggo and Seberry, 1964). Citrus fruit tolerate ammonia gas well, unlike many other fresh commodities (Phillips, 1985). Bottini (1927) first used ammonia gas, sublimed from ammonium bicarbonate, to control green and blue molds and diplodia stem end rot, caused by *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., on citrus fruit. Control of ammonia gas concentration from sublimed salts was difficult and rind injury of oranges occurred and this approach was not implemented for this reason (Tomkins and Trout, 1931; Grasovsky and Shiff, 1934; Eckert, 1967). Later, ammonia was applied as a fumigant from

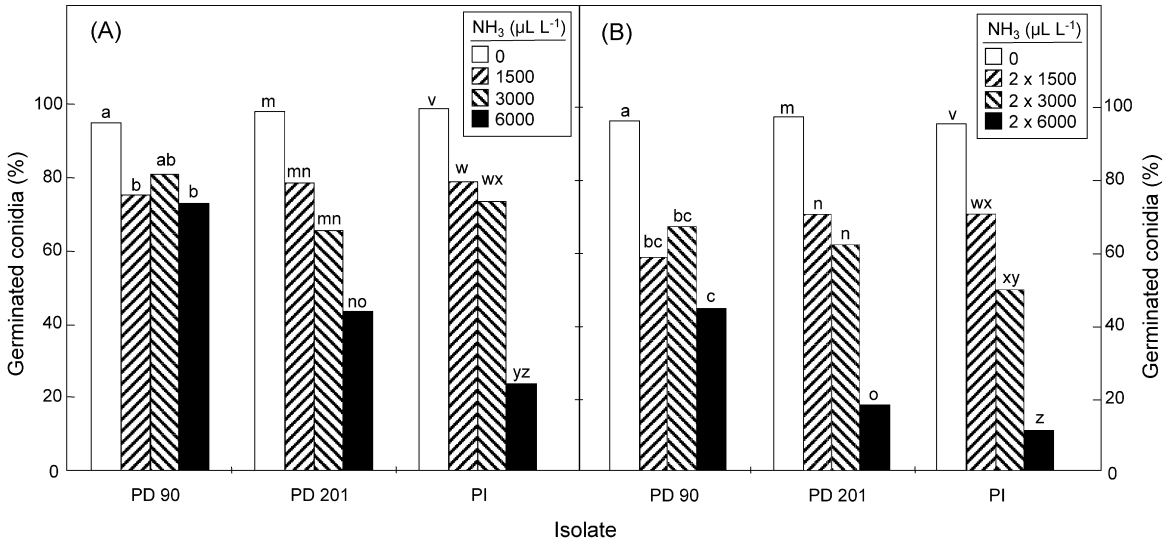

Fig. 6. Germination of conidia of *Penicillium digitatum* (imazalil-sensitive isolate PD90 and imazalil-resistant isolate PD201) and *Penicillium italicum* (PI) after fumigation for 6 h at 22 °C with dosages of 0, 1500, 3000, or 6000 µL L^{-1} of ammonia injected only at the beginning of the treatment (A) or at the beginning and again after 2 h (B). Germination was determined after incubation for 12 h at 25 °C on potato dextrose agar. Values within each isolate, fumigated by a single or double injection of ammonia, with unlike letters are significantly different by Fisher's Protected LSD test ($P \leq 0.05$). Each value is the mean from three experiments of three replicates of 150–200 conidia each.

Table 2
Color parameters of lightness (L^*; ±SD), chroma or saturation (C^*; ±SD), and hue angle (h°; ±SD) measured on non-treated lemons (control) and on lemons fumigated with 6000 $\mu L\,L^{-1}$ before (0) and 12, 20, and 22 d after fumigation and storage at 20 °C.

