Research Article Open Access

In Vitro and *In Vivo* Inhibitory Effects of Gaseous Chlorine Dioxide against *Fusarium oxysporum* f. sp. *batatas* Isolated from Stored Sweetpotato: Study II

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Chlorine dioxide (ClO₂) has been widely used as an effective disinfectant to control fungal contamination during postharvest crop storage. In this study, *Fusarium oxysporum* f. sp. *batatas* SP-f6 from the black rot symptom of sweetpotato was isolated and identified using phylogenetic analysis of elongation factor 1-α gene; we further examined the *in vitro* and *in vivo* inhibitory activities of ClO₂ gas against the fungus. In the *in vitro* medium tests, fungal population was significantly inhibited upon increasing the concentration and exposure time. In *in vivo* tests, spore suspensions were drop-inoculated onto sweetpotato slices, followed by treatment using various ClO₂ concentrations and treatment times to assess fungus-induced disease development in the slices. Lesion diameters decreased at the tested ClO₂ concentrations over time. When sweetpotato roots were dip-inoculated in spore suspensions prior to treatment with 20 and 40 ppm of ClO₂ for 0-60 min, fungal populations significantly decreased at the tested concentrations for 30-60 min. Taken together, these re-sults showed that ClO₂ gas can effectively inhibit fungal growth and disease development caused by *F. oxysporum* f. sp. *batatas* on sweetpotato. Therefore, ClO₂ gas may be used as a sanitizer to control this fungus during postharvest storage of sweetpotato.

Keywords : chlorine dioxide, elongation factor 1-α gene, *Fusarium oxysporum* f. sp. *batatas*, Fusarium wilt, sweetpotato

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Chlorine has been widely applied to control postharvest diseases in various fruits and vegetables such as blueberries, cucumber, oranges, potatoes, strawberries, and tomatoes (Bhagat et al., 2011; Mahovic et al., 2007; Popa et al., 2007; Tweddell et al., 2003; Yuk et al., 2006). Chlorine has a strong oxidation activity on cellular constituents and combines directly with membrane proteins and enzymes (Tweddell et al., 2003). Chlorine, with these action traits, can destroy various microbes existing on fruits and vegetables. Owing to this ability, chlorine dioxide (ClO₂) could be used as an alternative sanitizer as its oxidation capacity is 2.5 times higher than that of chlorine (Beuchat et al., 2004). ClO₂ works effectively and rapidly against microbes at low concentrations while its toxicity to human and animals is considered low (Vaid et al., 2010; Wang et al., 2016). Thus, ClO₂ as a sanitizer has been approved for agricultural and medical uses by the United States Environmental Protection Agency (EPA) (2006). Recently, the application of gaseous ClO₂ (e.g., ClO₂ concentration, time, and temperature) against pathogenic microbes on posthar-

vest stage and storage of various fruits and vegetables was well documented by Sun et al. (2019).

Sweetpotato (*Ipomoea batatas* Lam.) is one of the most important food crops worldwide (Sanusi et al., 2016). While the consumption of sweetpotato has increased, post-harvest diseases (especially fungal contamination) during crop storage are significant limitations (estimated quantity and quality loss, 15-65%) (Ray and Ravi, 2005). Sweetpotato is generally stored in storage houses, in which fungal contamination may spread throughout the storage facility, resulting in significant contamination of the stored crop (Wu and Rioux, 2010). Hence, it is important to apply effective control measures to prevent fungal infection during sweetpotato storage. Fungicides such as thiabendazole, dichloran, benomyl, and iprodione may be used to control root rots of sweetpotato in fields or to obtain disease-free 'seed' roots for plant production (Clark et al., 2013). However, application of agricultural chemicals is generally avoided during sweetpotato storage because the crop roots are directly used as a food source. Thus, ClO_2 gas, as described above, may be used as an alternative measure to control fungal contamination during crop storage.

Numerous *Fusarium* spp. have been reported to infect sweetpotato roots (Farr and Rossman, 2018); however, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *batatas* is one of the major diseases of sweetpotato (Clark et al., 2013). Fusarium wilt is usually widespread and shows higher infection levels in commercial fields (Thompson et al., 2011). In Korea, this disease was first reported by Park (1967) and has caused significant economic losses in sweetpotato production areas such as Haenam. In general, Fusarium wilt symptoms include yellowing of older leaves followed by wilting, abscission of older leaves, and stunting of vine growth (Clark et al., 2013). The infection of sweetpotato plants by the pathogen can occur from soil-borne inoculum such as chlamydospores. Thus, soil as a source of inoculum may also contribute to the infection of sweetpotato roots at harvest and root rot may follow during storage. In this sense, it is important to find measures to limit infection of the pathogen to sweetpotato roots (Clark et al., 2013).

