

How Tropospheric Ozone Influences the Allelopathy of Woody Species: Some Experimental Approaches

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Abstract: Plants undergo a high concentration of tropospheric ozone formed mainly as a result of industrial and automobile pollution that may act on allelopathic relations in biocenosis. The problem was considered in first experiments modeling in test reactions with leaf leachates from 10 woody species as plant-donors, exposed to ozone, and able to influence on herbs (plant-acceptors) grown under their canopy. The effects were dependent on the duration and the intensity exposure to ozone. The color and the autofluorescence of the woody plant leaves with secretory cells contained allelochemicals changed under the ozone treatment. The effects of water extracts (models of rain leachates) from leaves of woody species exposed to ozone on the seed germination of herb *Lavatera trimestris* (Malvaceae), plant-acceptor of allelochemicals, differed from untreated samples. This showed a possible transformation of allelochemicals or/and the formation of new similar exometabolites after the ozone treatment. In the fluorescence spectra of whole leaves, the maxima, peculiar to phenols, were found at different experiments, while peaks related to terpenes disappeared in ozonated samples. Under acute or chronic ozone exposure the formation of biogenic amines (dopamine and histamine) known as allelochemicals was observed in leaf cells. These test-reactions on tropospheric ozone stress could be used in the analysis of allelopathic relations in urban conditions.

Keywords: Allelopathy, Allelochemicals, Autofluorescence, Germination, *Lavatera trimestris*, Tropospheric Ozone, Woody Species

1. Introduction

The realization of allelopathic plant characteristics in Nature depends on many environmental factors. Among them appears to be tropospheric ozone both of natural and urban origin [1, 2]. However, this problem was not considered yet by scientists working in the field of Allelopathy. Ozone or O₃ is an allotropic modification of oxygen and is constantly present in the air [3, 4]. This gas is forming in the atmosphere under the influence of ultraviolet radiation, and its concentration increases, especially in the morning hours, when the flow of UV radiation is maximal. Ozone also occurs in thunderstorms, which is most noticeable in the forest. Technological progress has contributed to the formation of ozone, as many modern instruments are emitters of ultraviolet rays, and therefore

sources of ozone. Moreover, industrial and automobile pollution of the atmosphere leads to an increase of the oxidant concentration. Tropical and subtropical species may change their allelopathic characteristics more clearly than plants grown in a moderate climate [5]. Up to now, the soil nutrition and fire were known to influence on the allelopathic features of the genus [5], but no data connected with air-born conditions.

The herbal plants grown under or near trees/shrubs undergo the influence of leachates from trees that act on their development, especially on germination [5, 6]. Besides active components of leachates, external air factors as tropospheric ozone may act on the allelopathic interactions. The first example was considered earlier with an allelochemical coumarin esculetin that transformed under the ozone exposure and changed its allelopathic effect on the

germination of pollen *Hippeastrum hybridum* [7].

In a model system, tree/shrub-herb, changes that occurred in allelopathic plant characteristics could be highlighted by the use of test-reactions in laboratory conditions. The purpose of this paper is the analysis of changes that ozone induces in overground intact parts of plants and the leachate's content from trees and shrubs as well as leachate's effects on the herbs grown under or near the woody species.

2. Materials and Methods

2.1. Plant Material

Objects of the studies were tree plants grown in Sochi National Park (Dendrium) [8] as donors of allelopathically active compounds and decorative herb plant *Lavatera trimestris* var. Rubin as acceptor of these allelochemicals. Woody plants tested include *Eucalyptus cinerea* F. Muell. ex Benth. (fam. Myrtaceae), *Brachychiton diversifolius* R. Br. (fam. Malvaceae), *Ginkgo biloba* L. (fam. Ginkgoaceae), *Buddleja davidii* Franch. (fam. Scrophulariaceae), *Cassia floribunda* Cav. (fam. Caesalpiniaceae), *Casuarina equisetifolia* J. R. et G. Forst (fam. Casuarinaceae), *Citrus unshiu* (Yu. Tanaka ex Swingle) Marow. (syn. *Citrus reticulata* Blanco) (fam. Rutaceae), *Lantana camara* L. (fam. Verbenaceae) and *Nerium oleander* L. (fam. Apocinaceae),

