Different Photosynthetic Responses of Black Cherry (*Prunus serotina*) with Different Sensitivities to Ambient Ozone Concentrations under Natural Conditions

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자연상태에서 대기 중 오존 농도에 상이한 민감성을 가진 Black Cherry(*Prunus serotina*)의 상이한 광합성 반응

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ABSTRACT

Two different sensitivity classes of black cherry (*Prunus serotina*) under the natural growing environmental conditions were assessed adjacent to Air Monitoring Station located at Horton research center in Giles County, Virginia, USA. Ambient ozone concentrations, leaf gas exchange, and visible foliar injury were measured on-site during the growing seasons of 2000, 2001, and 2002. Ambient ozone exposures were sufficient to induce typical foliar visible injury corresponding with the reduction in photosynthetic activities only in sensitive black cherry. There were positive correlations between increasing cumulative ozone concentration and percent reduction in maximum net photosynthetic rates (Pn_{MAX}) under saturating light conditions and in quantum yield for carbon reduction (ΦCO_2) of sensitive black cherry compared to tolerant black cherry. There was a negative correlation between chlorophyll content and percent leaf injury in sensitive black cherry. Furthermore, Pn_{MAX} was inversely related to percent leaf injury.

Key words : Photosynthetic activity, Ozone, Sensitivity, Black Cherry

I. Introduction

Ozone is considered as one of the most widespread and serious air pollutants affecting forest trees (Simini *et al.*, 1992; Skelly, 2000). Ozone enters leaf through stomata, diffuses and reacts with the cellular membrane, and enters into metabolic processes (Reich, 1987). Therefore, ozone induced injury is closely related to physiological impairments with/without visi-

ble foliar injury. Samuelson (1994) reported the reduced photosynthetic rates and stomatal conductance accompanied with visible foliar injury when exposed to ambient ozone concentration.

Black cherry (*Prunus serotina*) is an economically and ecologically important in eastern forest of the United States (Rebbeck, 1996) and has been shown to be one of the most sensitive hardwoods to atmospheric ozone in terms of development of visible foliar injury

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(Neaufeld *et al.*, 1995). Palisade cells in the leaf mesophyll are particularly sensitive to ozone injury and the collapse of individual cells or groups of palisade cells produces visible symptoms on the leaf surface (Fredericksen, 1996). Older leaves are more severely affected with advancing symptoms of premature senescence and defoliation (Skelly *et al.*, 1998).

Genetic variations in sensitivity to ozone have been documented for several broad-leaved forest tree species. Reference showed that visible foliar injury among individual black cherry seedlings differed considerably. For example, the degree of visible foliar injury depended on sizes and ages of black cherry trees, suggesting the existence of sensitive and tolerant genotypes (Kolb *et al.*, 1997; Fredericksen, 1996).

It is challenging to assess direct and indirect effects of ozone under natural environmental conditions due to the diversity of environmental factors that affect exposure-response relationship (Schaub *et al.*, 2005). The present study was designed to determine the physiological processes that may cause the differences in foliar injury and growth response between sensitive and tolerant black cherry when exposed to ambient ozone concentrations under natural environment conditions. The main objectives were 1) to compare visible foliar injury and leaf gas exchange of black cherry with different sensitivities and 2) to better understand physiological mechanisms that may explain different sensitivity in foliar injury and growth response to ozone between genotypes.

II. Materials and Method

2.1. Experimental site and design

This study was conducted for three consecutive years, 2000, 2001, and 2002 during the growing season from May to September. The site was selected in the vicinity of Air Quality Monitoring Station located at Horton Research center in Giles County, Virginia, USA. A TECO ozone analyzer was used to measure ambient ozone concentrations. Weather variables including wind speed, wind direction, solar radiation, temperature, relative humidity (RH), and rainfall were monitored at the station. Two groups of black cherry (*Prunus Serotina* Ehl.) with contrasting sensitivities were selected on site. The sensitivity was determined as symptomatic foliar injury, occurring as upper leaf flecking and dead tissue, during the previous summer. Three trees were selected to represent two sensitivity

classes, sensitive and tolerant, respectively.