Previously, we often observed black rot symptoms on stored sweetpotato roots and frequently isolated *Fusarium* spp. from the infected roots. We suspected the causal agent for this symptom to be the pathogen causing Fusarium wilt on sweetpotato plants in fields. Therefore, the objectives of this study were (1) to identify the causal agent of the black rot symptom of stored sweetpotato roots and (2) to evaluate *in vitro* and *in vivo* inhibitory activities of gaseous ClO_2

against the causal agent isolated from sweetpotato at various ClO_2 concentrations and treatment times.

Materials and Methods

Fungus and sweetpotato. Diseased sweetpotato roots (cv. 'Juwhangmi') supplied from the Bioenergy Crop Research Center, National Institute of Crop Science, Rural Development Administration (Muan, Korea) in August 2015 were used in this study (Fig. 1A and B). For fungal isolation, small pieces of tissues from the margins of the diseased root were cut and sterilized with 1% NaOCl for 2 min. The tissue pieces were then washed with sterile distilled water three times and blotted on a sterile filter paper (Whatman No. 1). The pieces were placed on acidified potato dextrose agar (PDA) supplemented with lactic acid (600 μl/l) at 25°C. Two to three days after placement on the medium, hyphal tips of mycelia grown from tissue pieces (one per lesion) were transferred to acidified PDA. Obtained isolates were further stored on PDA slants at 4°C until use. A representative isolate SP-f6 from isolated cultures was selected and subjected to further identification. Isolate SP-f6 was grown on Spezieller Nährstoffarmer agar (SNA) for macroconidium and microconidium formation (Leslie and Summerell, 2006). Healthy sweetpotato roots (cv. 'Juwhangmi') from the same center mentioned above were used in this study and these were stored in a moist chamber with 60% relative humidity at 15°C until use.
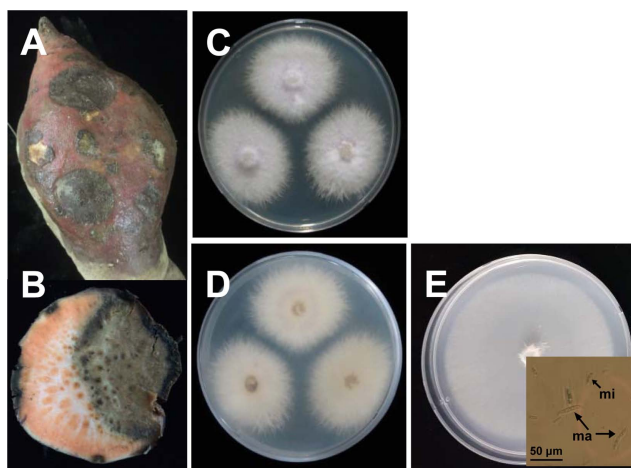

Fig. 1. (A) Outer and (B) inner black rot symptoms on sweetpotato (cv. 'Juwhangmi'). (C) Obverse and (D) reverse cultural morphologies of isolate SP-f6, isolated from a diseased sweetpotato, on potato dextrose agar at 25°C for 5 days in the dark. (E) Macroconidia and microconidia (inset) of isolate SP-f6 on Spezieller Nährstoffarmer agar.

Molecular identification of isolate SP-f6. For molecular identification of isolate SP-f6 obtained from the diseased root tissue, the genomic DNA was extracted from fungal mycelia grown on PDA at 25°C for 7 days using an i-genomic BYF DNA Extraction Kit (iNtRON Biotechnology, Seongnam, Korea) according to the manufacturer's instructions. The internal transcribed spacer (ITS) regions of isolate SP-f6 were amplified using the universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Glass and Donaldson, 1995); the elongation factor 1-α gene of the isolate was amplified with primers EF-1 (5′-ATGGGTA-AGGAAGACAAGAC-3′) and EF-2 (5′-GGAAGTAC-CAGTGATCATGTT-3′) (Kim et al., 2005). Polymerase chain reaction and DNA sequence analysis were conducted as described by Kim et al. (2009) and Sang et al. (2013). The sequenced data were analyzed using the Basic Local Alignment Search Tool (BLAST) at the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A phylogenetic tree was constructed using the neighbor-joining (Saitou and Nei, 1987) and maximum-likelihood (Fitch, 1971) algorithms using Molecular Evolutionary Genetics Analysis (MEGA) version 6.0. The topology of the tree was assessed by analyzing bootstrap with 1000 replications (Felsenstein, 1985). The experiments were performed twice for confirmation.

