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Ozone: Changing Anthracnose (caused by *Colletotrichum acutatum*) Severity and Accelerating Hypersensitive Response in Pepper

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The interaction effects of ozone (O_3) and anthracnose (*Colletotrichum acutatum*) disease were examined in green fruits and seedlings of pepper (*Capsicum annuum*). Pre-treatment with O_3 as a factor causing predisposition to the disease prior to infection was investigated in green fruits and stems using an O_3 concentration of 150 nL/L, which is easily reached in summer in Korea. O_3 treatment increased antioxidative responses in pepper foliar tissues, and defense against anthracnose was examined in fruits and stems. Anthracnose severity on stems of the O_3-treated, ozone-sensitive 'Dabotop' cultivar was always lower than that on untreated plants, but the difference was not always significant ($p = 0.147$). Significantly lower anthracnose severity was found on O_3-treated green 'Dabotop' fruits as compared to untreated green fruits in three of eight replicate experiments. In contrast, hypersensitive responses in O_3-treated seedlings were significantly accelerated compared to those in untreated seedlings by about 7.8 h ($p < 0.001$). This confirmed previous evidence of increased transcription of plant defense genes with O_3 treatment. O_3 treatment significantly decreased chlorophyll concentrations in the leaves in four replicate experiments ($p < 0.01$). O_3 increased hypersensitive responses in the leaves of pepper seedlings, but this increase did not contribute to the control of anthracnose severity on fruits. Antioxidant reactions to O_3 were limited to chlorosis and changes in hypersensitive responses in leaves.

Keywords **:** *Capsicum annuum, Colletotrichum acutatum,* hypersensitive response, ozone, predisposition

Levels of tropospheric ozone (O_3), a phytotoxic air pollutant, have increased worldwide, including in Korea (Reich, 1987; Yun and Kim, 2004). O_3 has negative effects on a number of plant physiological responses, including photosynthesis, water use efficiency, rate of senescence, dry matter production, flowering, pollen tube extension, and yield (Krupa, 1997). O_3 reacts with water in the leaf, changes the physiological activity, and oxidizes proteins and membranes (Heath, 1980; Mehlhorn et al., 1990). Acute O_3 injury in sensitive genotypes is usually observed as the development of foliar lesions, which resembles the hypersensitive response (HR) of plants to pathogen attack. An oxidative burst occurs as the initial plant reaction to both O_3 exposure and pathogen assault, and similar signal molecules have been implicated in the induction of HR and O_3 injury (Sandermann, 1998; Schraudner et al., 1997).

In hot pepper (*Capsicum annuum*), defense genes were transcribed after O_3 treatment (Lee and Yun, 2006). Most of the 84 ozone-stress-regulated genes identified were specifically up-regulated in the ozone-sensitive pepper cultivar 'Dabotop' (Yun, 2004), and were the same genes as are transcribed in plant defense in reaction to non-host pathogen infection. However, confirmation is needed that the effect of O_3 treatment at the tissue and organ levels supports the molecular evidence. The use of O_3 as an alternative control for economically important diseases should be considered because it leaves no chemical residuals.

Colletotrichum and its teleomorph *Glomerella* are considered major plant pathogens worldwide. They cause significant economic damage to crops in tropical, subtropical, and temperate regions. Pepper anthracnose, caused by *Colletotrichum acutatum*, is one of the most important diseases in pepper cultivation in Korea (Kang et al., 2005). Because of its ability to cause latent or quiescent infections, protective chemical control is recommended for good disease management. However, alternative methods are needed because of the widespread use of numerous chemicals to control this pathogen. O_3 was recently recognized as generally safe for application in the food industry and its application was tested (Palou et al., 2001).