2.2. Water Extracts Modeling Leachates

Water extracts from the intact whole leaves of woody plants were prepared according to ratio 1: 10 w/v during 1 h, where mainly surface components have leached, as it occurs under strong rain. The extracts filtered from leaf plates through glass filter 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 were leached. This time was enough to wash the water-soluble allelochemicals from leaves surface [5].

2.3. Ozone Treatment

Exposures of air-dried leaves from tested plant species to ozone were in the following procedures: 1) under ultraviolet irradiation (ozone 1), 2. acute – short time variant at high gas concentration (ozone 2) or 3. chronic - long-time variant at smaller gas concentration (ozone 3).

In the variant ozone 1, ultraviolet irradiation as the origin of ozone was done by apparatus OUVb-04 "Solnyshko" EAF (Russia). The amount of ozone produced was 0.1 µl/L per 30 min.

In the variant ozone 2, this gas was generated by electric charge Orion-Si ozonator (Russia) during 10 min in total dose of 0.7 µl/L. Chronic exposure in variant ozone 3 occurred with electric charge by ozone generator KPMZ (Russia) in plastic cell volume 439 cm³. The duration of chronic treatment was 5 h per day (5 h ozone + 12 h break without the gas) for 3 days (total dose of 0.05 µl/L).

2.4. Test for Seed Germination

The seeds of the test-species, *Lavatera trimestris*, decorative species, served as a model of plant grown under or near trees and shrubs in the park zone. *Lavatera* was used as an acceptor of leachates of the woody plant-donors of allelochemicals. Seeds germinated according to the standard method [5] in Petri dishes at 20-22°C. Ten seeds of acceptor plant *Lavatera trimensis* were put in each Petri dish (10 cm diameter) with filter paper irrigated with 5 ml water extract from leaves of donor woody plants or pure water (control without any additions). In order to know the effect of ozone on leaves, water extract from non-ozonated leaves, in its turn, served as second control in the comparison with the gas effects. The experiments with every variant repeated 4-times. Seed germinated were counted 24 - 36 h after water extracts application (observation and counting continued for 3 days).

2.5. Test for Microscopic Images in Transmitted Light of Microscope

The visual changes in the leaves' samples induced by ozone were observed on subject glasses (slides) under luminescence microscope Leica DM 6000 B in transmitted light, using various objectives 10, 20 and, 40 magnitude.

2.6. Test of Autofluorescence

Autofluorescence of leaf surface with secretory structures served as an indicator of the effect at ozone exposure. It was observed at the excitation by light 360- 380 nm and photographed using luminescence microscope Leica DM 6000 B as described earlier [9-12]. The fluorescence spectra of whole leaves also were recorded with the spectrofluorimeter Perkin-Elmer 350. Registration of such spectra carried out with three repetitions.

2.7. Test for Fluorescent Determination of the Appearance of Biogenic Amines

At fluorescent histochemical determination of biogenic amines, glyoxylic acid (reagent for catecholamines, in particular, dopamine) and o-phthalic aldehyde (reagent for histamine) given from Sigma, were used according to the method described for animal cells [13, 14]. Leaves were put on subject glasses (slides) and moistened by drops of 1% aqueous solutions of glyoxylic acid to determine dopamine [13] or 0.5-1% solutions of o-phthalic aldehyde to determine histamine [14]. After 10-20 minutes of staining with the reagents, samples were dried at 50-80°C during 5-10 min. Fluorescence reaction of forming products in whole leaves was studied under luminescence microscope Leica DM 6000 B at the excitation by light 360-380 nm. Fluorescence of the samples also measured at 475-485 nm using the spectrofluorimeter Perkin- Elmer 350 (excitation light from 360-380 nm or 430 nm). Histochemical reactions repeated (up to three times) for control variants and variants with exposure to ozone.

