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Control of Turf Grass Seedborne Pathogenic Fungi by Ozone

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ABSTRACT

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Ozone was evaluated to reveal its efficacy for controlling turf grass seedborne pathogenic fungi, *Bipolaris australiensis*, *Curvularia pallescens* and *Exserohilum rostratum*. Fumigation of seeds infested by these fungi with ozone at 240 ppb for 6 consecutive days (4 hr/day) failed to increase seed germination. Ozone-saturated water, however, was found to be highly inhibitory on the conidial germination of three tested fungi. The correlation curve between percent inhibition of conidial germination and duration of treatment in ozonated water was polynomial regression for all three fungi. The duration needed to completely kill the conidia of *B. australiensis*, *C. pallescens* and *E. rostratum* was approximately 10, 13 and 30 min, respectively. *E. rostratum* was obviously the most tolerant to ozone; because its conidia have the thickest wall as comparing to that of other tested fungi. The control efficacy of turf grass seedborne fungi by application of ozonated water was found as good as that by application of antagonistic *Trichoderma* sp. and *Bacillus megasperium* and by two conventional fungicides tested. The seedling growth vigor, however, was apparently better from seeds treated with ozonated water. The potential application of ozonated water in seed treatment is herein discussed.

Key words: ozonated water, turf grass seedborne fungi, control.

INTRODUCTION

Turf grass coverage of green land is becoming more and more important nowadays. It is used for vegetation on hill slopes, the courts of various athletics and entertainment, etc. To cope with the increasing demands of green land establishment, approximately 700 tons of turf grass seeds were imported in 1996 to Taiwan (2). Some of these seeds were found infested or infected with certain plant pathogenic fungi, predominantly the *Bipolaris australiensis* (Bugn.) Ellis, *Curvularia pallescens* Boedijn. and *Exserohilum rostratum* (Drechsl.) Leonard & Suggs. (10). An obvious effect of these seedborne pathogenic fungi was the deterioration of seed quality and the significantly decreased germination rates. In our previous studies we have shown that two fungicides, Difenoconazole 25% EC and Imazalil 21.2 % EC and two biological control agents, *Trichoderma* sp. and *Bacillus megaterium* (9) could successfully control these seedborne pathogenic fungi.

However, use of various fungicides to control seedborne pathogenic fungi has become an issue of great concern to the general public, especially application in areas close to the drinking water sources. In addition, the use of chemicals poses potential harmful effects on the ecosystem. The application of biological control agents was effective to some extent in controlling the fungal infection, however it was not comparable to that by chemical application (9). Therefore, it is important to find an alternative way to control these seedborne pathogenic fungi and reduce the dependency on fungicide use.The objectives of this study were to determine the toxicity of ozone gas and aqueous ozone solution to conidia of *B. australiensis*, *C. pallescens* and *E. rostratum*; to determine the effectiveness of ozone-saturated water in the control of seedborne pathogenic fungi of Bermuda grass; and to compare ozone-saturated water with fungicides and antagonistic microorganisms in controlling the disease.

MATERIALS AND METHODS

Sources of pathogenic fungi

The three seedborne pathogenic fungi used for the experiment were isolated from turf grass seeds. Among them, *B. australiensis* was from Bermuda grass (*Cynodon dactylon* L.), *C. pallescens* was from centipede grass (*Eremochlo aphiuroides* Hack.), and *E. rostratum* was from love grass (*Eragrostis curvula* Schrad.) (5). They were grown and kept on potato dextrose agar (PDA) slants at room temperature about 25±3 C. Periodically about one month culture was renewed on the same medium.

Artificial inoculation of turf grass seeds with seedborne pathogenic fungal conidia

Bermuda grass seeds were surface-sterilized with 1% sodium hypochlorite for 10 min and then rinsed with sterile distilled water twice. The conidia were harvested from 7 days' culture of *C. pallescens* and *B. australiensis*, or from 2 weeks' culture of *E. rostratum* on PDA plates with sterile distilled water. Conidial concentration in suspension was adjusted to 10^4conidia/ml. The disinfested seeds were immersed in conidial suspension for 30 min and then spread on tissue paper. The seeds were air-dried in a 30 C incubator.

