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Effects of Ozone (O_3) on leaf secretory cell characteristics related to allelopathy of woody plants: Modelling allelopathic interactions

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ABSTRACT

We studied the effects of ozone (O_3) on the allelopathic medicinal trees/shrubs that influenced other species growing under their canopy in National Park Sochi, Russia (Caucasus region). Using model reactions, the action of rain leachates from these allelopathic trees/shrubs on the plants growing under their canopy was determined. Depending on the duration and intensity of exposure, the O_3 treatment of test woody plants changed their (i) characteristics of leaves and their water extracts, (ii) colour and (iii) autofluorescence of secretory cells. Water extracts from the allelopathic woody donor plants served as model of rain leachates, and their effects were tested on the seeds and pollens germination of acceptor plants (*Lavatera trimestris* L. *var.*Rubin and *Hippeastrum* (Herb.) *hybridum*). The effects of water extracts from the ozone treated plants differed from the untreated plants. The allelochemicals in donor plants stimulated, inhibited or had no effects on the recipient plants compared with control. The test-reactions in tropospheric ozone stress should be used in Modelling System of tree/shrub-herbaceous plants interactions, to find the allelopathic interactions in urban conditions.

Key words: Autofluorescence, *Actinidia chinensis* Planch., *Albicia julibrissin* Durazz., *Citrus unshiu* (Marc.), colour, *Eucalyptus cinerea* F.Muel., *Ficus carica* Grossh.. , *Gleditsia triacanthos* L,, *Heimia salicifolia* Link., *Hippeastrum* (Herb.)*hybridum, Lavatera trimestris* L., *Nerium oleander* L., *Philadelphus caucasicus* Koehne., pollen, secretory cells, seeds, *Taxus baccata* L., ultraviolet irradiation

INTRODUCTION

The plants undergo various atmospheric effects, for example, high concentration of ozone formed naturally or due to industrial and automobile pollution (2,14,30). Tropospheric O_3, released from the electric discharges of thunderstorm, or under the Sun UV- irradiation at air pollution near the ground (4), adversely affects the health of living organisms, both human (2,14) and plants (30). To study this problem, it should keep in mind that trees may be biomarkers of pollution in the nature (7). In natural and artificial plantations, the higher O_3 content at the ground-level may change the normal allelopathic interactions. However, till now, except review of Roshchina (22), there are no reports on this aspect. Tropospheric ozone in high concentration is dangerous even to Moscow and Northern Regions in Russia (14), but we do not have this information for subtropical Russia. Presently, the problem has arisen due to the adverse effects of O_3 on subtropical invasive plants and on endemic plant species in Sochi National Park, Sochi, Russia (32). This region of Caucasus mountains (Longitude 39.7256900 ^{o} E and Latitude 43.5991700^o N) is about 200-1000 m above the sea level, total annual precipitations (rain and snow):

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1000-1200 mm. It has many introduced subtropical woody species that influences the climate and human health.

Presently, we do not know anything about these allelopathic interactions and how the tropospheric O_3 influences them in resorts' zone of Caucasus region. This study aimed to prepare the model reactions to analyze the effects of rain leachates from allelopathic woody medicinal trees/shrubs of Sochi (Caucasus region) on the herbal plant species grown underneath their canopy in Sochi National Park.

At first, we need to determine, which tests for ozone are suitable in our study. We studied the woody donor plants species with visible secretory structures, growing in Sochi National Park. Test healthy donor trees/shrubs leaves were exposed to ozone, afterwards, their leaves water extracts (as model of rain leachates) were used in our studies to find their allelopathic effects on germination of seeds or pollens of Acceptor test plants.

One of the approaches to study the allelopathic effects of O_3 is to use those plants, containing the allelochemicals in their secretory structures (26,29), e.g. allelopathic medicinal species from *Eucalyptus* genus. Secretory cells of allelopathic plant species contain compounds that participate in the chemical interactions in biocenosis, and their allelopathic effects are due to their allelochemicals' content in rain leachates (18-21). Leachates from the allelopathical trees and shrubs affects the plants growing nearby or under their canopy (21). Such interactions occurs in various species of *Eucalyptus* genus (1,12,18,19,20,35). Their leachate caused up to 60 % inhibition in the intercropped alley crops (*Phaseolus aureus* Roxb., *Lens esculentum* Moench,, etc.) compared with control (12) and the allelopathic potential was due to volatiles (cineol and terpenes) and phenolic compounds, in particular syringic acid. The excretions from various species of *Eucalyptus* genus inhibits or stimulates the crops depending on their taxonomic class (18-20,35). The application of *Eucalyptus* leaf extracts inhibited the seeds germination of grass (6,16).