2.2. Gas Exchange

Gas exchange analysis was performed on the 3rd basal leaf every month from May to September each year. Gas exchange analysis was conducted with a Li-Cor 6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE). Net photosynthetic rate under saturating light conditions (P_{nMAX} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at a light intensity of $900 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ PAR and 350 ppm CO_2) and stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) were determined monthly from May to September on the 3rd leaf position each year. Assimilation-Irradiance response curves (A-I) were obtained by measuring assimilation at 350 ppm CO_2 concentration and irradiance ranging from 0 to $1200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR monthly from May to September during the growing season. From the initial slope of A-I curve, the apparent quantum efficiency for net CO_2 assimilation (CO_2) was determined. Assimilation - CO_2 response curves were also generated at maximum light condition of $1100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR and CO_2 concentrations ranging from 0 to 1600 ppm.

2.3. Visible foliar injury

Visible injury was evaluated at two week intervals beginning in mid-May until the end of the season in mid-September each year. Leaf positions two through six in sensitive trees were examined. The percentage of visible injury of total leaf area was assessed on the upper leaf surface.

Leaf discs were used to determine total chlorophyll concentrations. Extraction was performed using pure DMSO solvent. Concentrations of chlorophyll a and b were determined spectrophotometrically at wavelengths of 663 and 645, respectively, according to Barnes *et al.* (1992) and related to the fresh weight of the leaves. Total chlorophyll concentrations were quantified and compared between sensitive and tolerant black cherry on the 3rd leaves during the growing season, June to August. In September from sensitive trees, the relationship between total chlorophyll concentrations and percentage leaf injury was evaluated.

2.4. Data analysis

Two branches per tree and three trees per sensitivity class (i.e., sensitive and tolerant) were chosen on site. Each tree was designated as an experimental unit and each branch represented a pseudoreplicate. Statistical

analysis of the data was performed with the Statistical Analysis System (SAS, Inc., NC, USA). Photosynthetic data as well as chlorophyll fluorescence data were analyzed by analysis of variance with ozone sensitivity as class variable. Statistical significance was designated at the $P \leq 0.05$ probability level by a single asterisk and the $P \leq 0.01$ level by two asterisks

III. Results

3.1. Ozone exposure

Black cherry trees were exposed to ambient ozone for 7 hours (from 09:00 to 16:00 hrs). On average, the concentration of ambient ozone was 51, 51, and 53 ppb in 2000, 2001, and 2002, respectively. One-hour peak value of ambient ozone was higher in 2002 compared to those in 2000 and 2001 (Table 1). The highest one-hour peak in 2000 and 2001 were recorded in June at 90 and 95 ppb, respectively. The highest one-hour peak in 2002 was recorded in July at 121 ppb (Fig. 1). The seasonal SUM00 and SUM40 ozone values were higher in 2002 compared to 2000 and 2001 (Table 1, Fig. 2).

3.2. Physiological gas exchange

For three growing seasons of investigation, photosynthetic activities differed between sensitivity classes in black cherry. The ambient ozone concentrations were high enough to induce the reductions of photosynthetic activity in sensitive class compared to tolerant class. The substantial reductions of Pn_{MAX} and ΦCO_2 in sensitive class were observed throughout the experimental seasons for the three years. Such reductions were associated with seasonal cumulative ambient ozone expo-

sure (SUM00). For example, R^2 value for Pn_{MAX} and ΦCO_2 were 0.80 and 0.82, respectively. The values of Pn_{MAX} and ΦCO_2 for sensitive class increased at first.

Fig. 1. Daily peaks of atmospheric ambient ozone concentration (ppb) at the Horton Research Center, Giles County, VA, during the growing season of three consecutive years from 2000 to 2002.

Table 1. Seasonal means of atmospheric ambient O_3 concentrations (ppb) and seasonal cumulative O_3 concentrations (ppm h) at Horton research center in Giles County, VA, for three consecutive years from 2000 to 2002. Values are means $\pm$ s.d.