Quantitating ozone in an aqueous phase

The method developed by Bader and Hoigne (3) using indigo as an indicator was applied. Stock solution of indigo was prepared using 1 ml phosphoric acid and 770 mg potassium indigo trisulfonate. The test samples were kept in 100-ml volumetric flasks for the experiment. For sample which contains 0.01-0.1 mg/L ozone, 10 ml of indigo reagent I (20 ml indigo stock solution, 10 g NaH_2PO_4, and 7 ml concentrated phosphoric acid, diluted to 1 L) were added. Whereas for that which contains 0.1-0.5 mg/L ozone, 10 ml of indigo reagent Ⅱ (100 ml indigo stock solution, 10 g NaH_2PO_4, and 7 ml concentrated phosphoric acid, diluted to 1 L) were added. The sample flasks were then filled to the mark with distilled water. Flasks containing sample without ozone were processed by the same protocol and used as the compared control. The A_{600} of both tested and control solutions was read by a Shimadzu UV-160A Spectrophotometer in a 1.0-cm cuvette.

Effect of ozone on spore germination

Ozone was generated by passing pure oxygen through an ozone generator (OREC model 03B1-D, 1.5 l/min). The ozone was mixed with stirring water at 24 C in a flask for 1 hr and the status of ozone saturation (approximately 3.52-4.76 mg/l) of the aqueous solution was examined by indigo test as above described. The pH of the solutions was approximately 6.0 which was not affected by the ozone concentration. Conidia of *B. australiensis, C. pallescen* and *E. rostratum* used for the experiment were harvested from 14-day-old culture growing on autoclaved seeds of Bermuda grass, suspended in sterile distilled water and centrifuged at 3,944 g (Sigma 3K30). After centrifugation, the spores were resuspended in sterile distilled water and adjusted to the concentration of 10^7 conidia/ml. This stock suspension was added to the ozone-saturated water to make a final concentration of 5×10^3 conidia/ml. After exposure for 0 (control), 1, 3, 5, 10, 15, 30 and 45 min, a 10-ml sample was removed and passed through a 0.22-m millipore membrane. The conidia retained on the membrane were then washed with 10 ml of sterile distilled water, placed on a glass slide in the moisture chamber and incubated at 25 C. Conidial germination of *C. pallescens* and *B. australiensis* were examined microscopically 2 hr after incubation while that of *E. rostratum* was examined 6 hr after incubation. A conidium was considered germinated when the germ tube length exceeded half the diameter of the conidium. All the experiments conducted were repeated at least four times.

Control of seedborne fungi by ozone fumigation

Gaseous ozone produced from ozone generator was introduced into a small glass chamber measured 2×2×2.5 m. The concentration of ozone in the glass chamber was adjusted with flow meter and ozone monitor (Dasibi Environmental Corp., Model 1008-PC) to 60±10 ppb, 120± 20 ppb and 240±40 ppb, respectively. Bermuda seeds infested with seedborne pathogenic fungi were wrapped with double layers of cheesecloth. Half of the treated seeds were kept dry; and the other half were kept wet by dipping one end of cheesecloth in a beaker filled with sterile distilled water. Ozone fumigation started from 10:00 am to 14:00pm for 4 hr daily which was proceeded for 5 consecutive days. After the fumigation seeds were placed on moist filter paper in a Petri dish and examined for germination and fungal infestation. Control seeds were also wrapped in cheesecloth and placed in glass chamber likewise except that ozone was not applied. All the treatments had three replications and the rate of seed germination and fungal infestation were recorded one week after treatment.

Control of seedborne pathogenic fungi by ozone-saturated water

Ozone gas produced from generator was introduced into beaker with l L of distilled water by a teflon tube. The water become saturated with ozone in 1 hr; with concentration approximately 5.23—6.43 mg/L at 18 C. Seeds which were artificially inoculated with tested seedborne fungi were immersed in this ozone-satruated water for 10, 20, 30 and 40 min. Infested seeds immersed in water saturated with common air for the same periods of time were used as compared control. After treatment, seeds were placed on three layers of filter paper in Petri dishes (9 cm diameter) and incubated at 25 C. Each dish contained 100 seeds and each treatment had 3 replicates. The number of seeds colonized by the pathogenic fungi and number of seed germinating were recorded 7 days after treatment.