Effects of ozone on allelopathic interactions have not been studied yet. This paper aimed to determine the effects of ozone on allelochemicals in model system in tree/shrub-herb species of subtropical Caucasian regions of Sochi, Gagra and Batumi. In this region of the health resorts, the automobile transport pollution caused the formation of tropospheric ozone.

MATERIALS AND METHODS

Our experiments had many stages: (i). Selection of woody plants with leaf surface secretory structures (containing allelochemicals) and suitable for analysis of microscopic and spectral characteristics, (ii). Exposure of some selected test plants to ozone, (iii). Use of microscopic and spectral methods to observe the changes on the leaf surface after O_3 -treatment and in untreated samples *in situ* and (iv). Preparation of water extracts that may be models of the rain leachates from ozonated and untreated whole leaves of woody plants (donor of allelochemicals) and testing their allelopathic activity on the seed or pollen germination of Acceptor plants.

I. Selection of Donor and Acceptor plants: The experiments were carried out in model system: woody species (donors of allelochemicals) – herbs (acceptors of allelochemicals). Ten healthy adult donor woody plants (> 10 years old and taller than 2-3 meters) grown in Sochi National Park (Dendrarium) (32) were used as test plants (Table 1), and their samples were collected on September 10-11, 2019. Fresh leaves were air-dried for better microscopic and spectral analysis, because water in the tissue quenches fluorescence and decreases the visible allelochemicals concentration in secretory structures (25). To study the effects of ozone on plants, we also prepared the aqueous extracts from the treated and untreated intact leaves of plants, having the intact secretory structures. Soaking in water

Table 1. Economic Importance of woody donor plants and ornamental acceptor plants in National Park, Sochi, Russia

S.No.	Botanical Name	Family	Economic Importance
	Woody Donor Plants		
1	*Actinidia chinensis* Planch	Actidiniaceae	Fruits, nutritional, and healthy
2	*Albicia* (*Albizia*) *julibrissin* Durazz	Mimosaceae	Ornamental, medicinal health-resort spp
3	*Citrus unshiu* var. unshiu (Marc.)	Myrtaceae	Fruits, nutritional and Ornamental,
4	*Eucalyptus cinerea* F.Muell.	Rutaceae	Medicinal (anti-inflammatory), marsh spp
5	*Ficus colchica (carica)* Grossh.	Moraceae	Ornamental, medicinal, nutritional
6	*Gleditsia triacanthos* L.	Fabaceae	Ornamental, medicinal
7	*Heimia salicifolia* Link.	Lythraceae	Ornamental, medicinal (relaxation)
8	*Nerium oleander* L.	Apocinaceae	Ornamental, medicinal (anticancer)
9	*Philadelphus caucasicus* Koehne	Saxifragaceae	Ornamental, aromatic and good for health
10	*Taxus baccata* L.	Taxaceae	Ornamental, medicinal (anticancer)
	Ornamental Acceptor Plants		
1	*Hippeastrum hybridum* Herb.	Amaryllidaceae	Ornamental plant
2	*Lavatera trimestris* L.var. Rubin	Malvaceae	Ornamental plant

Adult donor woody plants (> 10 years old and taller than 2-3 m) were used.

lasted for 1.0 h for the extracts. This time was enough to washing the water soluble allelochemicals from leaves surface (25). The water extracts, imitating the rain leachates, were tested on the seeds or pollen germination of model indoor ornamental Acceptor plants. As the model acceptor herb species (Table 1), two ornamental (decorative) plants grown in Sochi Park were used*:* *Lavatera trimestris* L.var. Rubin (seed germination test) and *Hippeastrum (*Herb,) *hybridum* (pollen germination test). Pollens of room-grown *Hippeastrum hybridum* (fam. Amaryllidaceae) had been used earlier for various analyses (22).

Selection of cells and tissues of donor plants for microscopy: Among the 10-donor woody plants, only 5- species (*Albicia julibrissin* Planch,, *Citrus unshiu* (Marc.), *Eucalyptus cinerea* F.Muel.., *Ficus colhica* Grossh. and *Heimia salicifolia* Link.) had leaf surface secretory cells, suitable for light or luminescence microscopy, hence, selected (Fig. 1). All samples fluoresced, but secretory cells were most visible in leaves of two trees *Citrus unshiu* and *Eucalyptus cinerea* (5) and thus chosen for microscopy. However, remaining 8 woody species were used to prepare water extracts to test their allelopathic activity.