Pathogenicity test of isolate SP-f6. For pathogenicity tests of isolate SP-f6, healthy sweetpotato roots (cv. 'Juwhangmi') were washed with tap water and rinsed with distilled water. The roots were surface-sterilized with 70% ethanol for 5 min. Then, these roots were rinsed with distilled water twice and dried for 10 min at 15°C. The roots were hole-inoculated with isolate SP-f6 grown on PDA at 25°C for 7 days. Holes in roots were made using a sterile 4-mm cork-borer. Mycelial plugs (5 mm in diameter) from the edges of the growing cultures of isolate SP-f6 or PDA plugs (uninoculated control) were inoculated into holes made in the outer layers of the sweetpotato roots [six holes: three (sub-replicates) for SP-f6 and three for PDA plug per root (replicate)]. The inoculated roots were then wrapped up in polyethylene film (Cleanwrap Co., Seoul, Korea) and placed in moist plastic containers [23 (length) × 13 (width) × 16 (height) cm] with three layers of wet paper towels (100% RH) and incubated at 28°C. After 10 days of incubation, lesion diameters (mm) were measured. At the same time, fungus was re-isolated from the symptomatic tissues to fulfill Koch's postulates. The edges of lesions were cut, sterilized with 1% NaOCl for 1.5-5 min, washed three times with sterile distilled water, and blotted on a sterile

filter paper (Whatman No. 1). These pieces were placed on acidified PDA at 25°C for 3 days. The mycelia grown from the pieces was transferred to PDA and the identity of the isolate reconfirmed by the elongation factor 1-α gene sequence analysis, as described above.

***In vitro* effect of gaseous chlorine dioxide against isolate SP-f6 on medium.** To examine the *in vitro* inhibitory effect of gaseous ClO_2 against isolate SP-f6 on the medium, spore suspensions (200 µl) of the isolate were spread on acidified PDA. Spores from cultures of isolate SP-f6 grown on PDA at 25°C for 7 days were harvested with 0.03% Tween 20 and adjusted to 5×10^3 spores/ml using a hemocytometer. Next, these spore-inoculated plates were treated with various concentrations (1, 5, 10, and 20 ppm) of ClO_2 gas for 0, 1, 10, 30, and 60 min. The gas treatment for various treatment times was conducted using a ClO_2 generator (PurgoFarm Co. Ltd., Hwaseong, Korea) with an electrochemical method (Gates, 1998), as described in our previous study (Lee et al., 2019). The gas-treated plates were further incubated at 25°C. After 2 days of incubation, numbers of colony-forming units (CFUs) on the plates were counted.

***In vivo* effect of gaseous chlorine dioxide against isolate SP-f6 on root slices.** To examine the *in vivo* inhibitory effect of gaseous ClO_2 against isolate SP-f6 on root slices, healthy sweetpotato roots prepared as described in the pathogenicity test were cut into 1-cm-thick slices, and placed in Petri plates (90 mm in diameter). The centers of the slices were drop-inoculated with the spore suspension (10 µl of 5×10^6 spores/ml) prepared as described above. The inoculated slices were treated with various concentrations (5, 10, and 20 ppm) of ClO_2 gas for 0, 10, 30, and 60 min, as described above. Next, the gas-treated slices were put in the plastic containers containing wet paper towels as described above and incubated at 28°C for 14 days. Then, lesion diameters on the gas-treated, inoculated slices were assessed as follows: longest lesion length (mm) + shortest lesion length (mm) were divided by two.

***In vivo* effect of gaseous chlorine dioxide against isolate SP-f6 on sweetpotato roots.** To examine the *in vivo* inhibitory effect of gaseous ClO_2 against isolate SP-f6 on sweetpotato, roots were dipped in spore suspension (5×10^6 spores/ml) prepared as described above, for 10 min. The 0.03% Tween 20 was used as an uninoculated control. The inoculated roots were treated with different concentrations (20 and 40 ppm) of ClO_2 gas for 0, 30, and 60 min as described above. Then, the surface layers (approximately 1.5

mm in thickness) of the gas-treated roots were peeled using a vegetable peeler. Samples (2 g) were finely ground using an analytical mill (IKA A11 basic, IKA Works) in sterile distilled water (20 ml). After serial dilutions of the ground sample solution, the samples were spread on acidified PDA and further incubated at 25°C. After 2 days of incubation, colonies that appeared on the medium were counted; these were expressed as CFUs per g dry weight.