3. Results and Discussion

We analyzed how water extracts imitated rainfall leachates from woody plant leaves untreated or treated to ozone act to allelopathic activity of herb- acceptor (measured as seed germination). The induced by O₃ visual changes in the leaves with well-seen surface secretory structures (which mainly contained allelochemicals leached by rain) were estimated under transmitted light or at excitation by actinic light of luminescence microscope.

3.1. Seed Germination of Plant–Acceptor

With or without the ozone treatment, water extracts demonstrated stimulatory or inhibitory activity on the seed germination and in some cases, there was no influence. Extracts of leaves from *Eucalyptus cinerea* and *Brachychiton diversifolius* contain stimulatory compounds that increased germination of the test-species, *Lavatera* by 4-5 times in a comparison with only water in medium (Figure 1). For bottle tree *Brachychiton*, main known biological activity belongs to caffeine [15], this purine compound also is a stimulator of human nervous system. Genus *Eucalyptus* has many allelochemicals - flavonoids such as quercetin-3 glycoside and myricetin-3-rhamnoside and volatile aromatic terpenes such as cineol, carvone or α - and β -pinenes [15]. However, chemical nature of stimulators in rain leachates was not

known yet. Ozone significantly decreased stimulatory effects of the leachates either in acute or chronic experiments, for both *Eucalyptus* and *Brachychiton*.

Lesser stimulation of the seed germination (up to 50% of the variant with water only) was in control for *Ginkgo* (Figure 1). Among biologically active components of the allelopathic species, besides flavonoids (apigenin) and coumaric acid, specific bilobalide and ginkgolide A were reported [15]. Unlike *Brachychiton* and *Eucalyptus*, in experiments with *Ginkgo biloba*, there was no difference between the ozone and control variants.

There was no effect for control for *Buddleja davidii* and *Nerium oleander*, but chronic exposure to ozone led to the stimulation of the seed germination by more than 200% for the first species and by 20-30% for the second one (Figure 1). Interestingly, that as biologically active compounds for butterfly bush *Buddleja* genus flavonoids quercetin, kaempferol and linarin are known, while for *N. oleander* - steroids (cardenolides) gitoxygenin, karabin, cornerin and neriodorin were reported [15]. Perhaps, chronic exposure in ozone made transformations in these compounds. Water extracts from leaves of *Casuarina* and *Lantana* had no marked allelopathic activity at all, neither without nor with ozone (Figure 1). In variant with *Citrus unshiu*, under ozone the 100% inhibition was seen (non-illustrated) in a comparison with weak allelopathic activity in the control.