Studies of the interactions between ozone and the development of plant diseases were recently reviewed (Manning and Tiedemann, 1995). However, there is no information on the joint effects of O_3 and anthracnose disease on hot pepper. In general, O_3 can decrease the incidence of diseases caused by obligate parasites, whereas it increases the problems associated with facultative parasites. Ozone is unlikely to have direct effects on fungal pathogens; rather, the effects are mediated by the host plant (Krupa et al., 2000). Conversely, the occurrence of disease can alter the

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foliar response to O_3. Consequently, we hypothesized that O_3 stimulates an oxidative burst, accelerates hypersensitive responses, and decreases anthracnose severity. Our aim was to confirm that O_3 accelerates hypersensitivity in pepper leaves and determine whether O_3 decreases or delays anthracnose disease on pepper fruit.

Materials and Methods

Ozone treatment and inoculation of anthracnose fungus. Hot pepper plants (*Capsicum annuum*) were fumigated with 150 nL/L of ozone for 8 h on each of three consecutive days in growth chambers under fluorescent light at 26 ± 1°C and with a 12-h light/dark photoperiod. The O_3 concentration was maintained using a PID controller from a discharge-tube-type generator. Ozone was measured and recorded using an O_3 analyzer (Model 400E, API, USA).

An isolate of *Colletotrichum acutatum* was obtained from a naturally infected pepper fruit exhibiting typical anthracnose symptoms. The isolate was grown on potato sucrose agar (PSA) plates at 25°C for 1 week. The culture surface on the PSA slants was flooded with 10 mL of sterilized distilled water and scraped to make a conidial suspension. The inoculum concentration of the conidial suspension was adjusted to 1.0×10^6 conidia/mL using a hemacytometer.

Inoculation of seedling stems. Seedlings of the hot pepper cultivars 'Dabotop' (ozone-sensitive) and 'Buchon' (ozone-tolerant; Yun, 2004), were prepared in 50-hole trays with commercial horticultural soil. Half of the 4- to 6-week-old seedlings were fumigated with O_3, after the stem of each seedling had been inoculated with spore suspension (10^6 spores/mL) using a pin. To accelerate disease progression, all plants were placed in a dew chamber at 100% relative humidity (RH) for 24 h. In each treatment, 10-20 plant stems were inoculated using 5-6 wound holes. Disease severity on the stem was quantified 2 weeks after inoculation by taking digital images of the stem and determining the percentage of damaged tissue using an image analyzer (Access, APS USA). The disease severity on plants pre-treated with O_3 and on those not treated was compared using paired t-tests with each experimental event (composed of 10-20 plants per treatment) as a replication. Disease severity on ozone-sensitive and ozone-tolerant peppers was similarly compared. Five replications were analyzed.

Inoculation of green fruits. Pepper plants that had at least four or five green fruits were planted in 20-cm diameter pots. Half of the plants were fumigated with O_3, after which the green fruits were inoculated with spore suspension using a needle. The plants were placed in 100% RH for 24 h and then transferred to a greenhouse. The typical symptoms appeared on the fruits 4-5 days after inoculation. Disease severity was quantified by measuring the diameter of the disease necrosis.

Disease severity on plants that were pre-treated with O_3 and on those not treated was compared using one-way analysis of variance (ANOVA). Although the disease severity differed in each replication, we were able to follow the progression of the disease in each replication.

Hypersensitivity test on the leaves. Four- to six-week-old seedlings with two to three leaves were used in this test. Half of the seedlings were pre-treated with O_3, after which the leaves of 10 seedlings were inoculated with a *Xantomonas axonopodis* pv. *glycins* bacterial suspension adjusted to an optical density of 0.5, while the leaves of another 10 seedlings were inoculated with distilled water (control). The time taken for plants to show a hypersensitive response in the first 5 of 10 leaves among the 10 seedlings was measured for six replications. In addition, the amount of chlorosis in leaves was measured using a SPAD (Soil and Plant Analyzer Development, Minolta, Japan) meter to compare inoculated and control leaves. The SPAD meter measures light at 650- and 940-nm wavelengths, which are sensitive and insensitive to chlorophyll, respectively, and determines the chlorophyll content (from –9.9 to 99.9) from the difference between the two wavelengths produced by light-emitting diodes and passed through the specimen to a silicon photodiode. SPAD differecnces between water-injected and non-water-injected tissues should be measured because the chlorophyll contents of water-soaked tissue would be changed without any treatment in control.