Measurement of conidial wall thickness by electron microscopy

The fungi were grown on Bermuda grass seeds for 20 days. Conidia were washed off by sterile distilled water and centrifuged at 3944 g (Sigma 3K30) for 10 min. The conidia in pellet were fixed in 5% glutaldehyde over night at 4 C. After discarding fixation solution by centrifugation, conidia in pellet were mixed with 2% water agar and washed with 0.1M phosphate buffer (pH 7.0) for 4 times. Post fixation was done by 2% osmium tetraoxide, dehydrated in a 50 to 100% ethanol series, embedded in LR White acrylic resin, and then ultrathin sectioned by an ultramicrotome. The sections were stained by uranyl acetate and lead citrate according to Reynolds' method (13). The measurement of conidial wall thickness was done by a transmission electron microscope (JEOL, model JEM-200CX).

Comparison of disease control efficacy by ozonated water, antagonistic microorganism and fungicide treatment

Two antagonistic microorganisms, including *Trichoderma* sp. (isolates TS1 and TA1) and *Bacillus megaterium* de Bary (isolates 5 and 7), and two fungicides including 25% Difenoconazole EC and 21.2% Imazalil EC, as selected from previous works (9) were used as compared control treatments. Bermuda grass seeds infested with pathogenic fungi were treated with either saturated ozone water for 30 min, or 3,000x diluted fungicides for 10 min, coated with *Trichoderma* sp. spores at rate of 10^7/ml or coated with *B. megaterium* at rate of 10^9cfu/ml in 0.1% Arabic gum. One hundred seeds sampled from these treatments were then sown in 15cm plastic pots containing sand, loam soil and peat moss at proportion of 3:1:1 (v:v:v). Each treatment had three replicates. The clean and infested seeds without the applied chemical or biological treatment were also included as the compared control. Percent of seed germination and dry weight were recorded 10 and 30 days after sowing, respectively. At harvest, the potted soil was thoroughly wetted and the whole turf grasses were carefully removed from the soil. They were weighted after oven dried at 80 C for 36 hr.

RESULTS

Effect of ozone-saturated water on conidial germination

The ozone-saturated water treatment significantly inhibited the conidial germination of three tested seedborne fungi. The correlation between percent inhibition of conidial germination and duration of the applied treatment appeared to be polynomial regression for all three fungi (Figs. 1 & 2). These regression data indicated that the duration needed to

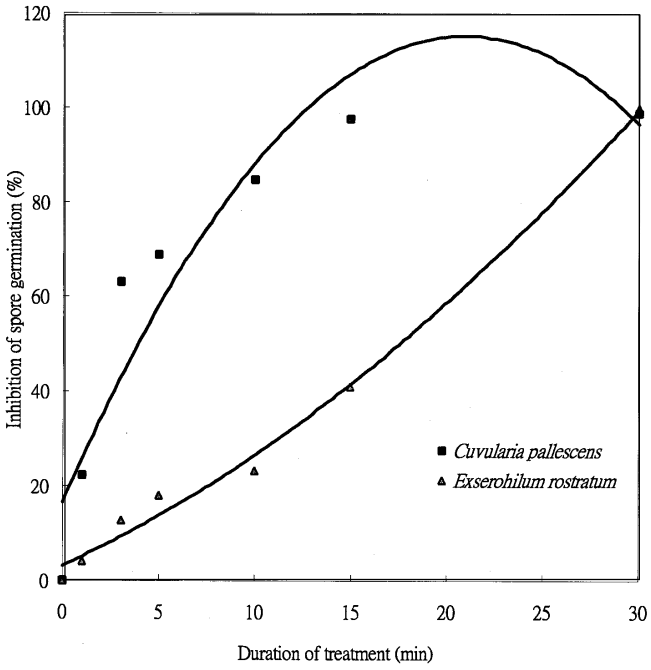

Fig. 1. Inhibition of spore germination of *Curvularia pallescents* and *Exserohilum rostratum* after treated in saturated ozonated water. regression equation for *C. pallescents* is $Y=-0.2253\ X^2+7\ X+16.553$ ($R^2=0.8939$) and for *E. rostratum* is $Y=0.0438\ X^2+1.9028\ X+3.1158$ ($R^2=0.9926$)