Days after fumigation	L^*		C^*		h°	
	Untreated	Fumigated	Untreated	Fumigated	Untreated	Fumigated
0	50.2 (0.6)	53.2 (3.0)	39.3 (0.4)	42.6 (2.4)	117.7 (0.5)	115.9 (1.4)
12	52.7 (0.3)	59.4 (2.6)	46.0 (0.9)	48.0 (0.7)	114.2 (0.3)	108.6 (0.7)
20	54.7 (4.8)	61.0 (4.7)	45.9 (4.5)	47.8 (2.7)	113.1 (3.0)	106.7 (3.5)
22	59.1 (5.2)	62.4 (4.8)	49.2 (4.0)	48.1 (3.2)	108.0 (3.7)	102.6 (4.5)

compressed cylinders where control of its concentration was facilitated. Constant storage in 50–200 $\mu L\,L^{-1}$ of ammonia controlled decay and stopped sporulation from decay lesions yet usually did not injure the fruit rind, although rind defects occurred and the calyxes were darkened in some lots (Eckert, 1967). In most prior studies, ammonia was used for relatively long periods, from 2 to 14 d. Only Roistacher et al. (1955, 1957, 1958) evaluated briefer treatments, and found a single shorter treatment (9–10 h) with an ammonia concentration of 100 $\mu L\,L^{-1}$ controlled green and blue molds. Effective ammonia *C–T* products were 500–3000 $\mu L\,L^{-1}\,h^{-1}$, while injuries occurred with higher *C–T* products (Roistacher et al., 1957). However, the application of this low concentration of ammonia constantly during treatment, as suggested by Roistacher et al. (1957), entailed repeated measurements and ammonia injections and special equipment was required for this task. In order to facilitate its commercial adoption, ammonia fumigation should be as uncomplicated and brief as possible, so we evaluated single or double injection ammonia applications for periods of 6 h.

On Valencia oranges, treatments with double injections and higher dosages of ammonia more effectively controlled green and blue molds than single injections and lower dosages. When comparing fumigations with similar *C–T* products, i.e., 3303 $\mu L\,L^{-1}\,h^{-1}$ of ammonia from two injections of 1500 $\mu L\,L^{-1}\,h^{-1}$ of ammonia compared to 3650 $\mu L\,L^{-1}\,h^{-1}$ of ammonia from a single injection of 3000 $\mu L\,L^{-1}$, double injections of a lower concentration more effectively controlled decay than a single injection of a higher dose. Roistacher et al. (1957) reported repeated dosages of the gas were believed to be more effective than single injections, and that it should be applied within 24–30-h after harvest, before the pathogens had penetrated into the rind to a depth no longer reachable by the gas. In the initial treatment, they speculated ammonia concentrations that were lethal to moist conidia within wounds may not have been sufficient to inhibit the relatively dry conidia not within wounds. Later, in the moist storage environment, most of the conidia were sufficiently hydrated to be controlled by the second ammonia dose. In the present work with Eureka lemons, double injections were always more effective than single ones, but higher dosages were not always more effective. Lemons were apparently damaged by treatments with single (*C–T* product = 7953 $\mu L\,L^{-1}\,h^{-1}$) or double injections of 6000 $\mu L\,L^{-1}$ of ammonia while the tissue darkening in wounds was absent or minor after treatments with two injections of 3000 $\mu L\,L^{-1}$ of ammonia (*C–T* product = 8564 $\mu L\,L^{-1}\,h^{-1}$). This shows the sensitivity of Eureka lemons to single high concentrations of 6000 $\mu L\,L^{-1}$ of ammonia was greater than to a similar *C–T* delivered by two injections with lower ammonia concentrations.