	2000	2001	2002
7-h means	51 $\pm$ 13	51 $\pm$ 12	53 $\pm$ 13
12-h means	51 $\pm$ 12	51 $\pm$ 11	53 $\pm$ 13
Peak means	58 $\pm$ 13	62 $\pm$ 12	66 $\pm$ 16
Seasonal peak	90	95	121
SUM40 (ppm h)	NA	67.46	75.77
SUM00 (ppm h)	80.47	81.38	85.02

*NA: not available

Fig. 2. Seasonal cumulative O_3 concentrations (SUM00, ppm h) for three years from 2000 to 2002 in Giles County, VA.

Fig. 3. Percent changes in (A) Pn_{MAX} (maximum net photosynthetic rates, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at $900 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and (B) apparent quantum yield for carbon reduction (CO_2 , $\mu\text{mol CO}_2 \mu\text{mol quanta}^{-1}$) in sensitive black cherry compared to tolerant black cherry related to cumulative O_3 concentration (ppm h). Data collected during the growing seasons from 2000 to 2002.

As SUM00 increased throughout the experimental growing seasons, Pn_{MAX} and ΦCO_2 for sensitive class decreased (Fig. 3). This implies that, in the beginning, sensitive class had greater photosynthetic activities than tolerant class. However, as seasonal ambient

cumulative ozone exposures increased, Pn_{MAX} and ΦCO_2 for tolerant class were relatively consistent.

Gas exchanges measured on the 3rd basal leaf from May to September each year are summarized in Table 2 for 2000. Pn_{MAX} was significantly higher in sensitive black cherry ($9.26 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) than in tolerant black cherry ($7.42 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at the beginning of the experimental season (e.g., May). Stomatal conductance (g_s ; $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) was also significantly higher in sensitive black cherry ($0.17 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) compared to tolerant black cherry ($0.10 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) in May. In June, Pn_{MAX} in both sensitive and tolerant black cherry were similar at 11.27 and $10.85 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively. From June to September, stomatal conductance was not significantly different between sensitive and tolerant classes. However, Pn_{MAX} started to decline in sensitive black cherry in July and was significantly reduced by 60% compared to tolerant black cherry in September. Pn_{MAX} in tolerant black cherry remained relatively high at $9.77 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ until August, and then declined slightly as leaves naturally senesced in September.

In May, values of ΦCO_2 were significantly higher in sensitive black cherry compared to tolerant black cherry corresponding to Pn_{MAX} and stomatal conductance. The values of ΦCO_2 reduced significantly in sensitive black cherry compared to tolerant black cherry in September. The values of Pn_{MAX} and ΦCO_2 for tolerant black cherry declined slightly as leaves naturally senesced.

During the experimental season, A-I response curves of black cherry were generated monthly and compared between sensitive and tolerant black cherry (data shown in Fig. 4 for 2002). Net CO_2 assimilation rates (P_n ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) increased as irradiance increased in both sensitive and tolerant black cherry (Fig. 4). In

Table 2. Maximum net photosynthetic rate (Pn_{MAX} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at $900 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), internal CO_2 concentration (C_i , ppm) and apparent quantum yield for CO_2 assimilation (CO_2 , $\mu\text{mol CO}_2 \mu\text{mol quanta}^{-1}$) on the 3rd basal leaf of sensitive and tolerant black cherry during the growing season of 2000. Values are mean $\pm$ s.d. Asterisks indicate the significant differences between cultivars. * at $P < 0.05$ and ** at $P < 0.01$

	May		June		July		August		September	
	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant	Sensitive	Tolerant
Pn_{max}	9.16 ± 0.67	$7.42 \pm 0.42^{**}$	11.27 ± 0.94	10.85 ± 1.50	8.93 ± 0.25	9.81 ± 0.42	7.26 ± 0.93	$9.77 \pm 0.93^{**}$	2.85 ± 0.67	$8.07 \pm 0.77^{**}$
g_s	0.17 ± 0.02	$0.10 \pm 0.01^{**}$	0.30 ± 0.02	0.26 ± 0.04	0.21 ± 0.02	0.16 ± 0.06	0.21 ± 0.04	0.22 ± 0.01	0.22 ± 0.04	0.21 ± 0.02
C_i	234 ± 7.2	$206 \pm 9.1^{**}$	259 ± 4.2	254 ± 5.1	256 ± 6.1	200 ± 10.8	298 ± 9.5	$278 \pm 5.5^*$	308 ± 10.0	$273 \pm 4.0^{**}$
CO_2	0.050 ± 0.002	0.046 ± 0.003	—	—	0.047 ± 0.001	0.048 ± 0.002	0.042 ± 0.002	$0.049 \pm 0.002^*$	0.031	$0.039 \pm 0.002^{**}$