Statistical analysis. Experiments were conducted using factorial designs to recognize the effects of ClO_2 gas concentration and treatment time with various levels on lesion diameters and fungal populations. All experiments except molecular identification of the isolate were conducted twice with three replicates per treatment. Fungal population data from repeated experiments of the *in vitro* ClO_2 inhibitory tests on the medium were pooled after confirmation of homogeneity of variances, using Levene's test (Levene, 1960). In addition, fungal population data in this study were analyzed after logarithmic transformation. Analysis of variance (ANOVA) was conducted using general linear model procedures; the means were separated using least significant difference (LSD) test at $P < 0.05$. Statistical analysis of all data was conducted using Statistical Analysis Systems (SAS) software (SAS Institute, Cary, NC, USA).

Results

Fungal isolation and identification of isolate SP-f6. The potential causal fungus was consistently isolated from the margins of symptomatic lesions of diseased sweetpotato roots. Isolate SP-f6 randomly selected from isolates obtained from diseased sweetpotato roots (cv. Juwhangmi) showed white floccose mycelium and pink to purple reverse color (Fig. 1C and D); consequently, it was considered as the genus *Fusarium*. In addition, macroconidia and microconidia of isolate SP-f6 were observed on SNA medium (Fig. 1E).

For the phylogenetic analysis of isolate SP-f6, partial sequences (469 and 608 bases) of the ITS region and elongation factor 1-α alpha genes, respectively, were analyzed. In the neighbor-joining analysis using the ITS region sequence, isolate SP-f6 was clustered with *Fusarium oxysporum* CBS 132475 (100% similarity), *Fusarium inflexum* NRRL 20433[T] (99.28% similarity), *F. oxysporum* f. sp. *batatas* CBS 258.50 (98.08% similarity), and *F. oxysporum* f. sp. *batatas* CBS 172.30 (98.08% similarity) (Supplementary Fig. 1). On the other hand, based on the neighbor-joining analysis of elongation factor 1-α gene, isolate SP-f6 was clustered with *F. oxysporum* f. sp. *bata-*
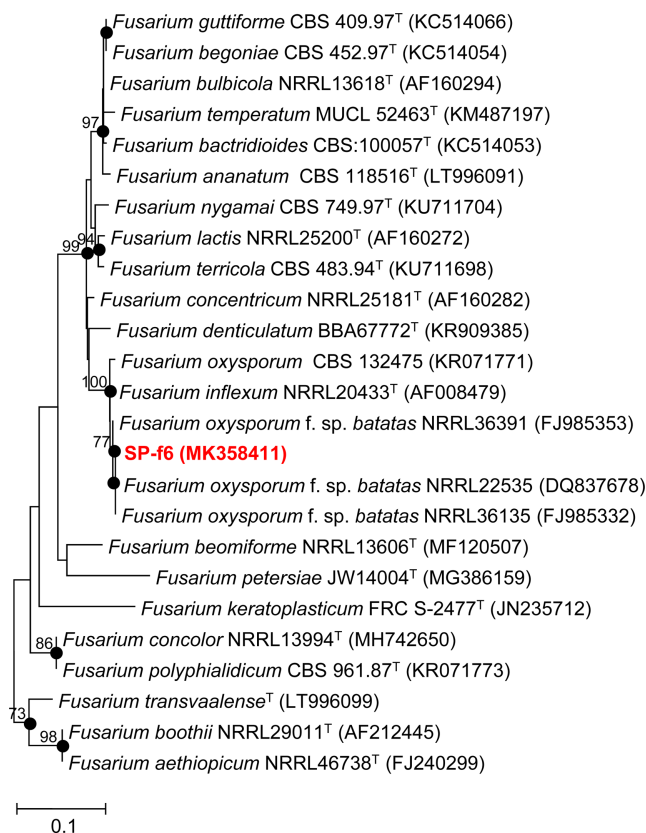

Fig. 2. Neighbor-joining tree that shows the relationships between isolate SP-f6 (MK358411) and related members of the genus *Fusarium*, based on phylogenetic analysis of elongation factor 1-α gene sequence. Filled circles on the branches indicate that the nodes were also recovered in the tree constructed by the maximum-likelihood algorithm. *Microdochium nivale* 200118 (KT736151) was used as an outgroup (not shown). Scale bar, 1 nt substitution per 10 nt of the sequence.