Risk of product injury must be established for any horticultural fumigant. Ammonia fumigations did not harm fruit with blemish-free rinds, but the exposed albedo tissue of wounded fruit darkened. The rind of Eureka lemons was more sensitive to ammonia fumigations than that of Valencia oranges. The rate of natural transition of green to yellow color of lemons was slightly accelerated by ammonia fumigation. Wounds in lemon rinds that exposed the albedo became dark brown in color after exposure to one or two injections of 6000 $\mu L\,L^{-1}$ of ammonia, while the same treatment applied to Valencia oranges caused only a slight browning of this tissue. Similar injuries were described by Roistacher et al. (1957) on lemons and navel oranges treated with two daily injections of 5000 $\mu L\,L^{-1}$ of ammonia applied daily for 4 d. Tomkins and Trout (1931) observed that neither the appearance nor taste of oranges treated with 1000 $\mu L\,L^{-1}$ of ammonia was impaired; however, after storage for 15 d, a treatment with 2000 $\mu L\,L^{-1}$ of ammonia caused considerable damage to the fruit. Leggo and Seberry (1964) observed no injuries on the rinds of Washington Navel oranges treated with 6000 $\mu L\,L^{-1}$ of ammonia for 78 h. Injuries reported in both the literature and in this study are primarily peripheral browning of fresh wounds in the rind. This effect could even have a positive aspect, since it makes fruit with injured rinds more visible, which would facilitate their detection and removal on packinglines.

Other aspects of the influence of ammonia on fruit quality were reported. Eaks (1959) examined the effect of repeated 10 h-long, 100 $\mu L\,L^{-1}$ ammonia fumigations on respiratory rate, juice composition, and appearance of oranges and lemons. Other than browning of fresh wounds, the only significant change was that premature browning and detachment of the calyxes ('buttons') on lemons, but not oranges, occurred after three weekly ammonia fumigations and subsequent storage for 4 weeks. Although not observed in our work, harm to the calyx has a negative influence on lemon quality, since intact, green 'buttons' are an important aspect of marketing quality and to retard alternaria stem end rot (Smilanick et al., 2006). Approaches to minimize calyx injury could be pre-treatment of the fruit with IMZ so lower ammonia concentrations could be used, incorporation of a low concentration treatment of 2,4-D before ammonia fumigation since 2,4-D prolongs the retention of calyx and is commonly used for that purpose (Smilanick et al., 2006), or if repeated ammonia fumigation of lemons was avoided.

Ammonia concentrations during fumigation declined rapidly during the first 2 h after injection, after which in many cases little ammonia remained within the chambers. In empty chambers, about 50% of the ammonia gas was present at the end of a 6-h fumigation. As the load of fruit increased, a faster decrease in the ammonia concentration occurred. Previous studies reported absorption of ammonia by fruit during fumigation (Roistacher et al., 1957; Gunther et al., 1959b) and it was deemed temporary since no significant ammonia residues were found in fruit after the treatments. Ammonia is a normal constituent of citrus fruit; Valencia oranges contained about 15 $\mu L\,L^{-1}$ in the clarified juice (Nelson et al., 1933). Ammonia at 50–700 $\mu L\,L^{-1}$ in air induced little, if any, measurable increase in the ammonia content of the fruit of treated citrus. Ammonia sorption was proportional to the ammonia concentration and the temperature of the fruit. Wounded, non-waxed, or immature fruit sorbed ammonia faster than non-wounded, waxed, or mature fruit (Gunther et al., 1959b). Roistacher et al. (1957) observed that *C–T* values of ammonia sufficient to cause chemically detectable increases in nitrogen residues in lemons usually resulted in severe fruit burns. Gunther et al. (1959b) reported that juice quality and organoleptic tests of ammonia-treated fruit revealed very little ammonia absorption in the juice and it was indistinguishable from juice samples of control fruit by standard

quality tests. In our limited organoleptic evaluations, no differences in flavor or aroma were detected.