— : data not available

Fig. 4. Net assimilation rate (P_n ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)-Irradiance (PAR; $\mu\text{mol m}^{-2} \text{ s}^{-1}$) response curves on the 3rd leaf of sensitive (closed circles) and tolerant (open circles) in black cherry during the growing season of 2002. Inserts are the initial slopes of the A-I response curves. Bars represent $\pm$ one standard deviation of the means and, where not apparent, are contained within symbols.

June, P_n values for sensitive and tolerant black cherry were not different significantly under all light conditions. From July, however, P_n under light conditions higher than $150 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR reduced significantly in sensitive black cherry compared to tolerant black cherry.

Net CO_2 assimilation rates in response to various CO_2 concentrations were measured during the experimental season in 2002. A- CO_2 response curves were generated and compared between sensitive and tolerant black cherry (Fig. 5). In 2002, P_n increased as CO_2 concentrations increased (Fig. 5). In June, P_n , at lower CO_2 concentration than ambient conditions of 350 ppm, was not significantly different in both sensitive and tolerant black cherry. However, P_n , at higher CO_2 conditions than 350 ppm, was significantly reduced in sensitive black cherry compared to tolerant black cherry. Under the doubled ambient CO_2 conditions (i.e., > 800 ppm), the reduction of P_n in sensitive black

cherry was 20% compared to that of tolerant black cherry. From July, P_n , at ambient CO_2 conditions of 350 ppm, was reduced in sensitive black cherry compared tolerant black cherry. By August, at even lower CO_2 conditions, P_n was significantly reduced in sensitive black cherry compared to tolerant black cherry. In August, reductions of P_n in sensitive black cherry compared to tolerant black cherry were getting greater as CO_2 concentrations increased.

The values of Φ_{CE} for both sensitive and tolerant black cherry were not significantly different in June. However, from July, reductions of Φ_{CE} in sensitive black cherry compared to tolerant black cherry were significant. By August, reduction of Φ_{CE} in sensitive black cherry was more than 40% compared to tolerant black cherry (data not shown).

3.3. Visible foliar injury

From late July to early August, visible foliar injury

Fig. 5. Net assimilation (P_n ; $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) - CO₂ concentrations (ppm) response curves (at $1100 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR) on the 3rd leaf of sensitive (closed circles) and tolerant (open circles) black cherry during the growing season of 2002. Bars represent $\pm$ one standard deviation of means and, where not apparent, are contained within symbols.

Fig. 6. Percent visible foliar injury of total leaf area estimated on the different leaf positions of sensitive black cherry in September 2001. Leaf position 10 (L10) is the youngest leaf estimated. Error bars represent standard deviations of the means.

Fig. 7. $P_{n\text{MAX}}$ (maximum net photosynthetic rates, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at saturated light condition of $900 \mu\text{mol m}^{-2} \text{ s}^{-1}$) related to percent visible foliar injury of sensitive black cherry in consecutive 2 years, 2000 (solid line and closed circles) and 2001 (dotted line and open circles).

became apparent on leaves of sensitive black cherry. Towards the end of growing season in September, most leaves showed visible injury in sensitive black cherry. The symptoms appeared as brown spots on the upper surface of leaves, which is typical foliar injury induced by ozone in black cherry. The estimated foliar injury was more severe in older leaves with 30 to 35% of total leaf area

than in younger leaves with 0 to 5% (Fig. 6). This visible foliar injury was well correlated with photosynthetic activity and chlorophyll contents. $P_{n\text{MAX}}$ was related inversely to percent visible foliar injury with an R^2 value of 0.83 in 2000 and 0.89 in 2001 (Fig. 7). Chlorophyll contents were also related inversely to percent visible foliar injury with an R^2 value of 0.95 (Fig. 8).