tas NRRL 22535 (100% similarity), *F. oxysporum* f. sp. *batatas* NRRL 36135 (99.84% similarity), *F. oxysporum* f. sp. *batatas* NRRL 36391 (98.84% similarity), *Fusarium inflexum* NRRL 20433[T] (99.04 similarity), and *Fusarium oxysporum* CBS 132475 (99.04 similarity) (Fig. 2). Thus, isolate SP-f6 was clearly identified as *F. oxysporum* f. sp. *batatas* based on the analysis of elongation factor 1-α gene sequence. The ITS (MK212928) and the elongation factor 1-α gene (MK358411) sequences of *F. oxysporum* f. sp. *batatas* SP-f6 have been deposited in GenBank.

Pathogenicity of *F. oxysporum* f. sp. *batatas* SP-f6 on sweetpotato. In the pathogenicity test, *F. oxysporum* f. sp. *batatas* SP-f6 caused dark grayish round lesions with white mycelia on sweetpotato (cv. Juwhangmi) roots 10 days after inoculation (Fig. 3A). Lesion diameters on outer layers of roots inoculated with isolate SP-f6 were 8.72 ±
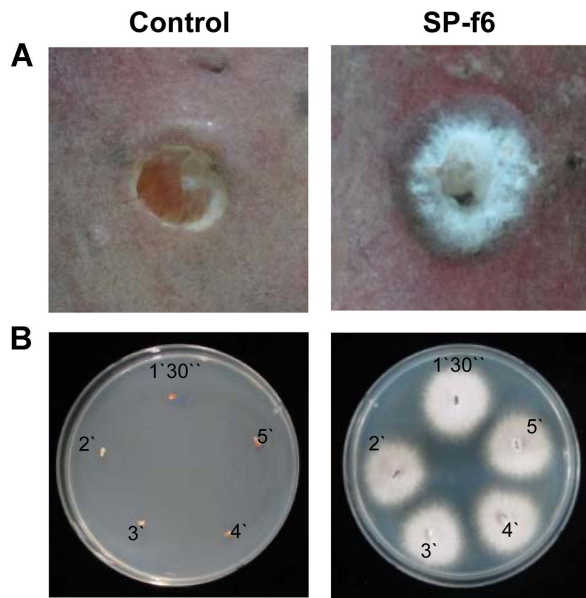
Fig. 3. (A) Symptoms of the sweetpotato roots at 10 days after hole-inoculation with *Fusarium oxysporum* f. sp. *batatas* SP-f6. Mycelial potato-dextrose agar (PDA) plugs were inoculated by placing them in 5-mm holes that were made by a sterile cork-borer on sweetpotato (cv. Juwhangmi). PDA plugs alone were used as uninoculated controls. (B) The inoculated fungal isolate was re-isolated on acidified PDA from (A) symptomatic tissues after surface-sterilization in NaOCl solution for 1.5-5 min; the recovered isolate was shown on acidified PDA 3 days after incubation at 25°C.
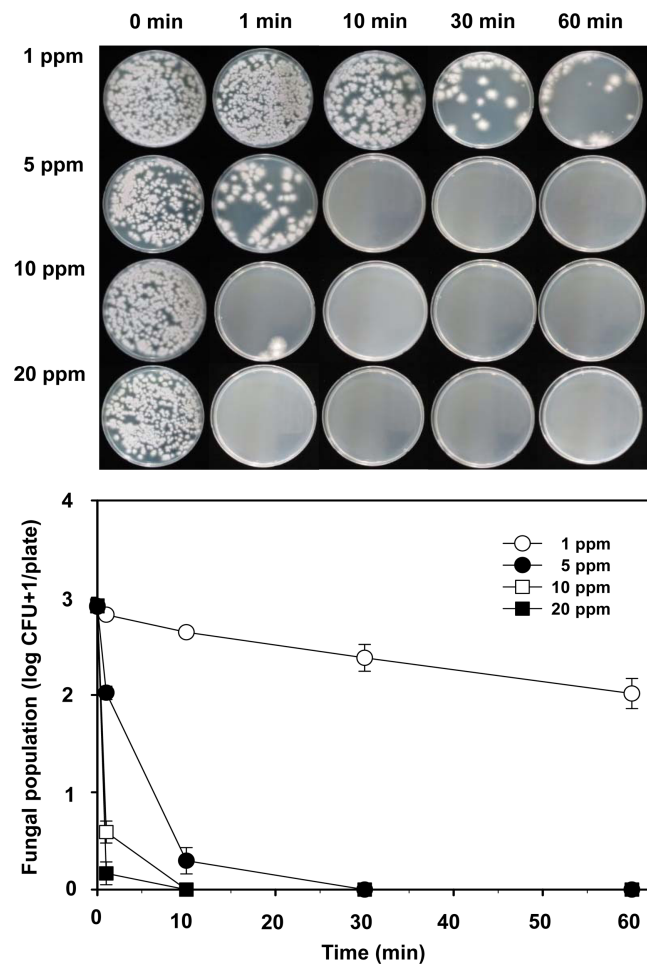