II. Water extracts modeling leachates. Water extracts from the leaves of 8-woody Donor plants were prepared. To prepare these aqueous extracts, the powdered leaves of Donor plants were soaked in water (1:10 w/v Ratio) for 1 h, filtered and used for experiments. This was imitation of leachates from the Donor trees leaf surfaces (with secretory structures) in 1 h-rain, where mainly water soluble components are leached. This time was enough to dissolve the water soluble allelochemicals from leaves surface (25).

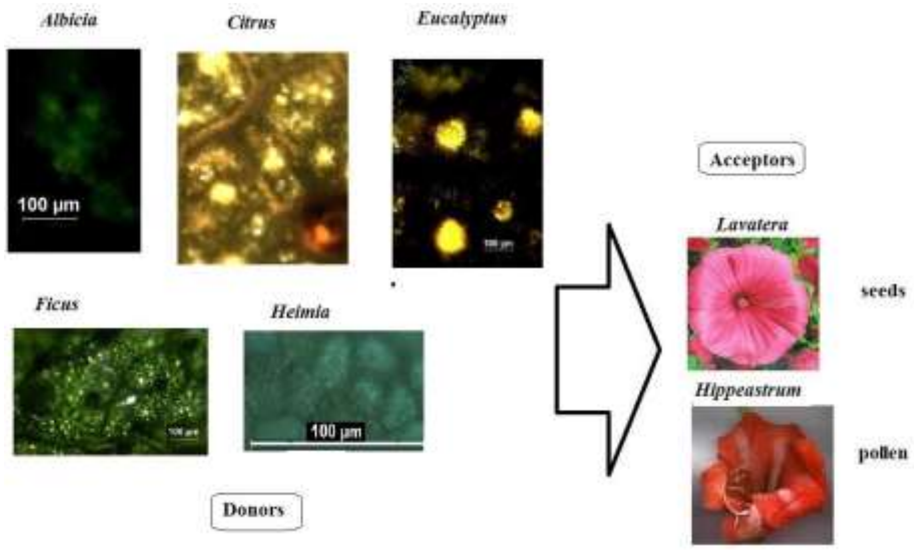

Figure 1. The microscopy of plant leaf surface in test woody donor plants (*Albicia julibrissin* Durazz, *Citrus unshiu* (Marc.), *Eucalyptus cinerea* F.Muell, *Ficus colhica* Grossh.and *Heimia salicifolia* Link.) and images of acceptor plants (*Lavatera trimestris* L. and *Hippeastrum hybridum*).

III. Ozone (O_3) was produced in following two ways:

(i). Electric discharge ozonators: It was used to apply acute and chronic doses of Ozone to Plants.

(ii). Ultraviolet irradiation: The ultraviolet bactericidal irradiator [OUVb-04 "Solnyshko" made by *EAS* (Russia)] was used for the Ozone exposure by ultraviolet irradiation. It was applied for antibacterial activity maximal 11 watt/m^2, and the amount of ozone produces was 0.1 µl /L per 30 min.

Ozone treatment: The air-dried leaves of test Donor plant species were exposed in ozone as under:

(i). **Acute Dose** (Short time exposure to High Concentration): Total O_3 dose : 0.5-0.7 to 5-50 µl/L for 10 min. The ozone was produced by *Orion-Si* ozonator (*Orion* company, Russia) in special polymer camera.

(ii). **Chronic Dose** (Long time exposure with Intervals to Lower Concentration): Total O_3 dose 0.05 µl/L, 5h per day for 3-days. The ozone was produced by ozone generator *KPMZ* (Russia) in plastic camera (volume 439 cm^3).

IV. Microscopy of non-ozonated and ozonated leaves

(i)Visual microscopic images of leaf surface: The changes in samples of leaves treated with ozone or controlled were observed under microscope *Leica* DM 6000 B (Germany-Austria-United States) in transmitted light.

(ii) Autofluorescence: Autofluorescence of leaf cells was observed and photographed using actinic light of luminescence microscope *Leica* DM 6000 B (Germany-Austria-United States) as described previously (31). The fluorescence spectra were recorded with the spectrofluorimeter *Perkin-Elmer* 350.