Fig. 8. Chlorophyll contents (mg m^{-2}) related to percent visible foliar injury of total leaf area in sensitive black cherry in September 2002. Each sample point has three samples.

IV. Discussion

In our study, level of exposure to ambient ozone in terms of concentrations and duration was high enough to cause visible foliar injury and to disrupt physiological activity only in sensitive black cherry. Our results were similar to those reported by Schaub *et al.* (2005). The seasonal 7-h and 12-h ambient ozone concentrations averaged 40-50 ppb (Table 1) and episodic peaks reached 90 to 120 ppb (Fig. 1), which typically occurs during the summer in Mid-Atlantic States (Skelly, 2000 and Schaub, 2003).

Our results showed that visible foliar injury of sensitive black cherry resulted from greater stomatal conductance and consequently greater ozone uptake than those of tolerant black cherry (Table 2). High ozone uptake with greater stomatal conductance in sensitive black cherry seedlings has been linked to greater ozone injury than tolerant black cherry seedlings (Kouterick *et al.* 2000). Our results confirmed that foliar injury was correlated with stomatal conductance in black cherry. For example, Ferdinand *et al.* (2000) reported that stomatal density of sensitive black cherry seedlings was greater than that of tolerant ones because the sensitive seedlings had greater potential of stomatal conductance than tolerant ones. Even though ozone tolerant mature black cherry showed greater leaf area, stomatal density was still significantly greater for ozone sensitive black cherry.

The response to ozone is underestimated, however, if sensitivity emphasizes only the differences in stomatal conductance (Schaub *et al.*, 2005). Not only stomatal density but other internal morphological leaf characteristic may play an important role in response to ozone of sensitive black cherry. Fredericksen *et al.* (1995) reported

thinner leaves and a lower palisade/spongy mesophyll thickness ratio in sensitive black cherry than tolerant black cherry. According to Ferdinand *et al.* (2000), leaves of R-12, ozone-sensitive seedlings, displayed greater spongy mesophyll with greater intercellular air space and thinner palisade mesophyll than MO-7, ozone-tolerant seedlings. These morphological characteristics in sensitive black cherry would provide less mesophyll resistance to ozone ingress to the palisade mesophyll and may lead dead palisade mesophyll cells and visible foliar injury. Evans *et al.* (1996) also supported that certain leaf morphological characteristics such as stomatal density and intercellular air space may lead ozone ingress into leaves and internal diffusivity among ozone target cells, such as palisade mesophyll cells. Although direct morphological characteristics have not been measured, a clear linear relationship between chlorophyll contents and foliar injury were observed in sensitive black cherry with R^2 value of 0.95 in this study (Fig. 8). As chlorophyll contents decreased, greater visible foliar injury was observed.

Kouterick *et al.* (2000) reported that the differences in ozone uptake and visible foliar injury between families did not correspond with family differences in photosynthetic activity to ozone exposure. They observed that the relative ozone sensitive family R-12 had more visible foliar injury as well as higher P_n than relative ozone tolerant family MO-7 throughout the growing season. In addition, all measurements of P_n were 12 to $17 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for sensitive family R-12 and 10 to $16 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for tolerant family MO-7 under $600 \mu\text{mol m}^{-2} \text{ s}^{-1}$ light conditions regardless to seasonal cumulative ozone concentration. However, they measured P_n of newly expanded young leaf throughout the growing season and cumulative effect of ozone was not considered. Such young leaves would have higher P_n and yet would not be severely affected by ozone. Our results clearly showed that reductions of P_n in sensitive black cherry compared to tolerant black cherry were correlated with seasonal cumulative ozone concentrations (Fig. 3).