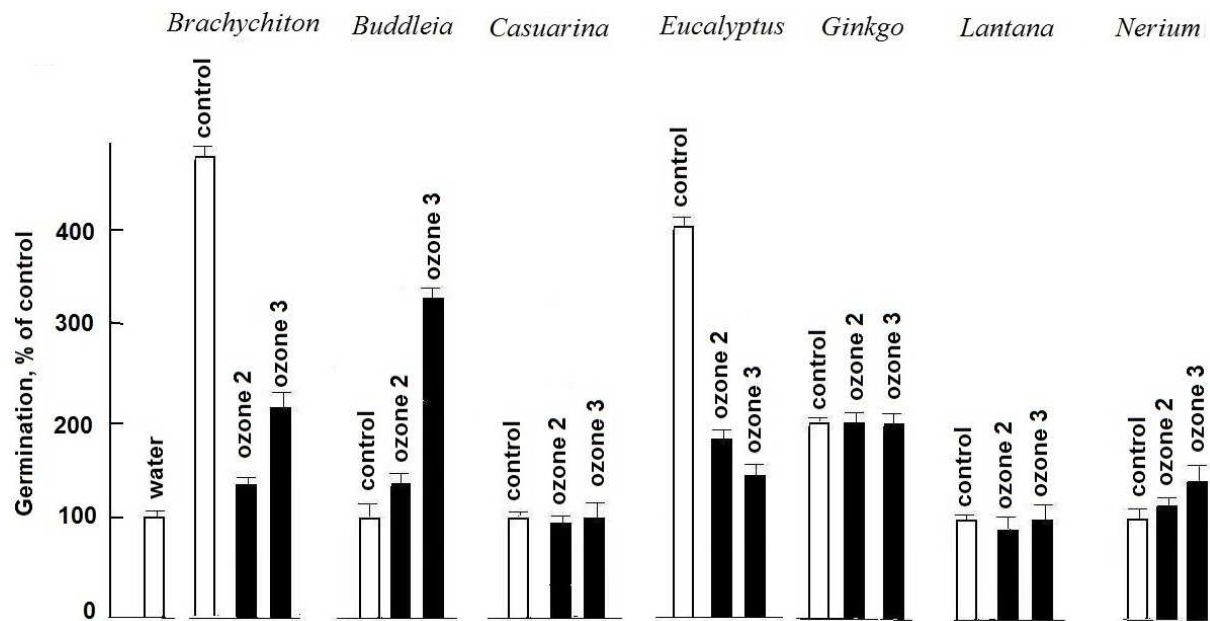


Figure 1. Allelopathic activity of water extracts from leaves of woody plants undergone to ozone estimated on seed germination of *Lavatera trimestris*, under pure water. Control (with water extracts from untreated leaves), water extracts from leaves treated with ozone 2 (acute ozonation), or ozone 3 (chronic ozonation). Results were expressed as mean \pm the standard error of the mean ($n = 40-50$ seeds per variant).

Among biologically active compounds possible to regulate the seed germination are flavonoids quercetin, kaempferol and linarin for *Brachychiton* spp. and for *Buddleja officinalis*; the monoterpene and sesquiterpene derivatives in essential oil for *Eucalyptus cinerea*, anacardic acid, bilobalide, riboflavin, triterpenoids- ginsengosides for *Ginkgo biloba*; adinerin, cardenolides, karabin for *Nerium*

oleander [15-17]. Essential oils of *Eucalyptus* genus, expressed a peculiar inhibitory action on the growth processes [17, 18].

Leachates from allelopathically active trees and shrubs are known for some species, for example for genus *Eucalyptus*. The leaching induced inhibition of agricultural plants like *Phaseolus aureus* and *Lens esculentum*. Most evident

allelopathic potential belonged to volatiles such as cineol and phenolic compounds such as syringic acid [17-20]. According to other findings, excretions from various species of the *Eucalyptus* genus may act on agricultural plants both as inhibitors and stimulators depending on the taxonomic position [18, 19, 21]. It is especially significant for Australian and Indian Species valuable for economic policy of these tropic countries [22]. The depressive influence of *Eucalyptus* leaf extracts on grass seeds was also reported [23].

3.2. Leaf Secretory Structures Seen Under Microscope

Plant-donors of allelochemicals may contain these compounds in secretory structures [7, 10, 11, 16]. In

transmitted light of usual microscope, the action of ozone is possible to see in leaf oil glands of *Eucalyptus cinerea* before and after exposure. Treatment by O₃ induced fast changes in colour of the structure (Figure 2). Orange, yellow and even red coloured glands in one moment became yellowish-green and dark green. The middle part of some visible glands seems pale yellow after the exposure (Figure 2). Phenolic pigments (flavones, anthocyanins) that contributed in the colour of the secretory cells fulfilled oil appears to bleach under oxidation. Out of secretory structures, the chlorophyll amount appears to decrease because in the control it looks dark green in a comparison with secretory structures treated with ozone (Figure 2).

