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Ozone-Induced Chlorophyll Degradation in *Populus tremuloides* Michx. Foliage

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Abstract

The effects of ozone (O_3) exposures on chlorophyll content of trembling aspen(*Populus tremuliodes*) rooted cuttings were studied. Ozone was treated with 100 ppb fumigation for 6 hours per day, for three consecutive days per week in open-top chamber. The ozone exposures caused severe foliage damage expressed in necrotic patches and extensive chlorosis. Degradation of chlorophyll was observed mostly in mature leaves. The chlorophyll response to ozone exposure maintained a similar pattern during all experimental seasons and plant materials. Significant genetic variation in O_3 responses was revealed by differences in sensitivities among clones.

Key words : *Populus tremuloides, ozone, chlorosis, clone*

Introduction

Studies concerning the impacts of ozone on plant tissue demonstrate that the damaging effect is associated with broad physiological and biochemical changes[1]. Two primary types of ozone injury patterns were described. Visible foliar injury manifested by loss of chlorophyll content is characterized as acute phase of injury induced by high ozone levels[2]. The second type of foliar injury associated with ozone was first reported for plants growing in the Los Angeles valley and consisted 'water-logging' and 'bronzing' and/or 'silvering'[3].

Chlorophyll degradation is a principal component of senescence in plants[4], and chlorophyll content is good bioindicator of air pollution level[5]. Ozone-induced chlorophyll reduction is considered to be symptomatic of premature aging[6]. Chlorophyll contents of whole plants and individual leaves were always significantly lower in ozone-exposed poplar leaves than in control, nonozonated leaves[7].

The close relationship between chlorophyll content and photosynthesis was recorded long ago. Kuno[8] found a close positive relationship between chlophyll content and photosynthetic rate in clonal poplar trees exposed to 70-150 ppb ozone for three months of fumigation.

Trembling aspen(*Populus tremuloides* Michx.) is described as a one of the most sensitive tree species to ozone[9,10]. This experiment was conducted to examine the effects of seasonal ozone exposures on aspen growth and development. In this paper, the effects of ozone on foliar chlorophyll content of aspen rooted cuttings are described.

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Materials and Methods

Plant material

The clonal material originated as softwood cuttings from greenhouse-grown trembling aspen stock plants representing eight genotypes (Table 1) previoulsy chracterized for sensitivity by amount of visible foliar injury following short-term ozone fumigation[11]. Cuttings were dipped in Rootone F and rooted in 40cm×20cm×8 cm plastic trays in a perlite-peat (1 : 1) mix placed in mist chamber. Rooting generally occured within four weeks. Rooted plants were then removed from the mist chamber and placed on a greenhouse bench.

Table 1. Clonal tolerance of trembling aspen clones to ozone determined based on chlorophyll reduction

Clone code No.	Sensitivity[2]	Chlorophyll reduction (%)[1]
216	Tolerance	48
253	Sensitive	60
259	Sensitive	59
271	Tolerant	30

[1] Comparison based on fully developed older leaves (LPI 25).
Chlorophyll content reduction is computed as compared to leaves from plants grown in charcoal-filtered-air chamber. Each value ismean of 9 plants.

[2] Based on references 9, 10, 11.

In early May, plants were transplanted into 37.5 cm deep×15 cm plastic pots in a media of peat perlite-topsoil (1 : 1 : 1), supplemented with 8 g of Sierra Osmocote (17-6-12 N,P,K formulation), plus micronutrients (four month slow release formula). Tlansplants were grown in the greenhouse for approximately two weeks until they were transferred to the fileld site and placed under 50 percent shade cloth. After field acclimatization for 1 week, plants about 40 cm tall were placed in fumigation chambers at the begining of June of growing season for ozone treatments. Open-top chambers modified with rain-exclusion caps were used for ozone treatment.

Ozone exposure

Ozone was generated using a Griffin Model 0.5A Ozone Generator. The seasonal fumigations consisted of 100 ppb ozone exposure for hours per day, for three consecutive days per week.

Plant measurements

The Leaf Plastochron Index (LPI)[12] was used to select leaves at comparable stages of development of sampling. Chlorophyll samples were taken from recently mature leaves (LPI 8) and mature leaves (LPI 25).

Chlorophyll samples for the plants were taken monthly as 1 cm diameter circular punches of leaves (2 punches per leaf). Chlorophyll was extracted in N,N-dimethylformamide[13] and determined spectrophotometrically.

Results

Foliar injury was visible within two weeks after the start of the ozone exposure. Black bifacial necrosis appeared first on the fully developed older leaves on the lower part of the stem. At the end of the fumigation season chlorosis and black bifacial stipple on the upper surface of the leaves were common on all but the youngest leaves. Premature leaf abscission occured commonly for older leaves (from LPI 1 to LPI 8) maintained a green color and healthy apperance in all treatments during the fumigation season.

Clones exposed to ozone responded in a pattern similar to their leaf injury sensitivity ratings. Clones 253 and 259 (considered as sensitive) showed high chlorophyll reduction (Table 1) in LPI 25 leaves of 4 clones tested. Chlorophyll reduction was also observed in LPI 8 leaves but to a significantly lower degree.