Fig. 4. Photographs (top) and populations (below) of *Fusarium oxysporum* f. sp. *batatas* SP-f6 on acidified potato dextrose agar (PDA) treated with various ClO_2 concentrations (1, 5, 10, and 20 ppm) for 0, 1, 10, 30, and 60 min. Colony-forming units (CFUs) were determined 2 days after incubation. Spore suspension (200 μl of 5×10^3 spores/ml) of isolate SP-f6 was spread on acidified PDA before ClO_2 gas treatments. Error bars = standard deviations of the means ($n = 6$).

0.70 mm (mean ± standard error) and 10.22 ± 0.49 mm in repeated experiments. However, PDA plugs (uninoculated control) did not produce any symptoms on the root (Fig. 3A). To fulfill Koch's postulates, isolate SP-f6 was isolated again from the symptomatic tissues of the inoculated roots, in which the tissues were surface-sterilized with NaOCl for 5 min (Fig. 3B). This isolate was further confirmed as *F. oxysporum* f. sp. *batatas* SP-f6 by elongation factor 1-α gene sequence analysis. However, fungal mycelia were not isolated from the PDA-inoculated controls (Fig. 3B).

***In vitro* effect of gaseous chlorine dioxide against *F. oxysporum* f. sp. *batatas* SP-f6 on medium.** When the *in vitro* inhibitory effect of gaseous ClO_2 against *F. oxysporum* f. sp. *batatas* SP-f6 was tested on PDA, gaseous ClO_2 at various concentrations and exposure times affected the growth of isolate SP-f6 (Fig. 4). Fungal population was significantly inhibited at 1 ppm of ClO_2 for 30 min and was almost completely suppressed at 1 ppm for 60 min and 5 ppm for 5 min. Furthermore, ClO_2 gas treatment at 5 ppm for 30-60 min and 10 and 20 ppm for 10-60 min completely inhibited the fungal population on medium (Fig. 4).