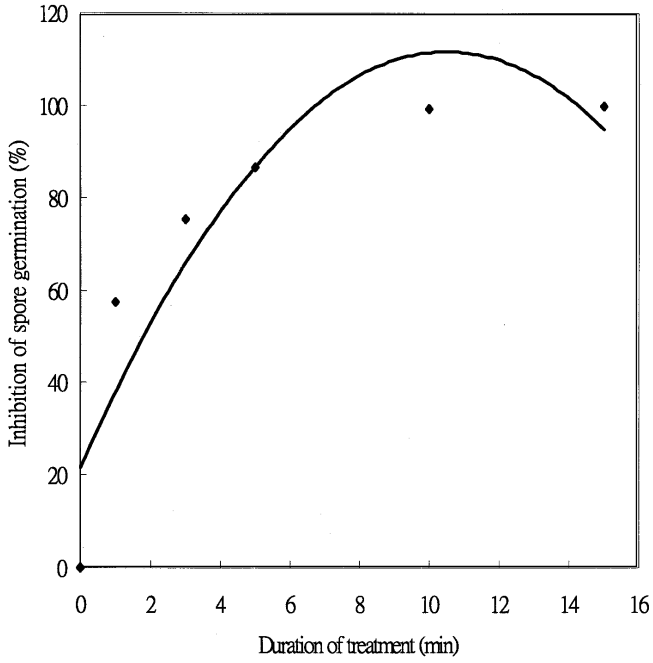

Fig. 2. Inhibition of spore germination of *Bipolaris australiensis* after treated in saturated ozonated water. Regression equation is $Y = -0.8171 X^2 + 17.16 X + 21.546$ (R^2=0.8458)

achieve 100% killing of conidia of *B. australiensis, C. pallescens* and *E. rostratum* was about 10, 13 and 30 min, respectively. It was apparent that among three tested fungi, *B. australiensis* was the most sensitive, while *E. rostratum* was the most tolerant. Approximately 50% of *E. rostratum* conidia remained viable after treated in the ozone-saturated water for 15 min.

Control of tested seedborne fungi by ozone fumigation

The applied ozone fumigation apparently failed to control the three tested seedborne fungi on Bermuda grass seeds. For seeds which were fumigated for 6 days (4 hr/day) at concentration of 240 ppb, neither the increase of seed germination, nor the decrease of colonization were observed.

Control of tested seedborne fungi by ozone-saturated water

The application of ozone-saturated water was not able to completely eliminate tested fungi infested on Bermuda grass seeds. However, as shown in Table 1, colonization of seeds by these fungi during the germination process was found significantly decreased by immersing the seeds in ozone-saturated water for 10 min. With the increase of treating time the decrease of colonization became more apparent. In case of infestation by *B. australiensis* a 21.3 % fungal colonization was detected from seeds treated in ozone-saturated water for 10 min. Whereas when the treating time increased to 40 min, the rate of fungal colonization decreased to 13.6%. As a contrast, the rate of fungal colonization of the compared control seeds without ozone treatment was more than 79%. The rate of colonization by *C. pallescens* on seeds treated with only water was between 12.7-21.3%. After treated with ozone-saturated water for 10, 20, 30 and 40 min, the colonization rate was reduced to 6.7, 5.3, 4.3 and 2%, respectively. Similarly, the rate of colonization by *E. rostratum* among seeds treated with only water was between 15.7-17.7%. After treated with ozone-saturated water for 10, 20, 30 and 40 min it was reduced to 8.3, 5.7, 4,7 and 1.7%, respectively (Table 1). The application of ozone-saturated water apparently reduced the activity of the test fungi on grass seeds and improved the seed germination rate (Table 2). Germination rates of all infested seeds treated in ozone-saturated water were significantly higher than the compared control seeds, which were treated with only water, but the 10-min treatment seems to be the most adequate. The prolonged treating time did not further improve the rate of germination. On the contrary, as treating time prolonged to 30 or 40 min,

TABLE 1. Colonization rates of infested Bermuda grass seeds after treated with saturated ozonated water