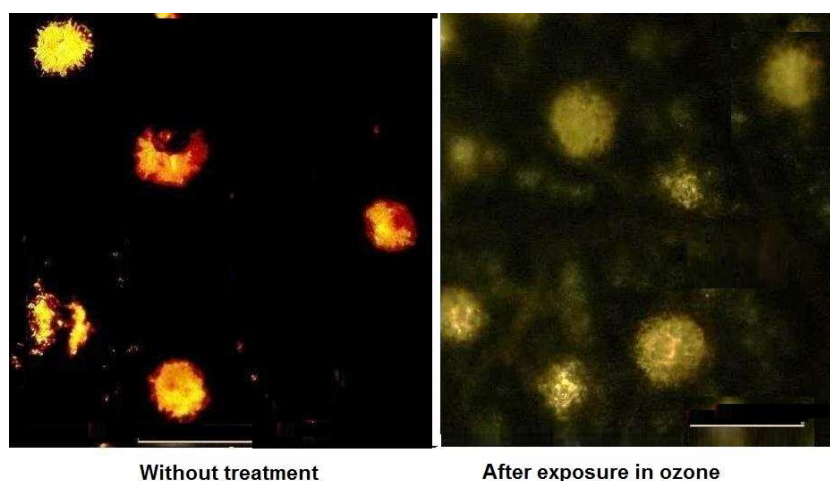


Figure 2. The colour changes in the secretory structures of the leaf of *Eucalyptus cinerea* after acute exposure to ozone (0.5-0.7 $\mu\text{L/L}$) during 10 min, observed under transmitted light of microscope. Bar = 200 μm .

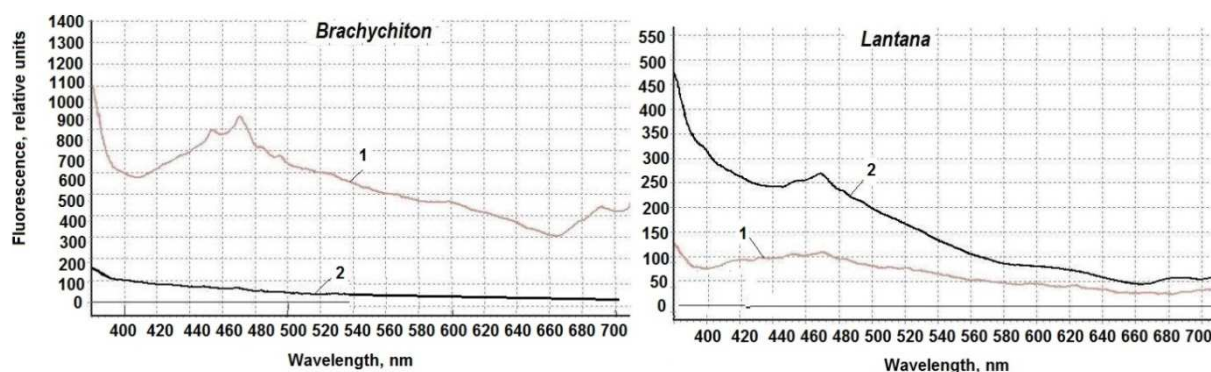
According to some authors [24], oxygen, ozone and Ultra-violet that form ozone, induced bleaching of the tissues of *Eucalyptus*. The decrease in chlorophyll a fluorescence of *Citrus* also has reported by Calatayud with co-workers [25].

3.3. Autofluorescence of Leaves

Most intact secretory cells showed multicomponent fluorescence spectra, and it is necessary to know the chemical composition of the prevailing components by comparing with the emission of individual known components [10-12, 26]. The leaf autofluorescence has changed after ozone exposure (Figure 3).