V. Bioassays

(i) Seeds germination: Ten seeds of acceptor plant *Lavatera trimensis* L.were put in each Petri dish (10 cm diameter) with filter paper as per standard method at 20-22 ^{O}C (21) and were irrigated with 5 ml water extract from leaves of donor woody plants. The treatments were replicated 4- times in Complete Randomized Design. The visual effects of water extracts on seedlings growth were recorded 24 -36 h after their application. The Experiment continued for 3 days.

(ii) Pollens germination: The pollen germination of *Hippeastrum hybridum* (Herb.) was studied *in-vitro* on the slides (subject glasses) at room temperature (20-22 ^{O}C) in the nutrients medium asper our earlier method (27). The nutrients medium contained (μg/L): potassium phosphate 6.63, calcium chloride 6.51, sodium chloride 3.47, magnesium chloride 5 in 10 % sucrose solution + test compound. One drop of test solution (0.05 ml = 1 drop) was added to pollen grains on each slide. Each slide was kept in the Petri dish with filter paper moistened with 5 ml water. Using light microscope, 2 h after moistening pollens with test solution, we analyzed 100 pollens grains per slide, to determine their microspores germination (%), as number of pollens with pollen tubes. The experimental duration was 2-3 h. The treatments were replicated 4- times in Complete Randomized Design.

VI. Statistical analysis: Results were expressed as mean ± SEM. The relative standard deviation was 5-6% (n = 40-50 seeds or 400-500 microspores per treatment; P =0.95).

RESULTS AND DISCUSSION

After O_3-treatment, we studied the changes in leaves of woody plants as donors of allelochemicals. The studies consisted of two experiments: (i) Microscopic and spectral analysis of the leaf surface with secretory structures, from which the compounds may be washed by rain and (ii) Testing of allelopathic activity of water extracts from leaves of donor woody species (imitated rain leachates).

1. Ozone-induced changes in leaf secretory structures of donor woody species containing allelochemicals

We studied the secretory and non-secretory cells in leaves of *Eucalyptus cinerea* and *Citrus unshiu* (5,9,10,34), which were suitable for microscopy analysis.

(i). *Microscopy images in transmitted light*: Fig. 2 showed the leaf oil glands of *Eucalyptus cinerea* F.Muell before and after exposure to chronic ozone treatment and oil glands of *Citrus unshiu* in control and after acute dose of ozone. In both cases, the bleaching of primarily orange, yellow and even red coloured glands were observed in samples treated by O_3. In *Eucalyptus*, the acute dose of ozone gas also affected the colour of glands similar to *Citrus*. Acute exposure to ozone (total dose 0.5-0.7 μl/l for 10 min) quickly changed the colour of glands. For one moment, secretory cells became yellowish- green and dark green. The middle part of some visible glands of *Eucalyptus* were pale yellow, perhaps, due to the presence of phenolic compounds in secretory cells (5,9). The changes in the