The chlorophyll content was measured to determine whether the typical hypersensitive response (HR) was accelerated, causing an increase in chlorosis, after ozone fumigation. We used a non-pathogenic bacterium to show typical hypersensitive symptoms. The chlorophyll content of uninoculated leaves was compared between leaves that were pre-treated with O_3 and leaves that were not treated. In addition, the chlorophyll content was compared in untreated, uninoculated leaves and ozone-treated, inoculated leaves. The experiment was conducted four times using the 'Dabotop' cultivar.

Results

Anthracnose on the stem. Anthracnose symptoms appeared on the stems of pepper seedlings as necrotic spots that spread from the site of the puncture. Because infection was caused by an artificial wound, we examined the progression of the disease after infection. The disease severity on stems of 'Dabotop' without O_3 treatment was 10-30%, depending

Table 1. Anthracnose disease severity (% damaged area) on stems of ozone-sensitive (cv. 'Dabotop') and ozone–tolerant (cv. 'Buchon') pepper seedlings after treatment with 150 nL/L ozone for 8 h/day for 3 days. Differences in anthracnose severity (% damaged area) on stems and pair-wise comparisons for the effects of ozone treatment and ozone sensitivity in two pepper cultivars.

Cultivar	Experiment[a]	O_3 pre-treated[b]		O_3 untreated[c]		Differences in severity[e]	Effects of O_3 treatment[f]
		No. of stems	Dis. severity (%)[d]	No. of stems	Dis. severity (%)		
Dabotop	1	7	9.44	9	10.80	1.36	
	2	14	6.70	11	10.56	3.86	
	3	12	10.43	10	15.27	4.84	
	4	8	16.78	12	18.59	1.81	
	5	11	15.70	5	36.65	20.95	
							0.147 ns
Buchon	1	6	6.69	9	5.09	-1.60	
	2	13	9.24	11	14.12	4.88	
	3	13	7.07	15	3.61	-3.46	
	4	9	13.48	13	16.45	2.97	
	5	11	28.79	9	20.51	-8.28	
							0.664 ns
Effect of cultivar[f]			0.714 ns		0.138 ns		

[a]Experiments 1-5 were conducted on Jul 14, Jul 18, Jul 22, Jul 26, and Aug 12, respectively, in both cultivars simultaneously.
[b]Pepper seedlings were treated with O_3 prior to inoculation with anthracnose fungus.
[c]Pepper seedlings were not treated with O_3.
[d]The percent of damaged tissue was measured using image analysis computer software.
[e]Difference in disease severity between O_3 treatments for each cultivar = (disease severity in untreated plants) – (disease severity in O_3 pre-treated plants). Positive values indicate that ozone pre-treatment reduces disease severity and vice versa.
[f]Pair-wise comparisons for five replicate experiments. "ns" means non significant.

on the experiment, and was higher than that of plants that received O_3 pre-treatment in all five experiments (Table 1). However, the difference was not statistically significant (pair-wise t-test, $p = 0.147$). In contrast, the disease severity on stems of 'Buchon' without O_3 treatment was 3-20%, which was lower and more variable than that in 'Dabotop' (Table 1). In addition, there was no tendency for O_3 treatment to reduce the disease severity in this cultivar.

Anthracnose on green fruits. We examined the progress of anthracnose disease on green pepper fruits with and without O_3 pre-treatment (Table 2). A total of eight replicate experiments were conducted in growth chambers over 1 year. The necrosis ranged in size from 10 to 30 mm in diameter, and those of plants receiving the O_3 pre-treatment were somewhat smaller than those of untreated plants. With the exception of the first, third, and eighth experiments, the severity of anthracnose disease was not significantly different between O_3 treated and untreated seedlings. Significantly reduced disease severity was found in O_3-treated plants in only the first and eighth experiments, while increased disease severity was found in O_3-treated plants in the third experiment (Table 2). Interestingly, the standard deviation of symptom size in O_3-treated plants was 4-6 mm, whereas that in untreated plants was 5-8 mm for the same replicate experiments.