If autofluorescence in control, without ozone, was weak

(in *Lantana* and *Citrus*), after the exposure in O₃ common emission increased significantly, and clear maxima arose in blue (450 and 470 nm) spectral region and sometimes in red (675 or 685 nm). On the contrary, autofluorescence of *Brachychiton*, in the control marked maxima 450 and 470 nm in blue; after ozonation it demonstrated the lack of any peaks in the emission spectrum and drop in common fluorescence intensity. Although leaves of *Nerium* showed small maxima in the control emission spectrum, clear peaks, and the increase in common fluorescence intensity were observed at chronic exposure to ozone. In the fluorescence spectra of *Citrus*, after both acute and chronic exposure to ozone, there were maxima in blue.



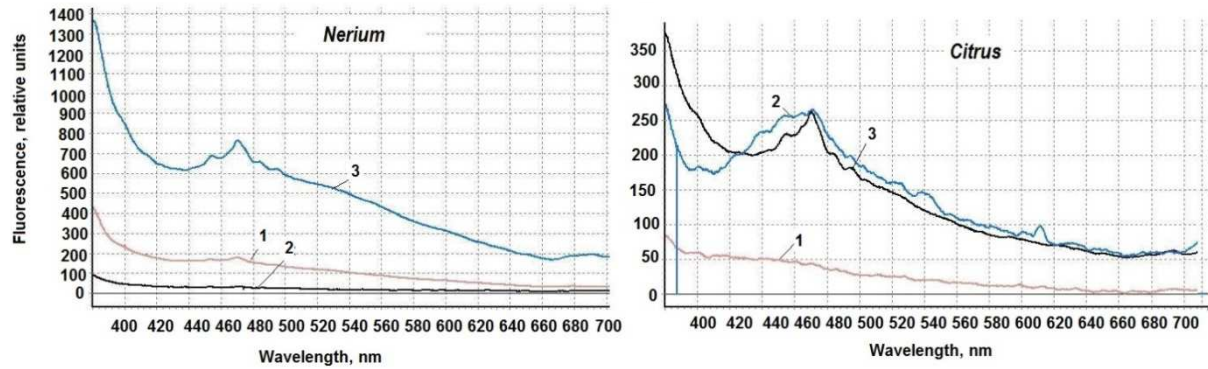


Figure 3. The fluorescence spectra of leaves from *Brachychiton davidii*, *Lantana camara*, *Nerium oleander* and *Citrus unshiu*. (1) – control, without treatment; (2) – ozone 0.5-0.7 µl/L; (3)– ozone 0.05 µl/L.

Usually, the compounds that lie on the surface and in secretory cells may be extracted by hydrophilic or /and hydrophobic compounds that changes the fluorescence characteristics of the surface [26]. One of example is on Figure 4 for leaf image of *Eucalyptus* before or after extraction with water or ethanol. When the sample was not treated, the leaf surface of *Eucalyptus cinerea* looks brightly in pale green; only cells of sheath around dark glands

fluoresce are in darker green (Figure 4, a). Water extraction of possible phenolic compounds made the surface more green emitted (Figure 4, b). Only after extraction with ethanol, red fluorescence, peculiar to chlorophyll, lying deeply in parenchyma was observed (Figure 4, c). It was due to the ethanol extraction of terpenoid fluoresced in blue, and the red emission of chlorophyll was seen clearly (Figure 4, c).