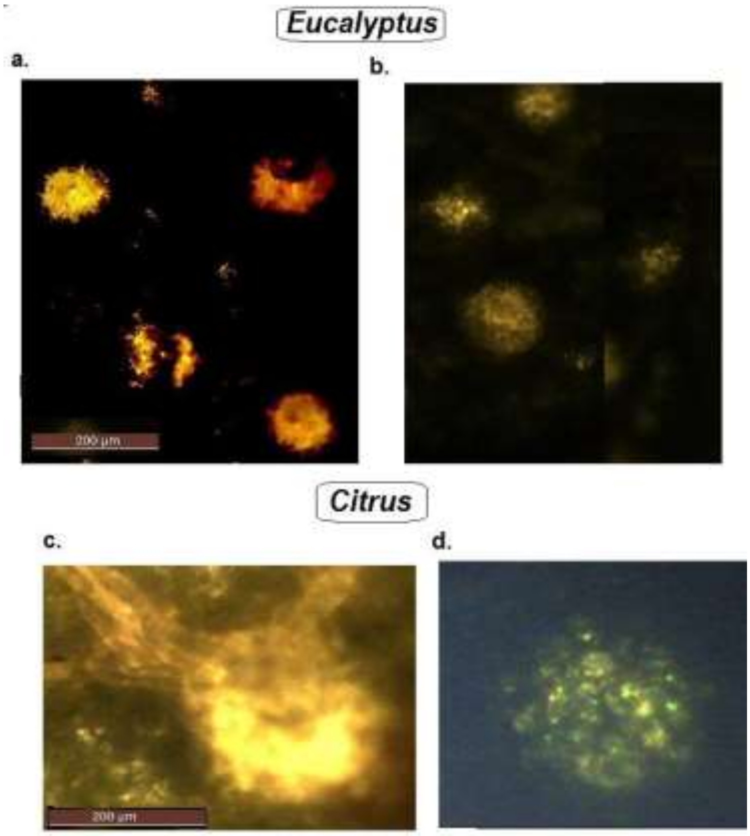

Figure 2. The colour changes in the secretory structures of leaf from (a) *Eucalyptus cinerea* F.Muell. and (c) *Citrus unshiu* (Marc.) before and after exposure to ozone (0.05 µl/L) during 3 days (b) or (0.5-0.7 µl/L) during 10 min (d).

glands' colours due to ozone, depends on the phenols contents in the glandular oil (9,10,26). Transformation of phenolic compounds (flavonoids, for example anthocyanin pelargonidin) may be a cause the loss of red colour under acute (5 min) and chronic (>25 h with intervals) action of ozone in *Saintpaulia ionantha* Wendl.petal hairs (31). The ozone treatment markedly changes the accumulated flavonoids in fruits of *Citrus unshiu* (37). There were also changes in the non-volatile components (monoterpene esters) or flavanones of essential oil secretory cells and cavities of *Eucalyptus* leaves (9).

We also found the loss of chlorophyll green colour in our experiments. Other studied plants (*Ficus carica Grossh., Actinidia chinensis* Planch.and *Philadelphus caucasicus* Koehne.) showed similar bleaching of the pigment, but there were no visible changes in their glandular secretory cells (mainly trichomes). The oxygen, ozone and ultra-violet light that produces the O_3, causes the bleaching of *Eucalyptus* leaf tissue (15).

(ii). ***Autofluorescence*****:** Autofluorescence of secondary metabolites (allelochemicals) is the biosensor and bioindicator reaction of plants (23-25). The drastic changes in the autofluorescence is cellular signal of plants for survival under unfavourable conditions (33). Based on the autofluorescence characteristics data of some allelochemicals (24-26,36), we determined their possible accumulation, transformation or disappearance in our test woody plants: *Eucalyptus cinerea* F.Muell and C*itrus unshiu* (Marc.) treated with ozone gas (Figure 3, Table 2). The maxima of fluorescence spectra in leaves were analyzed by spectrofluorimetry (Table 2). This helped us to see the changes induced by ozone. We found under the luminescence microscope, that (i). the leaves of *E. cinerea* fluoresced at the excitation light 360 - 380 nm, (ii). the glandular structures did not emit and looked as dark spots surrounding the blue-emitted non-secretory cells and (iii). *C. unshiu* leaves had no fluorescence in control (without ozone treatment). In the fluorescence spectra, peaks were better seen in dry leaf samples than in fresh leaves. In *Citrus*, after chronic (3 days exposure with intervals) or acute (10 min) exposure to O_3, the autofluorescence of high intensity with many peaks was visible in the emission spectra: 454, 479, and 690 nm or 470 and 610 nm, respectively. The phenols and chlorophyll maxima are 470-479 and 690 nm, respectively (25)**.** The peaks of NADH/NADPH also are known in the region of 450-460 nm. This data showed that various groups of compounds present in the glandular secretory cells of leaves were transformed to new metabolites. The changes occurs in the flavonoid contents in of *Citrus* fruits exposed to ozone (37). Unlike *C. unshiu*, the *Eucalyptus* leaves fluoresced in control with maxima in blue region (400 and 413 nm, peculiar to terpenoids, sesquiterpenes), at 450-470 nm (NADH/NADPH and phenols) and 680-685 nm (chlorophyll). Most allelopathic plants containing the secondary metabolites/allelochemicals (8), may autofluorescence, depending on their development stage (23-25).

Table 2. Influence of ozone or ultra-violet irradiation on fluorescence of dried leaves of test plants. Excitation360/380 nm

Plant species	Fluorescence maxima (nm)				
	Control (without treatment)	Ozone (dose 0.05 µl/L during 3 days)	Ozone (dose 0.5 µl/L during 10 min)	UV-irradiation (Bactericide spectral energy, watt/m^2) 30 min. Analysed after 24 h	Water extract (1: 10 w/v) after acute treatment with ozone
Citrus unshiu (Marc.)	No emission	454, 479, small 690/454, 479, small 690 (high common emission)	470, small 610/470 ((high common emission)	460/460	450/460
Eucaliptus cinerea (F.Muell.)	400, 413, 450, 470, 680/450, 470, 685	425, 450,470, 675/ 454, 470, 680	430, 450, 470, 485, 490, 680/450, 470, small 680 nm	454, 470/454,470	440,450,470/450, 470