***In vivo* effect of gaseous chlorine dioxide against *F. oxysporum* f. sp. *batatas* SP-f6 on root slices.** Lesion diameters caused by *F. oxysporum* f. sp. *batatas* SP-f6 in the inoculated sweetpotato slices decreased with an increase in ClO_2 concentrations and exposure times in experiment 2, but not in experiment 1 (Fig. 5A, Supplementary Table 1). ClO_2 concentration ($P = 0.0013$) and exposure duration ($P < 0.0001$) significantly affected lesion diameters on the inoculated slices in experiment 2, but not in experiment 1 (Supplementary Table 1). Significant concentration and time interactions ($P = 0.9415$ for experiment 1 and $P = 0.1258$ for experiment 2) for lesion diameters were not observed in either experiment (Supplementary Table 1). The
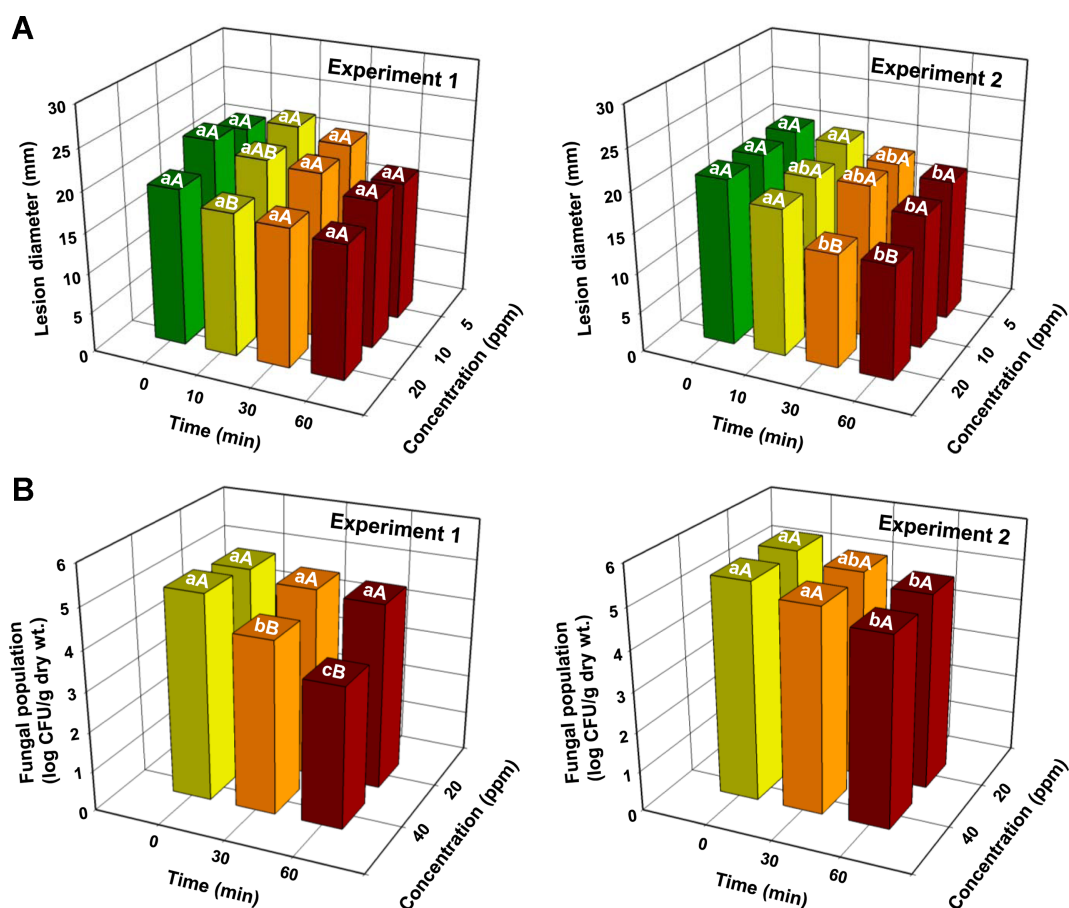
A

B

Fig. 5. (A) Lesion diameters and (B) fungal populations on sweetpotato slices and surface layers of roots inoculated with *Fusarium oxysporum* f. sp. *batatas* SP-f6, followed by treatments with gaseous chlorine dioxide (ClO_2), respectively. Sweetpotato slices were drop-inoculated with spore suspension (10 μl of 5×10^6 spores/ml) of isolate SP-f6, followed by treatment with various concentrations (5, 10, and 20 ppm) of ClO_2 gas for 0, 10, 30, and 60 min. Roots were dipped in spore suspension (5×10^6 spores/ml) for 10 min and then treated with different ClO_2 concentrations (20 and 40 ppm) for 0, 30, and 60 min. Different lowercase and uppercase letters on bars ($n = 3$) are significantly different between time at a given concentration and between concentrations at a given time according to the least significant difference test at $P < 0.05$, respectively.

degree of darkness of lesions declined over time, regardless of gas concentration. In addition, root slice tissues were lignified after gas treatment, especially at 20 ppm for 30-60 min (Supplementary Fig. 2). However, disease symptoms were not observed in uninoculated sweetpotato slices (Supplementary Fig. 2).

***In vivo* effect of gaseous chlorine dioxide against *F. oxysporum* f. sp. *batatas* SP-f6 on sweetpotato roots.** When the *in vivo* inhibitory effect of gaseous ClO_2 against *F. oxysporum* f. sp. *batatas* SP-f6 was tested on sweetpotato roots, growth of isolate SP-f6 was significantly inhibited by ClO_2 gas treatment at 40 ppm for 30-60 min in experiment 1 and at 20 and 40 ppm for 60 min in experiment 2 (Fig. 5B). ClO_2 gas treatment at 40 ppm inhibited more population of isolate SP-f6 than the gas treatment at 20 ppm for

30 and 60 min in experiment 1 (Fig. 5B). The concentration of ClO_2 showed significant ($P = 0.0001$) inhibition on fungal population in an experiment; treated time exhibited significant ($P = 0.0001$ for experiment 1 and $P = 0.0003$ for experiment 2) reduction of fungal populations in both experiments (Supplementary Table 1). Significant ($P = 0.0002$) concentration and time interactions for fungal populations were observed in experiment 1 (Supplementary Table 1).