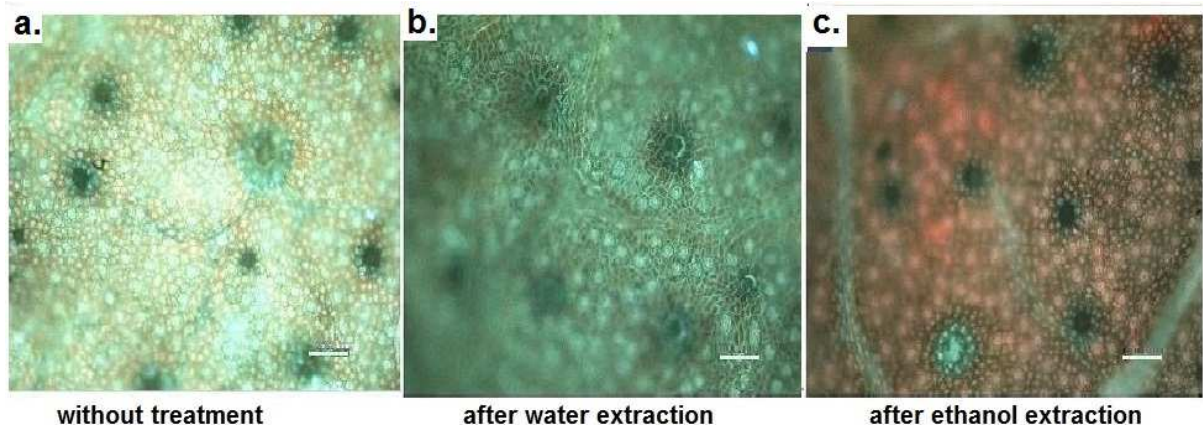


Figure 4. Fluorescent images of the leaf surface of *Eucalyptus cinerea* after extraction by water (b) or ethanol (c), compared to control (a). Excitation 430 nm. Bar = 100 µm.

Autofluorescence of plant leaves' surface studied linked with the terpenoids and flavonoids maxima seen at 400 -440 nm and 470-480 nm for an excitation at 360 nm (Figure 3, Table 1). Some weak maxima 670-685 nm, peculiar to chlorophyll, were also observed; but were well seen at the excitation of 430 nm after extraction from intact whole leaf by water or ethanol in ratio 1:10 w/v per 1 h, when some flavonoids or terpenes from the surface released, relatively. Blue colour autofluorescence (420-430 nm) of the leaf surface was, perhaps, as related to the presence of blue pigments azulenes found in *Eucalyptus cinerea* [27]. Summed data in Table 1 showed different autofluorescence characteristics of the leaf surface. Among species studied, leaf autofluorescence of *Brachychiton diversifolius* and *Buddleja davidii* were absent at chronic and acute exposures to ozone formed by electric

charge, except ultraviolet irradiation, where maxima 456 and 470 nm were seen (Table 1). For *Nerium*, similar effect was seen only at acute exposure, while in chronic variant of experiment additional new maxima 450 and 680 nm (in control was only one 470 nm in blue region) were observed. In *Citrus unshiu*, which has no fluorescence in control, under O₃-exposure the emission arose (maxima 454, 479, 690 nm at chronic exposure or 470 and 610 nm at acute exposure) and strongly increased. *Eucalyptus cinerea* was especially sensitive to ozone: new peaks 425 and 430 nm appeared in the chronic and acute exposures with ozone. In variants with *Eucalyptus*, maximum 470 nm, peculiar to phenols, was both in control and after all treatments. However, under ozone related to terpenoids peaks in region 400 and 413 nm disappeared (Table 1).

Table 1. Influence of ozone or ultra-violet irradiation on the fluorescence maxima of the dried leaves with allelochemicals. Excitation 360 nm.

Plant species	Fluorescence maxima, nm			
	Control (without treatment)	Ozone (dose 0.05 $\mu\text{L/L}$ during 3 days)	Ozone (dose 0.5-0.7 $\mu\text{L/L}$ during 10 min)	UV-irradiation ($\text{O}_3 > 0.1 \mu\text{L/L}$) 30 min. Analysis after 24 h
<i>Brachychiton diversifolius</i>	452, 470, 600, 685	0	0	456, 470
<i>Buddleja davidii</i>	470, 680	0	0	450
<i>Citrus unshiu</i>	No emission	454, 479, 690 (high common emission)	470, 610 (high common emission)	460
<i>Eucalyptus cinerea</i>	400, 413, 450, 470, 680	425, 450, 470, 675	430, 450, 470, 485, 490, small 680 peak	454, 470
<i>Nerium oleander</i>	470	450, 680	0	470

For *Nerium*, action of ozone led to arise new maxima 450 and 680 nm at chronic exposure and the absence of any peaks after acute treatment. Generally, the maximum 680-685 nm, peculiar to chlorophyll, decreased or disappeared in studied plants under ozone exposure like for *Citrus* leaves [25] and flower petals of *Saintpaulia ionantha* [28].