The mode of action of ammonia to inhibit fungi has not been conclusively determined. Its toxicity to the microsclerotia of *Verticillium dahliae* increases with increasing pH and it is fungistatic at sub-lethal concentrations (Tenuta and Lazarovits, 2002). It conceivably inhibits green and blue mold infections by both direct action, due to the toxicity to conidia, and indirect action, by modifying the wound environment where infection occurs. In previous tests on oranges fumigated with ammonia during up to 4 d, a direct toxic effect of ammonia on the pathogens was observed, since many conidia could no longer germinate after fumigation (Roistacher et al., 1957; Leggo and Seberry, 1964). A single injection of 6000 $\mu L\,L^{-1}$ of ammonia was lethal to 25, 60, and 80% of conidia of *P. digitatum* isolates D90 and D201 and a *P. italicum* isolate, respectively, and a double injection of ammonia at this concentration further increased mortality. Differences in sensitivity of conidia to ammonia among the isolates may explain why ammonia fumigation controlled green mold caused by *P. digitatum* isolate D201 and blue mold better than they controlled green mold caused by *P. digitatum* isolate D90. Quantification of ammonia toxicity and the influence of humidity on its toxicity to *P. digitatum* and *P. italicum* are needed. However, the reduction in disease incidence by ammonia fumigation was very high and greater than the reduction in conidial germination. This supports the existence of an indirect effect of ammonia to inhibit these pathogens, probably by the induction of changes in the tissues of the wounds where infections are initiated. The pH of albedo exposed to ammonia through wounds was significantly increased by the fumigation, although the increase was reversible and typical pH values were recovered 1 d after treatments. It is conceivable that ammonia imparted a thin layer of alkalinized tissue within the wounds that significantly delayed or stopped infection, while wound healing mechanisms within the rind continued or were even stimulated to retard infection from the surviving conidia. Treatment of several *Citrus* spp. and cultivars with an alkaline solution of sodium carbonate, which reduced green mold infections by more than 90%, also temporarily raised the pH of the albedo tissue in wounds about 3 units, altered its structure to a "melted" appearance, and induced the accumulation of the phytoalexin scoparone from four to ten times in the tissue (Venditti et al., 2005). Elevated pH alone could inhibit the growth of these pathogens. The germination of *P. digitatum* is inhibited at pH 8 and above (Smilanick et al., 2005).

Local pH modulation by pathogens may be a common mechanism for increasing their virulence during infection (Prusky et al., 2001). Colonization by *Penicillium* spp. on citrus and apple fruit, a pathogen that acidifies host tissue, was enhanced by citric acid and retarded by alkalinization with $NaHCO_3$ (Prusky et al., 2004). *Penicillium* spp. acidify the tissue within lesions on citrus fruit tissue by the production of organic acids, mainly citric and gluconic, and by the utilization of ammonium ions that was correlated with an efflux of protons. Tissue pH was positively correlated with ammonium ion content, and presumably, ammonia fumigation would greatly increase ammonium ion content in this tissue, raise its pH, and retard pathogenesis by acidifying pathogens such as the *Penicillium* spp. Citrus fruit pathogens such as *Colletotrichum* spp. and/or *Alternaria alternata*, which raise the pH of lesion tissue by ammonia secretion, may not be controlled by ammonia fumigation.

The combination of IMZ treatment followed by ammonia fumigation was usually additive or synergistic in effectiveness to control green mold; IMZ concentrations as much as 50 times lower than those used commercially effectively controlled the disease. These very low rates would not be recommended; we used them to enable the interaction between these treatments to be evident. Siegel et al. (1977) showed the fungicide was more toxic to *P. italicum* at pH 7 than at pH 5, and observed that little IMZ entered the mycelium at pH 5 compared with pH 7. They ascribed this difference in potency to the charge present on the molecule and concluded that reduced toxicity of IMZ at lower pH, when the molecule is charged, occurs because it is less incorporated into the mycelium. Lukens (1971) reported that neutral forms of fungicides penetrated membranes and were more toxic than charged forms. Relatively small differences in pH can have a significant effect on the concentration of the neutral (un-dissociated) IMZ (Siegel et al., 1977; Guan et al., 1989). Above the p*K*a of IMZ (pH 6.53), the imide nitrogen of the molecule is primarily un-dissociated, the molecule is not charged, and it is more lipophilic and soluble in membranes. At pH values below the p*K*a, the imide nitrogen is primarily protonated, the molecule is charged, and it has less lipid solubility and poor penetration into membranes. Smilanick et al. (2005) empirically quantified IMZ toxicity associated with pH and reported increased pH enhanced both the activity of IMZ to inhibit conidial germination and its effectiveness to control green mold on fruit using an IMZ-sensitive or IMZ-resistant isolates, and in this study, we found in the present study ammonia fumigation increased pH of the albedo in the wounds markedly. The pH values we measured within the wounds were 5.9 and 7.2, respectively, before and after ammonia fumigation with an initial dose of 6000 $\mu L\,L^{-1}$. Applying formulas from Smilanick et al. (2005) that predicted IMZ toxicity as a function of pH, in the present study the EC_{50} of an IMZ-sensitive isolate of *P. digitatum* at pH 5.9 would be 20.0×10^{-3} mg L^{-1}, while at pH 7.2 the EC_{50} would be reduced to 5.7×10^{-3} mg L^{-1}; an increase in IMZ activity of approximately threefold. It is conceivable that the synergism between ammonia and IMZ treatments occurred in part due to the increase in IMZ toxicity at higher pH values in the wounds where IMZ is in contact with the pathogens. Other possibilities to explain the synergy between IMZ and ammonia include alteration and migration of IMZ residue within the wounds at higher pH; if a larger portion of the IMZ was neutral and more oil soluble, its distribution within the wound site may have been altered. Treatment with sodium bicarbonate, an alkaline solution, induced natural resistance and structural changes within wounds on lemons when co-applied with IMZ; ammonia may induce similar host defense responses (Dore et al., 2009).