Hypersensitive response. The time between inoculation and the appearance of a typical hypersensitive response was significantly greater in untreated plants (49.8 h) than in O_3-treated plants (42.0 h; t-test, $p < 0.01$; Fig. 1). SPAD measurements of chlorophyll content were conducted on healthy (injected with sterilized water) and inoculated (injected with bacterium) tissues of the same leaf. At 50 h after injection, the chlorophyll content (SPAD) of healthy tissue ranged from 28 to 36, and that of O_3-treated tissue ranged from 25 to 31 (Table 3). The chlorophyll content decreased in infected tissues in both O_3-treated and untreated leaves (Table 3).

The differences in chlorophyll content (SPAD) between inoculated and uninoculated tissue on the same leaf (inoculation column) for all replicate experiments ranged from 3 to 9, but the differences between water injection and no injection on the same leaf (uninoculated column) were < 1 (Table 4). This indicates that chlorophyll content data are applicable in quantifying hypersensitive responses.

In the examination of the interactive effects of ozone and inoculation on the chlorophyll content of leaves, chlorosis caused by O_3 was statistically significant in the first experiment at 43 h, the third experiment at 41, 49, and 50 h,

Table 2. Size (mm) of anthracnose necrosis on hot pepper fruits after inoculation.

Experiment	Days[a]	No. of fruits	O_3 pre-treated[b] (mm)	No. of fruits	O_3 untreated[b] (mm)	P-values[c]
1	5	10	8.41 ± 11.59	20	9.88 ± 7.41	0.675
	11	13	19.8 ± 13.99	15	30.20 ± 4.20	**0.022**
2	7	12	20.61 ± 13.99	11	19.44 ± 8.31	0.772
3	2	17	2.63 ± 0.69	16	2.22 ± 0.78	0.117
	4	17	13.73 ± 4.91	16	7.58 ± 2.57	**0.000**
	6	17	19.63 ± 8.22	16	13.64 ± 4.67	**0.016**
	7	17	23.78 ± 10.41	16	14.43 ± 5.01	**0.003**
	8	17	31.12 ± 12.49	16	15.13 ± 5.06	**0.000**
4	5	9	12.16 ± 3.95	7	16.56 ± 8.70	0.250
	7	9	19.62 ± 6.01	7	19.87 ± 9.33	0.948
	9	9	24.57 ± 5.61	7	26.15 ± 8.69	0.666
	11	9	29.78 ± 4.26	7	28.84 ± 7.15	0.747
	13	9	32.44 ± 4.04	7	31.15 ± 6.00	0.613
5	7	10	10.18 ± 3.81	10	11.56 ± 5.41	0.521
	8	10	14.32 ± 4.02	10	15.48 ± 4.18	0.534
6	5	11	21.41 ± 5.14	10	16.66 ± 7.58	0.107
	6	11	25.15 ± 5.71	10	22.65 ± 8.53	0.435
7	5	12	14.91 ± 4.67	12	13.27 ± 7.21	0.513
	6	12	19.92 ± 4.83	12	15.60 ± 6.08	0.067
8	7	12	12.45 ± 4.45	12	14.57 ± 5.85	0.329
	8	12	15.11 ± 4.77	12	20.12 ± 5.62	**0.028**
	9	12	19.45 ± 4.60	12	24.46 ± 5.36	**0.023**

[a]Number of days after inoculation when measurements were made.
[b]Size of anthracnose necrosis (mm) on green fruits in each experiment (mean ± standard deviation). Seedlings were pre-treated with ozone at 150 nL/L for 8 h/day for 3 days before anthracnose inoculation.
[c]One-way ANOVA between ozone treatments for anthracnose disease progression.