The chronic or acute exposure to ozone, shifted the maxima to 400 and 413 nm (Table 2), characteristic to terpenes and many oils, into long wavelength-region (at O_3 exposure 425 and 430 nm), perhaps, it occurred due to lipid peroxidation. Maxima in the range 456-479 nm related to phenols, were seen at applied ozone doses. In our experiments, the decrease of chlorophyll fluorescence (675-685 nm) was first observed in *Citrus* and thereafter in *Eucalyptus.* Under the long-time O_3 -exposure (3), bleaching in chlorophyll of *Citrus* leaves has been described earlier.

In nature, ozone is also produced under the ultraviolet irradiation. Thirty min exposure to ultraviolet light induced the visible changes after 24 h in the test objects

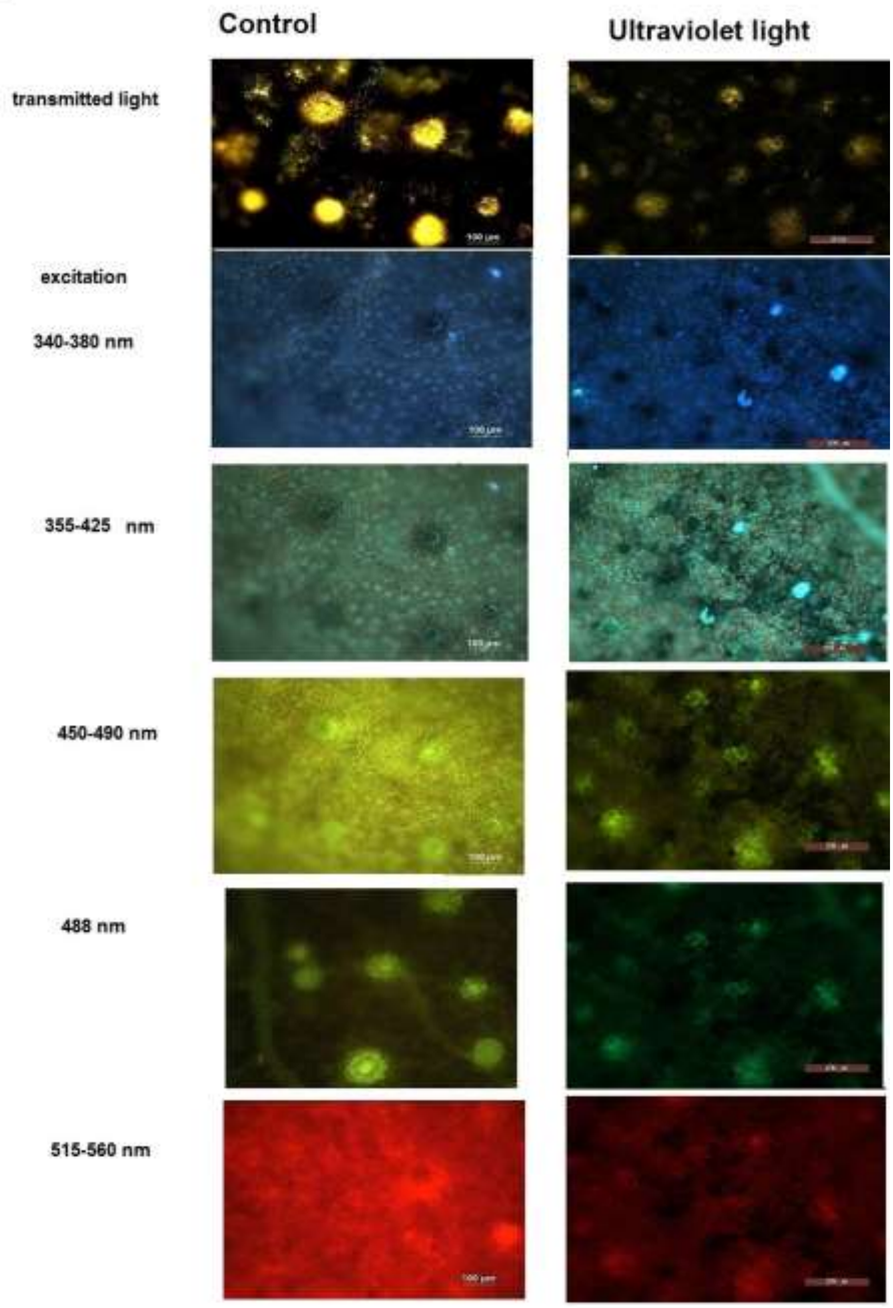

Figure 3. Effects of O_3 (total dose - 0.1µl/L producing by ultraviolet light) on the fluorescence of leaves of *Eucalyptus cinerea* excited by light of various spectral range.