Several issues must be addressed before using ammonia as a fumigant. Anhydrous ammonia is classified by the US Department of Transportation as nonflammable (Anon., 2006). However, ammonia vapor in high concentrations (16–25% by weight in air) will burn, although it is difficult to ignite and will not support combustion after the ignition source is withdrawn. In previous studies, ammonia fumigation and ethylene degreening were compatible (Roistacher et al., 1957; Leggo and Seberry, 1964). Ammonia fumigation could be applied within degreening chambers, either before, after, or perhaps during ethylene degreening, and the treatment would not greatly prolong the time fruit are in the chambers. In the present study, we employed short exposures and the temperatures used during degreening, in order to facilitate their combined use. The existing degreening chambers, present in most of the packinghouses in California and Spain, would need to be modified to contain the gas safely and to resist corrosion. Ammonia, especially in the presence of moisture, reacts with and corrodes copper, zinc, and many alloys (Anon., 2006). Only wood, iron, steel, certain rubbers and plastics, and specific nonferrous alloys resistant to ammonia should be used. In order to establish a standardized procedure to employ ammonia fumigation, further studies on the optimal dosages and methods of application of ammonia gas are needed that account for different citrus species and cultivars, and to account for the fruit load factor within fumigation chambers. Gunther et al. (1959b) suggested the use of fiberboard cartons, which are now the most popular packaging, should be avoided unless the amount of ammonia applied was increased to compensate for their large ammonia sorption capacity. Safe entry into

these chambers once ammonia fumigation is complete requires removal of the gas. Since rapid and complete sorption of ammonia occurs into the fruit and chamber during fumigation occurs, atmospheric release of ammonia may be minimal, although it is likely entrapment of the remaining gas would be required. Occasionally, entrapment of sulfur dioxide gas remaining after fumigation of table grapes is done using an alkaline solution in an air-washer (Nelson, 1991); with an acidic solution, similar air scrubbing could be done to entrap remaining ammonia after fumigation before chambers are opened.

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References

Anon., 2006. Ammonia as a Refrigerant. Position Document of the Board of Directors. American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., Atlanta, GA, http://www.ashrae.org/docLib/200622793710_347.pdf.

Adaskaveg, J.E., Förster, H., 2010. New developments in postharvest registrations for edible horticultural crops and use strategies in the United States. In: Prusky, D., Gullino, M.L. (Eds.), Post-Harvest Pathology. Springer, New York, pp. 107–117.

Bates, R., 2002. Pesticide residues and consumer risk assessments. Pestic. Outlook 13, 142–147.

Bond, E.J., 1984. Manual of fumigation for insect control. FAO Plant Production and Protection Paper 54 of the Food and Agriculture Organization of the United Nations.