Pathogen	Ozone treating time (min)				Water treating time (min)			
infested[1]	10	20	30	40	10	20	30	40
Ba	21.3 b[2]	22.7 b	16.0 b	13.6 c	79.0 a	82.0 a	83.3 a	82.3 a
Cp	6.7 cd	5.3 d	4.3 d	2.0 d	17.7ab	17.3 ab	21.3 a	12.7 c
Er	8.3 b	5.7 bc	4.7 bc	1.7 c	17.7 a	15.7 a	17.7 a	17.3 a
CK	1.0 ab	0.7 b	1.3 ab	1.3 ab	3.7 a	1.3 ab	0.3 b	2.3 ab

1. CK= seeds without inoculated with any seedborne pathogenic fungi; Ba = *Bipolaris australiensis*; Cp = *Curvularia pallescens*; Er = *Exserohilum rostratum*.
2. Means (n=3) of seeds colonized by the pathogenic fungus in percent, figures in the same row followed by same letter are not significantly different (P =0.05) according to Duncan's multiple range test.

TABLE 2. Germination rate of infested Bermuda seeds after treated with saturated ozonated water

Pathogen	Ozone treating time (min)				Water treating time (min)			
infested[1]	10	20	30	40	10	20	30	40
Ba	28.7 a[2]	27.7 a	29.7 a	22.3 a	4.7 b	5.0 b	5.7 b	4.3 b
Cp	28.0 a	26.3 a	26.0 ab	24.3 b	19.0 c	19.7 c	15.7 c	19.0 c
Er	31.3 a	25.7 a	21.3 b	24.0 ab	7.7d	15.7 c	7.7 d	12.6 c
CK	38.0 a	38.3 a	35.0 abc	29.0 c	36.0abc	33.3 bc	43.3 a	36.7abc

1. CK= seeds without inoculated with any seedborne pathogenic fungi; Ba = *Bipolaris australiensis*; Cp = *Curvularia pallescens*; Er = *Exserohilum rostratum*.
2. Means (n=3) of seed germination in percent, figures in the same row followed by same letter are not significantly different (P =0.05) according to Duncan's multiple range test.

slightly decreased seed germination was observed (Table 2).

Measurement of conidial wall thickness by electron microscopy

Transmission electron microscopy of the three tested fungi revealed that conidial wall was the thickest at both ends and the thinnest at places near septa. The measurement of wall thickness taken at middle of the conidia indicated that *E. rostratum* had the thickest wall among the three tested fungi. The average of 30 measurements obtained from *E. rostratum* was around 4.4 m, whereas those of *B. australiensis* and *C. pallescens* were around 2.1 and 2.0 m, respectively

Comparisons of disease control efficacy by ozonated water, antagonistic microorganism and fungicide treatments

The infested seeds without biological or chemical treatment had germination rates between 22.0 to 24.3%, while the compared control seeds without infestation by the 3 test fungi had germination rate of 33.3%. The efficacy of applied biological (*Trichoderma sp.* and *B. megaterium*) and chemical (Difenoconazole and Imazalil) in reducing the fungal infestation was manifested by the significantly increased rate of seed germination shown in Table 3. Among most of the applied treatments, rates of seed germination were raised to the level close to that of control seeds that were not artificially inoculated with the test fungi. Besides the improvement of seedling stands, the applied treatment also showed apparent effect in improving the growth vigor (Table 4). Among the applied treatments, the improvement of seedling growth seemed to be most prominent by ozone application. The measurement by dry weight indicated that for seeds with infestation by *E. rostratum* and B. austaliensis, treatment by ozonated water provided the best protection as regards to the resulted seedling growth vigor. Whereas for seeds infested with *C. pallescens*, growth vigor resulted from

TABLE 3. Control of Bermuda grass seedborne pathogenic fungi by saturated ozonated water, antagonistic microorganisms and fungicides

Treatment	*C. pallescens*	*E. rostratum*	*B. australiensis*
Saturated ozonated water for 30 min	36.33 abc[1]	36.00 ab	36.00 ab
Trichoderma sp. (TS1) [2]	32.33 bc	33.00 ab	33.00 ab
Trichoderma sp. (TA1) [2]	40.00 ab	36.67 ab	36.67 ab
Bacillus megaterium (B5) [3]	37.00 ab	37.33 ab	37.33 ab
Bacillus megaterium (B7) [3]	40.67 ab	30.67 bc	30.67 bc
Difenoconazole [4]	36.00 abc	31.67 ab	31.67 ab
Imazalil [4]	29.00 cd	38.67 a	38.67 a
CK(none treatment) [5]	22.00 d	24.34 c	24.33 c