Discussion

In this study, we isolated fungal isolate SP-f6 from the black rot lesion on a diseased sweetpotato root and identified it as *F. oxysporum* f. sp. *batatas* using phylogenetic analysis of the elongation factor 1-α gene. Furthermore,

we tested whether ClO_2 gas had inhibitory activity against the development and growth of *F. oxysporum* f. sp. *batatas* on sweetpotato. We found that ClO_2 gas suppressed fungal population and infection on sweetpotato roots. In addition, we observed that treatment time had a greater inhibitory effect than the ClO_2 gas concentration on disease development and fungal population on sweetpotato.

ClO_2 gas as a disinfectant has been widely used to control food-borne or postharvest microbial contamination on storage crops (Bhagat et al., 2011; Du et al., 2002; Yuk et al., 2006); thus, we examined the efficiency of ClO_2 gas in controlling *F. oxysporum* f. sp. *batatas*–mediated infection in stored sweetpotato. In *in vitro* medium tests, the growth of *F. oxysporum* f. sp. *batatas* SP-f6 was significantly inhibited when treated with 1 ppm ClO_2 gas for 30-60 min. Furthermore, growth of the fungus was completely inhibited following treatment with increased ClO_2 concentration of 5 ppm for 30-60 min and 10 and 20 ppm for 10-60 min. In our previous study, *Diaporthe batatas* was shown to have similar growth inhibition pattern against various ClO_2 concentrations and exposure times (Lee et al., 2019). There are several other studies that demonstrate *in vitro* inhibitory activity of ClO_2 on various fungal contaminations on crops. Chen and Zhu (2011) reported that treatment of chestnut with 7 mg/l of ClO_2 for 15 min led to a 4.6-log reduction of *Fusarium tricinctum* spores, whereas a 5.0-log reduction in *Dothiorella gregaria* spores was detected under the same treatment conditions. Wu et al. (2011) observed that spore germination rate of litchi anthracnose pathogens (*Colletotrichum* spp.) was significantly inhibited following treatment with 5 mg/l of ClO_2 solution. In addition, Sun et al. (2017) found that aqueous ClO_2 significantly inactivated spores of *Fusarium graminearum*, the causal agent of Fusarium head blight of wheat.

In *in vivo* inoculation tests on sweetpotato slices, lesion diameters were significantly restricted when the slices were treated with ClO_2 gas for 30 min at 20 ppm treatment. Treatment for 60 min led to decrease in lesion diameters at all concentrations of the gas tested. However, lesion darkness, which indicates fungal growth and development, on slices turned lighter over time, and the tissues were lignified after gas treatment especially following the 60 min-treatment, regardless of the ClO_2 concentration used. Tissue lignification resulting from gas treatment may not be a cause for concern as ClO_2 gas is usually applied on sweetpotato roots. In *in vivo* root dip-inoculation tests followed by ClO_2 gas treatment, fungal populations were reduced as observed in the slice tests. The inhibitory effect of ClO_2 gas in this study followed a pattern similar to that observed in our previous study (Lee et al., 2019). These previous

and present results imply that the ClO_2 gas may have wide antifungal or antimicrobial activities beneficial for stored crops. In this regard, Bhagat et al. (2011) showed that ClO_2 gas treatment inhibited Salmonella growth on oranges. They spot-inoculated a mixed culture of four *Salmonella* strains on the surface of orange and then treated the fruits with 0.1, 0.3, and 0.5 mg/l ClO_2 gas for 2-14 min at 22°C and 90-95% relative humidity. Consequently, a 5-log reduction of *Salmonella* per sample was observed with 0.1 and 0.3 mg/l ClO_2 gas treatments at 14 min, and 0.5 mg/l ClO_2 gas at 10 min. Moreover, Sy et al. (2005) also found similar reductions in populations of foodborne pathogens such as *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, yeasts, and molds when the inoculated fresh and fresh-cut produces were exposed to 4.1 mg/l ClO_2 gas.

Taken together, the results in this study showed that ClO_2 gas can effectively inhibit fungal population and infection by *F. oxysporum* f. sp. *batatas* on sweetpotato. The ClO_2 gas treatment with 40 ppm for 30-60 min may produce a desirable outcome by controlling the fungal growth and symptom development on the crop. Therefore, ClO_2 gas may be used as a sanitizer to control *F. oxysporum* f. sp. *batatas* during postharvest storage of sweetpotato.

Acknowledgments

Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

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