Bottini, E., 1927. Sulla concervazione della frutta. Ricerche sperimentali esquite sulle Arancie. Ann. Chim. Appl. 17, 129–163.

Bus, V.G., Bongers, A.J., Risse, L.A., 1991. Occurence of *Penicillium digitatum* and *P. italicum* resistant to benomyl, thiabendazole, and imazalil on citrus fruit from different geographic origins. Plant Dis. 75, 1098–1100.

Dezman, D.J., Nagy, S., Brown, G.E., 1986. Postharvest fungal decay control chemicals: treatments and residues in citrus fruits. Residue Rev. 97, 37–92.

Dore, A., Molinu, G.M., Venditti, T., Petretto, A., D'Hallewin, G., 2009. Host involvement in the improved decay control following $NaHCO_3$ co-application with imazalil. Commun. Agric. Appl. Biol. Sci. 74, 715–721.

Eaks, I.L., 1959. Effect of ammonia gas fumigation on respiratory rate, chemical composition of juice, and general appearance and vitality of lemon and orange fruits. Proc. Am. Soc. Hortic. Sci. 73, 237–241.

Eckert, J.W., 1967. Application and use of postharvest fungicides. In: Torgeson, D.C. (Ed.), Fungicides. An Advanced Treatise. Academic Press, New York, pp. 287–378.

Eckert, J.W., Brown, G.E., 1986. Evaluation of postharvest treatments for citrus fruits. In: Methods for Evaluating Pesticides for Control of Plant Pathogens. APS Press, St. Paul, MN, pp. 92–97.

Eckert, J.W., Eaks, I.L., 1989. Postharvest disorders and diseases of citrus fruits. In: Reuter, W., Calavan, E.C., Carman, G.E. (Eds.), The Citrus Industry. Division of Agriculture and Natural Resources, University of California Press, Berkeley, CA, pp. 179–260.

Grasovsky, A., Shiff, M., 1934. The effect of ammonium bicarbonate on the storage of oranges. Hadar 7, 168–172.

Guan, J., Kerkenaar, A., de Waard, M.A., 1989. Effects of imazalil on sterol composition of sensitive and DMI-resistant isolates of *Penicillium italicum*. Neth. J. Plant Pathol. 95, 73–86.

Gunther, F.A., Blinn, R.C., Barkley, J.H., Kolbezen, M.J., Staggs, E.A., 1959a. Fungicides for packaged foods, generation of ammonia for in-package fumigation of foodstuffs. J. Agric. Food Chem. 7, 489–496.

Gunther, F.A., Blinn, R.C., Kolbezen, M.J., Conkin, R.A., Wilson, C.W., 1959b. Fungicides for package foods, sorption of ammonia by fruits, vegetables, eggs, and fiberboard in dynamic systems. J. Agric. Food Chem. 7, 496–502.

Holmes, G.J., Eckert, J.W., 1999. Sensitivity of *Penicillium digitatum* and *P. italicum* to postharvest citrus fungicides in California. Phytopathology 89, 716–721.

Kinay, P., Mansour, M.F., Gabler, F.M., Margosan, D.A., Smilanick, J.L., 2007. Characterization of fungicide-resistant isolates of *Penicillium digitatum* collected in California. Crop Prot. 26, 647–656.

Leggo, D., Seberry, J.A., 1964. Ammonia fumigation for reducing green mould wastage in oranges during ethylene degreening. Austral. J. Exp. Agric. Anim. Husb. 4, 173–177.

Lukens, R.J., 1971. Chemistry of Fungicidal Action. Springer-Verlag, New York.

Montesinos-Herrero, C., Palou, L., 2010. Combination of physical and low-toxicity chemical postharvest treatments for integrated disease management of citrus fruit: a review. Stewart Postharvest Rev. 1, 1.

Montesinos-Herrero, C., del Río, M.A., Pastor, C., Brunetti, O., Palou, L., 2009. Evaluation of brief potassium sorbate dips to control postharvest Penicillium decay on major citrus species and cultivars. Postharvest Biol. Technol. 52, 117–125.