1. Means (n＝3) of percent germination of Bermuda grass seeds in soil. Numbers within the same column followed by same letter are not significantly different (P＝0.05) according to Duncan's new multiple range test.
2. Infested Bermuda grass seeds coated with 10^7 conidia/ml in 0.2% Arabic gum.
3. Infested Bermuda grass seeds coated with 10^9 cfu/ml in 0.2% Arabic gum.
4. Infested Bermuda grass seeds immersed in 3000x dilution of fungicide for 10 min.
5. Infested seeds without any treatment served as CK; while seeds, which were not artificially infested with the three tested fungi, had germination rate around 33.3%.

TABLE 4. Effect of the applied seed treatments on the growth vigor of the Bermuda grass seedlings

Treatments	*C. pallescens*	*E. rostratum*	*B. australiensis*
Ozone-saturated water for 30 min	0.55 abc[1]	0.70 a	0.67 a
Trichoderma sp. (TS1) [2]	0.61 a	0.35 de	0.41 bc
Trichoderma sp. (TA1) [2]	0.54 abc	0.50 bcd	0.66 a
Bacillus megaterium (B5) [3]	0.39 cd	0.37 de	0.43 bc
Bacillus megaterium (B7) [3]	0.45 a-d	0.39 cde	0.35 c
Difenoconazole [4]	0.58 ab	0.56 bc	0.67 a
Imazalil [4]	0.41 bcd	0.58 b	0.57 ab
CK (none) [5]	0.29 d	0.29 e	0.29 c

1. Dry weight (g)/pot, data are means of three duplicates obtained 30 days after sowing. Values within a column followed by same letter are not significantly different (P＝0.05) according to Duncan's new multiple range test.
2. Infested seeds coated with 10^7 conidia/ml suspended in 0.2% Arabic gum.
3. Infested seeds coated with 10^9 cfu/ml suspended in 0.2% Arabic gum.
4. Infested seeds immersed in 3000x dilution of fungicides.
5. Infested seeds without any treatment; seedling obtained from seeds which were not artificially infested with tested fungi had dry weight around 0.45 g/pot.

ozone treatment was second only to that from application of biocontrol agent *Trichoderma* sp. The increment of growth vigor provided by ozone treatment all appeared to be significant as compared to that from control seeds without any treatment (Table 4).

DISCUSSION

Ozone has very powerful oxidation capability and can disinfest or disinfect microorganisms. It has been used to disinfest drinking water as early as 1893 (14). In the past decades many efforts have been devoted by the environmental microbiologists to explore the potential application of its germicidal effects to reduce the contagious effects from bacteria, viruses and protozoa (7, 11). Ozonated water has been successfully used to disinfest postharvest pathogens of pear (17), contaminating microbes on poultry meat (19), shrimp pathogens in seawater (4), food-related microorganisms (12) and pathogens in plant nutrient solution (15, 18).

The conidial germination of three-tested seedborne fungi was greatly inhibited by ozone-saturated water. The inhibitory effect was evidently due to killing of conidia by ozone applied. For the purpose of disinfestation of turf grass seeds, ozone-saturated water appeared to be a rather convenient system for field practices. It was thus used in all experiments proceeded. In regard to the killing of target microorganism, the results as above provided showed that the concentration of ozone and duration of treatment required to kill fungal conidia are considerably higher and longer than that known to inactivate bacteria, viruses and certain fungal species (17). For example, the LD_{95} for conidia of *Botrytis cinerea* with a 5-min exposure was 0.99 μg/ml (17) and the LD_{99} for *Escherichia. coli* with a 0.33-min exposure is only 0.06 μg/ml (7). For conidia of *B. australiensis*, *C. pallescens* and *E. rostratum*, the exposure time needed to achieve LD_{100} using ozone-saturated water (5.23-6.43 μg/ml) were 10-, 13-, and 30-min, respectively. The morphology of the conidia was known to have some connections to the sensitivities to ozone. It was reported that most of sensitive conidia were relatively small in size and hyaline, whereas the most resistant ones were large and pigmented (5). The reason seemed to apply for the relatively greater resistance of *E. rostratum* to the applied ozone treatment as compared to that of *B. australiensis* and *C. pallescens.* Our studies showed that conidia of *E. rostratum* have relatively thicker wall, and were dark pigmented, large and multiple cellular. As a contrast, conidia of *B. australiensis* and *C. pallescens* have relatively thinner cell wall and therefore are less resistant to ozone.