(Table 2). The new maxima increased to 460 nm in the leaf emission spectrum of C. *unshiu* that showed the changes in their components (allelochemicals). On the contrary, maxima 450-454 and 470 nm in the spectrum of *E. cinerea* were not variable like in control (Table 2). The same spectral characteristics were observed for water extracts (possible leachates) from leaves after the acute ozone treatment. Significant changes in the autofluorescence (visible under luminescence microscope), occurred on the leaf surfaces of both test species. It was seen in the fluorescent images for *E. cinerea* (Fig. 3) under different excitations of fluorescence (from ultraviolet to red light) before and after the ozone exposure. First, unlike the colour images in transmitted light, glands had no fluorescence at short-wavelength blue excitation (seen as dark spots), only surrounding cells were emitted in blue. In control, the visible pale green emission of glands appeared at 355-425 nm excitation, usually the phenols and some alkaloids may fluoresce in this case (25). Besides, the autofluorescence increases under stress (33). There were marked differences between the control and exposure to ozone (formed at Ultra-violet irradiation) at excitation 450-490 nm. Yellow-greenish fluorescent glands on yellow background were seen in control, and after the ozone treatment, green fluorescence was seen in glandular cells and quenching the emission of surrounding non-secretory cells. If the emission was only 488 nm, secretory cells in control were green, while the surrounded cells had no emission, and after ozone exposure the visibility of glands decreased. Therefore, yellow fluorescence of non-secretory cells was excited by light < 488 nm. The excitation > 515 nm induced the red fluorescence, mainly due to chlorophyll, but only in control. In this case, secretory cells looked as black spots, surrounding the red-fluorescing cells. However, after the ultraviolet irradiation, only parts of non-secretory cells weakly fluoresced in red. After the ozone or ultraviolet irradiations, bleaching occurs in the leaves of *Eucalyptus* genus (15). Main visible responses to ozone are quenching of emission of glands and the surrounding non-glandular cells are excited by light 450 - 490 nm.

The exposure to ozone influenced the allelochemicals content (mainly terpenes, phenols and alkaloids) in leachates as shown by the changes in secretory cells. Many woody invasive plants are markers of pollution effects (7) or parasitic (11), contains allelochemicals in leachates that influences the plants growth (6,16,17).

2. Allelopathic activity of water extracts from leaves of woody plants undergone ozone treatment

Rainfall usually forms the leachates from the water-soluble compounds on surface of shoots, leaves and flowers. Water extracts from leaves and shoots of woody plants may serve as models of such leachates in following treatments: (i). Leaf water extracts/ leachates in control (without ozone treatment) and (ii) Leaf water extracts/leachates after treatment with ozone. The effects of these extracts were determined on the germination of seeds (21) or pollens (27), to know, how the ozone changed the activity of allelochemicals contained in the secretory structures of leaves and leached by rain.

***(i) Seed germination of acceptor plant* (*Lavatera trimestris*):** We used the seeds of ornamental plant *Lavatera trimestris* L. (grown under or near trees and shrubs in parks) as it is the acceptor of leachates from the test woody donors plants. Both the water extracts/leachates of donor plants (*Albicia, Citrus, Heimia, Philadelphus* and *Taxus*) were inhibitory to seeds germination (Table 3). While water extracts/leachates of *Eucalyptus, Ficus* and *Gleditsia* were stimulatory. The decrease in germination was 25-35%, but the stimulation was about 50 %, especially for *E. cinerea* was 4-times higher than control (water) (Table 3). The main inhibitory components in examined leachates were various phenolic compounds or alkaloid ephedrine in case of *T. baccata* (8). The oil components such as azulenes in *E. cinerea,* perhaps, caused stimulation (13).