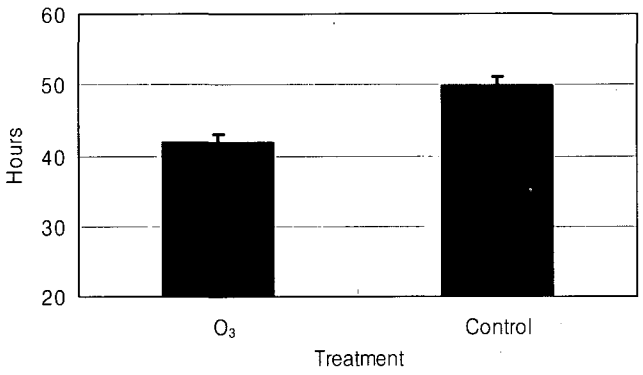

Fig. 1. Time between anthracnose inoculation and the appearance of typical symptoms on the first five of ten inoculated pepper leaves. Plants were pre-treated with ozone prior to inoculation with the bacterium *Xantomonas axonopodis* pv. *glycins*. Error bars indicate the standard deviation of six replicate experiments.

and the fourth experiment at 43 and 50 h, respectively (one-way ANOVA; Table 5). We analyzed the synergistic effects of O_3 treatment and inoculation on chlorophyll content by comparing the uninoculated control and the O_3 pre-treated inoculated condition. Except for experiment 2 at 41 h, all of the O_3-treated inoculated leaves had significantly ($p < 0.05$) decreased the chlorophyll contents of the leaves (Table 5). SPAD of uninoculated leaves decreased by 1-4 with O_3 treatment and did not change with time; in contrast, the SPAD of inoculated leaves was decreased to 0-7 with time.

Discussion

The ozone (O_3) level used was 150 nL/L, which is easily reached in the environment in summers in Korea (Yun and Kim, 2004). This may affect the severity of anthracnose disease on peppers experiencing increases in O_3 levels in

Table 3. SPAD chlorophyll measurements of O_3 pre-treated and O_3 untreated leaves of hot pepper seedlings. Each leaf was measured with water-injected and non-water-injected tissue to determine the severity of the hypersensitive response.

	O_3 pre-treated						O_3 untreated					
Leaf #	Inoculated		Diff[a]	Uninoculated		Diff	Inoculated		Diff	Uninoculated		Diff
1	29.0	34.0	5.0	26.4	26.8	0.4	31.1	36.4	5.3	36.4	36.6	0.2
2	27.0	30.9	3.9	31.0	31.7	0.7	22.1	28.5	6.4	29.2	29.9	0.7
3	26.3	28.8	2.5	28.9	28.8	-0.1	29.0	33.5	4.5	32.3	33.8	1.5
4	23.5	32.5	9.0	25.5	26.4	0.9	23.6	26.9	3.3	29.5	28.7	-0.8
5	27.3	33.5	6.2	30.8	30.5	-0.3	29.2	32.9	3.7	29.8	30.5	0.7
6	25.3	28.2	2.9	30.6	30.8	0.2	23.5	27.3	3.8	28.9	28.9	0.0
7	26.6	32.2	5.6	30.8	30.8	0.0	24.6	29.9	5.3	30.4	28.2	-2.2
8	26.5	29.3	2.8	24.0	24.4	0.4	25.6	31.6	6.0	28.3	28.4	0.1
9	25.0	28.8	3.8	27.3	27.7	0.4	26.0	30.1	4.1	32.0	30.9	-1.1
10	26.5	31.0	4.5	24.8	25.7	0.9	24.0	28.3	4.3	28.4	28.6	0.2
Average	26.3	30.92	4.62	28.01	28.36	0.35	25.87	30.54	4.67	30.52	30.45	-0.07
Variance	1.47	2.09	1.97	2.74	2.52	0.41	2.95	3.04	1.03	2.48	2.74	1.05

[a]Diff indicates the difference in SPAD (chlorophyll content) between water-injected and non-water-injected tissues in the same leaf.

agricultural fields in suburban areas near Seoul. Unfortunately, our results show that it will be difficult to predict changes in pepper anthracnose levels according to future increases in O_3 levels. From an ecological viewpoint, an increase in the interaction of natural O_3 levels and anthracnose on pepper plants may be beneficial. In general, O_3 can decrease the incidence of disease caused by obligate parasites; however, it increases problems associated with facultative parasites (Manning and Tiedemann, 1995; Krupa et al., 2000). O_3 has no effects on disease severity in other plant diseases, such as poinsettia-*Botrytis cinerea* (Manning et al., 1972) and corn-*Helminthosporium maydis* (Heagle, 1977).