The ozone fumigation treatment at applied dosage was ineffective in reducing infection of the 3 tested fungi on Bermuda seeds. The applied concentration of ozone at 24 pphm (parts per hundred million) might be simply too low in order to achieve the desired fungicidal activity. Hibben and Stotzky (5) reported that large pigmented fungal spores such as that produced by *Chaetomium* sp., *Stemphylium sarcinaeforme, S. loti,* and *Alternaria* sp. were insensitive to gaseous ozone at 100 pphm. For killing the conidia of the 3-tested seedborne fungi, the applied ozonated water was rather effective. The conidial germination was all totally inhibited

after a short exposure in ozonated water. However, the same ozonated water and treatment time was apparently not enough to completely eliminate their viability. An early report also showed that gaseous ozone was not able to reduce the wounding infection of apple by an artificially inoculated pathogen (16). Obviously, ozone is a strong oxidant, which can react readily with free particles in air or water. Whereas for inactivation of microorganisms attached to or embedded in plant tissue, the efficacy was greatly deterred by the complication of the in vivo system (17).

Approximately 90% of field crop productions are through seed propagation. As a consequence, seed qualities, including genetic basis, percent infestation or infection by a potential pathogen, and factors which affect rate of germination and seedling growth vigor are all very important in regarding to the subsequent growth and production of the crops. Among these influencing factors seed infestation or infection by pathogens was known to be most crucial and deserved great attention (8).

Due to small sizes, wind, rainfall and animals including human being easily disseminate seeds. In addition, the greatly increased international trading activities also greatly accelerated the speed of seed distribution around the world. By using seeds as a carrier, plant pathogens can be disseminated more readily and widely. Plant pathogens residing on or in seeds were known in many cases to be the main primary inoculum sources responsible for incidence of serious epidemic (3). The diseases can very easily spread out especially through this channel to the new areas. Therefore most of the countries all over the world are very concern about seed health. Strict regulation regarding to examination of seed health, quarantine, and seed treatments are generally practiced to reduce the potential threat of disease dissemination and occurrence due to the use of bad quality of seeds (1, 6). The great efficacy of ozonated water treatment in reducing the infection by the 3 tested seedborne fungi indicated that the same method might be also ideal to knock out the dangerous pathogens from most of the commercial seed products.

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摘　要

謝式坤鈺、甯順熙、曾德賜 1998. 臭氧對草種種媒病原真菌之防治。植病會刊 7:105-112. (臺中市國立中興大學 植物病理學研究所)。

本研究利用臭氧來防治草種種媒病原真菌 *Bipolaris australiensis, Curvularia pallescens* 和 *Exserohilum rostratum*，利用臭氧氣體在 240 ppb 濃度連續六天、每天四小時燻蒸處理無法減少種子帶菌率或增加種子發芽率。臭氧飽和水溶液則可有效抑制這三種真菌分生孢子之發芽，統計上臭氧水處理時間和分生孢子發芽之抑制率之相關性為多項式回歸關係。臭氧飽和水溶液導致 *B. australiensis, C. pallescens* 和 *E. rostratum* 分生孢子 100% 致死率處理時間分別為 10, 13 和30分鐘，顯示三種供試真菌中 *B. australiensis* 對臭氧最敏感，而 *E. rostratum* 最具耐性，三種真菌中之細胞壁也以 *E. rostratum* 最厚。臭氧水並無法完全消除草種上之病菌，但可降低種子發芽時之病菌纏據率，並因而提高發芽率。盆栽試驗比較結果顯示臭氧水、生物防治製劑 (*Trichoderma sp. 和 Bacillus megaterium*) 與兩種傳統化學藥劑對三種種媒真菌具同等之防治效果，唯經臭氧水處理之種子，其幼苗生長勢顯較其他處理為佳，本文一併討論以臭氧水處理在種傳病害防治上之應用潛力。

關鍵詞：臭氧水、草種種媒真菌、防治。