Analysis of chlorophyll content of plants showed that significant differences among clones occur even without ozone exposure (Table 2). The highest concentration

Table 2. Chlorophyll content changes during the grwoing season in leaves of potted Populus tremuloides plants of four clones exposed to ozone. Clonal means (± standard deviation) are the average of fifteen plants per clone and per treatment

Clone	chlorophyll content (μg/cm²)				
	Control[1]	Ozone treated			
		June 20	July 10	August 10	september 16
		LPI 8[2]			
216	42.0± 3.8 b[3]	42.7 ±3.8 b	51.3∓1.7 c	47.0± 1.4 b	41.8± 1.7 b
253	42.2± 2.2 b	44.2± 2.0 b	44.9± 1.6 b	46.8± 1.6 b	43.1± 1.5 ab
259	43.3± 2.3 b	44.3± 2.4 b	54.2± 2.0 c	51.0± 1.4 a	46.4± 1.8 a
271	55.9± 4.6 a	54.9± 4.4 a	38.4± 1.7 a	38.9± 1.9 b	39.3± 1.4 b
Mean	45.9± 1.7	46.3± 1.7	47.1± 0.9	45.9± 1.4	39.3± 1.4
		LPI 25			
216	56.0± 4.9 b	56.9± 5.1 b	36.8± 1.9 b	32.7± 1.6 b	20.5± 1.5 b
253	63.5± 6.5 b	66.7± 6.5 b	50.4± 2.1 a	33.3± 2.2 b	16.9± 1.1 c
259	60.1± 4.9 b	59.1± 4.7 b	40.1± 2.0 b	32.4± 1.6 b	20.5± 1.2 b
271	81.3± 5.3 a	83.5± 5.0 a	49.4± 1.9 a	38.6± 1.6 a	23.3± 1.1 a
Mean	65.2± 2.9	67.3± 2.9	44.2± 1.0	34.3± 0.9	20.3± 0.6

[1] Leaves were taken before ozone treatment.

[2] Clones with the same letter were not significantly different at the 0.05 level as determined by the Student-Newman-Keuls multiple comparison test.

[3] Chlorophyll samples were taken from recently mature (LPI 8) and mature (LPI 25) leaves.

was detected in clone 271. Significant differences in chlorophyll concentration among the clones were recorded during the entire season. Considering all plant material and treatments in general, there was a similar pattern in chlorophyll content decrease after exposure to ozone. Chlorophyll concentration decreases along the stem in upper leaves. There was a visible chlorophyll degradation after exposure to ozone in lower leaves while the concentration in upper leaves increased steadily throughout the season(Table 2).

Discussion

Ozone expose caused severe foliar symptoms expressed in necrotic patches and chlorosis. The phenomenon was more noticeable in mature leaves than in young leaves. The pattern of increasing chlorophyll content in young leaves and decreasing in aging leaves is described by Reich et al.[7] as a typical age-related consequence. The degradation of chlorophyll is the main criterion of leaves senescening[14]. The loss of chlorophyll and closely related decline of photosynthesis is a natural phenomenon during autumnal leaf senescence[15]. Reigh[16] reported that with increasing leaf age chlorophyll content declines more rapidly than in plants not exposed to ozone. Accelerated leaf aging due to ozone exposure and decline of chlorophyll content has other serious consequences such as decreased net photosynthesis[17].

In-depth studies showing accelerated leaf aging as a result of ozone exposure were performed by Ballach[6]. Our study confirms earlier studyies where increased chlorophyll content was observed in younger leaves while lower leaves showed significantly reduced chlorophyll

content under ozone exposure. Ballach[6] suggested that increase of pigment concentration in younger leaves in ozone exposed plants indicates the elevated anabolic reactions of the leaves. This may lead to a form of compensation for ozone effects on older leaves. Healthy, recently developing leaves in ozone treated plants may partially compensate for the dramatic loss of chlorophyll in older leaves.

Conclusions

The ozone exposures caused severe foliage damage such as necrotic patches and extensive chlorosis on the plants used in this experiment although there was a significant genetic variation in ozone responses by differences in sensitivity among clones.

Results obtained in this study were consistent during periods of experiments despite variability of environmental conditions and plant material.

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초록 : 오존처리에 의한 미국 사시나무의 엽록소 분해

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미국사시나무(*Populus tremuloides*)의 삽목개체에 오존이 엽록소함량의 변화에 미치는 영향을 조사하였다. 오존은 일일 6시간씩, 주 3일간 연속적으로 open-top chamber에서 fumigation 하였다. 오존처리는 괴사반점과 광범위한 chlorosis 등 잎 조직에 심각한 상해를 발생시켰다. 오존처리에 의한 엽록소의 분해는 완전히 성숙한 잎에서 현저하게 관찰되었으나 어린 잎에서는 분해가 거의 보이지 않았다. 오존처리에 대한 엽록소의 반응은 실험 전 기간에 걸쳐 모든 실험대상식물에 있어 일정한 양상을 보였다. 또한 clone별로 오존 민감성에 따라 엽록소함량의 변화에 상당한 유전적 변이를 보였다.