We performed O_3 fumigation before infecting plants because they are generally more severely affected by O_3 than by fungal pathogens (Krupa et al., 2000). Therefore, we investigated the effects of increasing antioxidant reactions in pepper plants using O_3 fumigation. A pathogen and non-pathogen were inoculated on pre-fumigated plant tissue. Studies of pre-fumigation before pathogen infection

Table 4. Differences[a] in chlorophyll content (SPAD) between inoculated and uninoculated tissue for each ozone treatment.

		O_3 pre-treated		O_3 untreated	
Experiment	Hours	Inoculated	Uninoculated	Inoculated	Uninoculated
1	43	3.05 ± 2.11 **	-0.09 ± 1.61 ns	1.35 ± 1.97 *	-0.44 ± 1.76 ns
	50	4.48 ± 2.54 **	-0.74 ± 1.36 ns	2.17 ± 1.97 **	-0.39 ± 1.12 ns
	67	5.85 ± 2.81 **	-0.51 ± 1.32 ns	3.20 ± 3.23 **	-0.47 ± 0.97 ns
2	41	1.97 ± 2.03 **	-0.06 ± 0.68 ns	1.37 ± 1.12 **	0.33 ± 1.49 ns
	48	3.21 ± 1.35 **	0.22 ± 1.13 ns	2.91 ± 1.04 **	0.08 ± 1.32 ns
	65	4.62 ± 1.97 **	0.35 ± 0.41 *	4.67 ± 1.03 **	0.04 ± 0.99 ns
3	41	3.14 ± 1.63 **	-0.24 ± 1.46 ns	2.50 ± 1.32 **	0.12 ± 0.71 ns
	49	4.63 ± 2.32 **	-0.42 ± 1.98 ns	3.94 ± 1.85 **	0.28 ± 0.86 ns
	65	5.77 ± 3.38 **	-0.74 ± 0.54 ns	4.85 ± 1.78 **	0.11 ± 0.63 ns
4	43	4.90 ± 2.39 **	-0.06 ± 0.67 ns	3.44 ± 1.10 **	0.15 ± 0.72 ns
	50	7.09 ± 2.85 **	0.28 ± 0.54 ns	4.32 ± 1.61 **	0.26 ± 0.78 ns
	67	9.10 ± 3.39 **	0.29 ± 0.45 *	7.74 ± 2.38 **	0.02 ± 0.63 ns

[a]Difference in SPAD chlorophyll measurements between water-injected and non-water-injected tissues in the same leaf for O_3 pre-treated or O_3 untreated seedlings (mean ± standard deviation). P-values of Statistical results are for paired comparisons of symptoms on ten leaves between treatments; *P < 0.05; **P < 0.01; ns, non-significant.

Table 5. Differences in SPAD chlorophyll content with ozone and inoculation (*Xantomonas axonopodis* pv. *glycins*) treatments. Ozone and ozone+inoculation effects were analyzed using one-way ANOVA of ten leaves for each treatment.