Table 3. Effects of water extracts from test woody allelopathic donor plant species treated with ozone (acute dose 0.5-0.7 μl/L for 10 min) on seeds germination of test recipient plant *Lavatera trimestris*.L. Pure water: 100 % control

Plant species	Extracts from	
	Untreated leaves	Ozone treated leaves
	Lavatera trimestris seed germination (% of control) germination	
Albicia julibrussin Durazz.	75±5	100±4
Citrus unshiu (Marc.)	66±6	0±1
Eucalyptus cinerea F.Muell.	400± 10	120±9
Ficus colhica (carica) Grossh.	130±5	50±4
Gleditsia triacanthos L.	153±11	82±4
Heimia salicifolia Link.	81±3	100±6
Philadelphus caucasicus Koehne.	75±7	53±4
Taxus baccata L.	67±5	151±2

However, after the ozone treatment in chronic and acute experiments, the above effects were, changed by oxidation. Weak inhibitory effects of water extracts from *A. julibrissin* and *H. salicifolia* seen in control, disappeared. Moreover, water extracts of *T. baccata* after the ozone exposure showed the stimulation, instead of inhibition in control. As for *Citrus unshiu*, water extracts from the ozonated leaves completely blocked the seed germination. While the leachates from leaves of *Philadelphus*, decreased the magnitude of inhibition than in non-ozonated sample. On the contrary, the water extracts after the leaves ozonation of *E. cinerea* decreased their stimulation and there was no stimulation in samples of *Ficus* and *Gleditsia*. We can propose that the fast oxidation by ozone transformed the allelochemicals, especially phenols (28,30), that needs to be considered for the urban biocenosis, where tropospheric ozone is formed in high concentrations. It is significant not only for human health, but also for allelopathic relations of plants in artificial culture (like in subtropical resorts with many introduced species collected in Sochi National Park). Here only *Taxus* and *Philadelphus* are endemic species, while others were introduced in Caucasus more than 100 years ago (Table 3).

Table 4. Effects of water extracts from leaves of woody allelopathic donor plants treated with acute dose of ozone (0.5- 0.7 μl/L for 10 min) on pollen germination of *Hippeastrum* (Herb.) *hybridum*

Plant species	Germination Index (Ratio: Germinated : 100 total analyzed pollens)	
	Untreated pollens	Ozone treated pollens
	Hippeastrum hybridum pollens germination	
Control (without extracts)	0.56± 0.02	0.0
Actinidia chinensis Planch.	0.28± 0.05	0.48± 0.04
Eucalyptus cinerea F.Muell.	0.52± 0.01	0.70± 0.05
Ficus carica Grossh,.	0.57± 0.04	0.58± 0.09
Heimia salicifolia Link.	0.60± 0.06	0.60± 0.07
Taxus baccata L.	0± 0.01	0.56± 0.03

(ii). *Pollen germination of acceptor plant* (*H. hippeastrum*): Reaction of pollen germination of *H. hippeastrum* served as test for many biologically active compounds, including ozone and allelochemicals (28,30). In our experiments with water extracts *in vitro* in control and after ozonation of woody plant leaves (Table 4) had variable effects on the pollens germination. Water extracts from untreated leaves of *Actinidia chinensis* and *Taxus baccata* were inhibitory. Ozone treatment of woody species leaves before extraction, stimulated the pollens germination, especially in water extracts of *Actinidia* and *Eucalyptus*. Water extracts from *Taxus* completely inhibited the pollen germination in control and stimulated after O_3 exposure. Water extracts from *Ficus* and *Heimia* had effects similar to control.

CONCLUSIONS

Role of tropospheric ozone in allelopathic relations has not been studied yet. We tried first time to study the problem by modelling the interactions in system consisting of woody species (donor of allelochemicals released in rain leachates) and ornamental herbal species grown under the canopy as Acceptor-plants of rain leachates. Exposure to O_3 changed the microscopic images and spectral characteristics of leaf surface with the secretory cells of donor allelopathic woody trees/shrubs that changed the allelopathic activity of their leaf water extracts (models of rain leachates). The ozone treatment of test woody plants changed their leaves (i) characteristics and their water extracts, (ii) colour and (iii) autofluorescence of secretory cells. The effects of water extracts from leaves exposed to O_3 before and after were tested on the seeds and pollens germination of acceptor plants (*Hippeastrum hybridum* and *Lavatera trimestris*, respectively). Thus, the ozone influenced the leachates from woody allelopathic donors plants that affected the germination of seeds and pollens of acceptors plants. In Future, these results shown influence the tropospheric ozone in artificial Parks biocenosis like in Sochi National Park, may be used for green planning of urban resort conditions and Agro-industry of subtropical regions.

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