Nelson, K.E., 1991. Quality and preservation of fruits. In: Eskin, N.A.M. (Ed.), The Grape. CRC Press, Boca Raton, FL, pp. 125–167.

Nelson, E.K., Mottern, H.H., Eddy, C.W., 1933. Nitrogenous constituents on Florida Valencia orange juice. Fruit Prod. J. 12, 231–235, 250.

Palou, L., Smilanick, J.L., Droby, S., 2008. Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. Stewart Postharvest Rev. 2, 1.

Phillips, D.J., 1985. Detecting ammonia injury on stone fruit. Int. J. Refrig. 8, 54–55.

Prusky, D., McEvoy, J.L., Leverentz, B., Conway, W.S., 2001. Local modulation of host pH by *Colletotrichum* species as a mechanism to increase virulence. Mol. Plant-Microbe Interact. 14, 1105–1113.

Prusky, D., McEvoy, J.L., Saftner, R.A., Conway, W.S., Jones, R., 2004. Relationship between host acidification and virulence of *Penicillium* spp. on apple and citrus fruit. Phytopathology 94, 44–51.

Ritcher, D.L., 1987. Synergism: a patent point of view. Pestic. Sci. 19, 309–315.

Roistacher, C.N., Eaks, I.L., Klotz, L.J., 1955. Ammonia gas to control blue-green mold decay of citrus fruits. Plant Dis. Rep. 39, 202–205.

Roistacher, C.N., Klotz, L.J., Eaks, I.L., 1957. Ammonia gas used in citrus packing plants as fumigant for control of blue-green mold on Valencias, navels and lemons. Calif. Agric. 11, 11–12.

Roistacher, C.N., Klotz, L.J., Kolbezen, M.J., Staggs, E.A., 1958. Some factors in the control of blue-green mold decay of citrus fruit with ammonia. Plant Dis. Rep. 42, 1112–1122.

Siegel, M.R., Kerkenaar, A., Kaars Sijpesteijn, A., 1977. Antifungal activity of the synthetic fungicide imazalil. Neth. J. Plant Pathol. 83, 121–133.

Smilanick, J.L., Brown, G.E., Eckert, J.W., 2006. Postharvest citrus diseases and their control. In: Wardowski, W.F., Miller, W.M., Hall, D.J., Grierson, W. (Eds.), Fresh Citrus Fruits, second ed. Florida Science Source, Inc., Longboat Key, FL, USA, pp. 339–396.

Smilanick, J.L., Mansour, M., Gabler, F.M., Sorenson, D., 2008. Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides. Postharvest Biol. Technol. 47, 226–238.

Smilanick, J.L., Mansour, M.F., Margosan, D.A., Mlikota-Gabler, F., Goodwine, W.R., 2005. Influence of pH and $NaHCO_3$ on effectiveness of imazalil to inhibit germination of *Penicillium digitatum* and to control postharvest green mold on citrus fruit. Plant Dis. 89, 640–648.

Tenuta, M., Lazarovits, G., 2002. Ammonia and nitrous acid from nitrogenous amendments kill the microsclerotia of *Verticillium dahliae*. Phytopathology 92, 255–264.

Tomkins, R.G., Trout, S.A., 1931. The use of ammonia and ammonium salts for the prevention of green mould in citrus. J. Pomol. Hortic. Sci. 9, 257–264.

Venditti, T., Molinu, M.G., Dore, A., Agabbio, M., D'Hallewin, G., 2005. Sodium carbonate treatment induces scoparone accumulation, structural changes, and alkalinization in the albedo of citrus fruits. J. Agric. Food Chem. 53, 3510–3518.

Wardowski, W.F., Miller, W.M., Grierson, W., 2006. Degreening. In: Wardowski, W.F., Miller, W.M., Hall, D.J., Grierson, W. (Eds.), Fresh Citrus Fruits, second ed. Florida Science Source, Inc., Longboat Key, FL, USA, pp. 277–298.