Greater foliar injury in the older leaves was observed in sensitive black cherry at the end of growing season due to a longer duration of ozone exposure than younger leaves produced later in the growing season (Fig. 6). Older leaves also showed more severe injury in $P_{n\text{MAX}}$ than younger leaves in sensitive black cherry (data not shown). Visible foliar injury was well correlated to photosynthetic activity in this study. The per-

cent leaf injury of total leaf area was negatively related to Pn_{MAX} in sensitive black cherry with R^2 value of 0.83 and 0.89 in 2000 and 2001, respectively (Fig. 7). Our results agree with other studies on other ozone sensitive trees and crops, which showed a clear inverse relationship between visible foliar injury and photosynthetic activity (Davis and Skelly, 1992; Flagler, 1994). Our results indicated that visible foliar injury was related to photosynthetic activity between black cherry families. Therefore, visible foliar injury could be used as a simple measure to study ozone impacts on black cherry in terms of genetic difference.

With limited access to biochemical mechanisms such as direct measure of production and activation of RUBISCO, carboxylation efficiency was determined from the assimilation- CO_2 response curves during the growing season in 2002 and compared between sensitive and tolerant black cherry (Fig. 5). Carboxylation efficiency in sensitive black cherry was reduced in July with other photosynthetic activity. When carbon is not a limiting factor under saturating CO_2 conditions, however, the maximum rate of electron transport used in the regeneration of RUBP was reduced in sensitive black cherry at first.

This study demonstrated the different genetic responses of black cherry to ambient ozone concentrations under the natural environment conditions. The genotypic differences in response to ozone have been clearly observed. Further researches in genetic variations of ozone sensitivity among black cherry should include examinations of biochemical analysis such as anti-oxidant production and RUBISCO activity levels which differ in sensitivity to foliar ozone injury.

적 요

오존은 식물에 가장 큰 피해를 주는 대기 오염원 중 하나로 알려져 있으며, 현재 대기 중 오존 농도만으로도 민감한 종에서는 피해가 관찰된다. 기공을 통해 유입된 실제 오존량이 식물의 잎에 상당한 피해를 입히며, 광합성률의 저하를 일으키는 것으로 알려져 있다. 식물의 오존에 대한 반응은 식물의 생리적 연령과 민감성에 따라 다양하며, 잎의 성숙과 밀접하게 연관되어 있다. 가장 최근에 성숙한 잎이 이미 성숙한 잎들이나 빠르게 성장하는 어린 잎들과 비교해 보았을 때 오존에 가장 예민한 것으로 알려져 있다. Black Cherry (*Prunus serotina*)는 경제적, 생태적으로 중요한 수종중의 하나이다. 또한 오존에 민감한 수종중의 하나이며,

대기 중 오존 농도에 의해 피해를 보여, 오존의 생물 지표로 이용되기도 한다. 상이한 오존 민감성은 유전적 차이에 기인하는 것으로 알려져 있으며, 이 점으로 인해 대기 중 오존 농도가 식물의 광합성 및 PSII 기능에 미치는 영향을 연구하기에 이상적인 수종으로 여겨진다. 이에 본 연구는 3년간 버지니아 주 Giles County 에 위치해 있는 Horton 연구센터 실험 숲에서, Black Cherry 중 상이한 두 민감성을 보이는 품종을 이용하여 대기 중 오존 농도가 민감한 품종과 저항성을 지닌 품종에 미치는 영향을 연구하였다. 대기 중 오존에 노출된 채로 식물의 생장이 가장 빠른 성장기를 거친 결과, 대기 중 오존에 의해 민감한 품종은, 가시적인 피해와 함께 최대 순 광합성이나 탄소 동화에 대한 양자 수득율이 크게 감소되었으나 저항성을 지닌 품종은 가시피해의 부재와 함께 소폭의 최대 순 광합성 및 양자 수득률의 피해가 관찰되었다. 오존에 민감한 품종에서 잎의 가시피해는 광합성 기능과 밀접하게 연관되어 있으며, 잎의 생리적 연령이 늘어남에 따라 더욱 커지는 것으로 관찰되었다. 오존에 저항성이 있는 품종에서도 잎의 연령 증가에 따른 광합성 기능의 저하가 관찰 되었으나, 가시피해는 관찰되지 않았다. 오존에 의한 총 광합성률의 감소는 오존에 민감한 품종에서 더욱 현저하게 관찰되었다.

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