Experiment	Hours	Inoculated		Uninoculated		P-values[a]	
		O_3 pre-treated	O_3 untreated	O_3 pre-treated	O_3 untreated	O_3[b]	O_3+inoculation[c]
1	43	21.41	26.60	24.36	26.46	**0.024**	**0.000**
	50	20.92	25.74	25.48	26.80	0.164	**0.000**
	67	18.41	25.12	24.89	26.76	0.085	**0.000**
2	41	29.94	29.36	28.94	30.73	0.085	0.267
	48	27.91	28.26	28.81	30.53	0.067	**0.012**
	65	26.30	25.87	28.36	30.45	**0.047**	**0.000**
3	41	24.13	25.21	26.00	29.06	**0.022**	**0.002**
	49	22.76	24.39	26.21	29.33	**0.029**	**0.000**
	65	21.00	23.14	25.38	29.00	**0.020**	**0.000**
4	43	22.97	26.97	28.06	29.98	**0.049**	**0.000**
	50	21.01	25.66	27.42	29.46	**0.039**	**0.000**
	67	18.31	22.32	27.91	29.22	0.130	**0.000**

[a]One-way ANOVA.
[b]O_3 effects were determined from the comparisons between uninoculated O_3 pre-treated and uninoculated O_3 untreated.
[c]O_3+inoculation effects were determined from the comparisons between inoculated O_3 pre-treated and uninoculated O_3 untreated.

are more common than those of post-fumigation after infection (Manning and Tiedemann, 1995).

Previous gene transcription data for pepper leaves (Lee and Yun, 2006) indicate that O_3 may predispose pepper plants to infection by economically important diseases. We confirmed that the hypersensitive response occurred 7.8 h earlier in O_3-treated plants than in untreated plants. This is consistent with the increase in transcription because O_3 turns on plant defense genes and accelerates the hypersensitive response. The measurement of chlorophyll content was easier and more reliable for quantifying leaf response to O_3 and inoculation. Chlorosis caused by O_3 was quite obvious and could be measured instantly, especially on the leaves of young seedlings.

As an eco-friendly alternative to chemical treatments, O_3 pre-treatment against anthracnose fungal infection is not applicable in the field. Artificial anthracnose infection of stems did not reach severe levels, and it was difficult to find any ozone pre-treatment effects because of the small difference between treatments. Although there were no significant differences between O_3 treatments in the ozone-sensitive cultivar 'Dabotop', there was a tendency for a decrease in anthracnose disease progression. In contrast, little effect of ozone on anthracnose disease progression was found in the ozone-tolerant cultivar 'Buchon'.

Again, in green fruit, we were not able to confirm whether pre-treatment with ozone turns on pepper defense mechanisms to control anthracnose infection. It seems that pre-O_3 treatment does not defense an anthracnose in fruits. However, we investigated the disease progression only after artificial infection, not natural infection. We could not easily quantify pathogenicity caused by O_3 effects because pathogenicity is a result of the integration of the host plant, pathogen, and environmental factors, as well as the ozone treatment. This caused high variation within treatments, resulting in a lack of statistical significance. The results of eight replicate experiments on green fruits and five replicate experiments on stems were highly variable but it may show that the effect of O_3 on anthracnose disease severity was not critical to decrease the disease.

Because the leaves of 'Dabotop' are sensitive to O_3, the stem would also be expected to be sensitive, as it was except in the second replication (Table 1). However, we did not detect a significant difference in ozone sensitivity between the two cultivars because the differences were within 10%, and at best, 13-16% (Table 1). In the experiments with fruit, the coefficients of variation in the O_3 treatment were 12-33%, whereas those without the O_3 treatment were 19-54% (Table 2) because of high variation in the pathogenicity. Although such high variation makes it difficult to detect statistical differences, it may indicate that O_3 treatment makes anthracnose disease less variable.

In leaves, the effects of ozone in accelerating the HR and chlorosis were obvious and statistically significant. These results support previous molecular evidence of increases in plant defensive gene expression. Statistically significant responses to O_3 in pepper were limited to the leaves. Antioxidant changes in the cellular tissue did not occur in either

fruits or stems.

In conclusion, the antioxidant effect of O_3 was observed in foliar hypersensitive responses and chlorophyll content, supporting previous evidence of increased transcription of plant defense genes. However, the effects of O_3 were restricted to foliar tissue. Pre-treatment with O_3 did not decrease anthracnose disease severity on green fruits and stems.

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