Treatment with ultra-violet irradiation, which also produced ozone, also resulted in marked changes in autofluorescence (Table 1). Like chronic and acute experiments with ozone formed by electric charge, here are maxima in blue at 400-413 nm, peculiar to terpenoids, also disappeared in leaves of *Eucalyptus*. After the UV- treatment, maximum 470 nm, seen in control and related to phenols, registered for three species (*Brachychiton*, *Eucalyptus* and *Nerium*) out of five. New maxima at region 450-456 nm arose (*Brachychiton* and *Buddleja*) or were the same as in the control (*Eucalyptus*). In *Citrus*, only peak 460 nm was after UV-irradiation. Autofluorescence of plant leaves' surface studied linked with the terpenoids and flavonoids maxima seen at 400-440 nm and 470-480 nm for an excitation at 360 nm (Figure 3, Table 1). Equal maxima seen under both ozone and UV-irradiation are at 450-456 nm (*Brachychiton*, *Buddleja* and *Eucalyptus*) or/and at 470 nm (*Brachychiton*, *Nerium* and *Eucalyptus*). At all ozone exposures, we can see changes may be related to Only long-term exposure of *Citrus unshiu* reported to induce a decrease in photosynthesis and chlorophyll fluorescence [25] transformations of

allelochemicals or even new compounds may arise.

The contribution of individual allelochemicals in the fluorescence that may be observed after the dried sample exposure to ozone and then dissolution in water or in ethanol (2 mg/ml) was confirmed by earlier works [3, 26, 28]. Some known allelopathic compounds – phenols, terpenoids and alkaloids - are given in Table 2. Among chosen compounds, flavonoid anthocyanin pelargonidin that has been dissolved in water or in ethanol after the exposure in ozone was most sensitive to ozone because characteristic maximum 665 nm disappeared first after 25 h exposure in ozone at small dose 0.05 $\mu\text{L/L}$ [28]. For terpenoids absinthin and azulene solved in ethanol, the changes occurred at higher dose of O_3 , the maximum 540 nm of the first compound shifted in blue spectral region, while maximum 400 nm of second substance disappeared (Table 2).

It is similar with our data of Table 1, where in the leaf spectra maxima at range 400-430 nm were absent after the ozone treatment, for example in variant *Eucalyptus* that contains azulenes [27]. Chlorophyll was not too sensitive to ozone in a comparison with flavonoid anthocyanin, with a decrease to a maximum of 675-680 nm after only to 100 h in chronic exposure (dose 0.2 $\mu\text{L/L}$) to ozone action (Table 2). Only long-term exposure of *Citrus unshiu* reported to induce a decrease in photosynthesis and chlorophyll fluorescence [25].

Tolerant compound to all used doses of ozone was alkaloid berberine. Like berberine, chlorogenic, ferulic and caffeic

Table 2. The effects of ozone on the fluorescence maxima of individual compounds solved in 96% ethanol (2 mg/ml). Excitation 360 nm.

Individual compound	Maximum, nm/ Fluorescence intensity changes			
	Control	+ dose of ozone ($\mu\text{L/L}$)		
		0.05 (25 h)	0.1 (50 h)	0.2 (100 h)
Anthocyanin pelargonidin (pH 5.5.)	450-460, 665	450-460	410-420/decrease	0
Absinthin	540	540	470, 450 nm	470
Azulene	380-430	400	400 (no maxima)	400 (no maxima)
Berberine	540	540	540	540
Chlorophyll	675-680	675-680*	675-680/increase	675-680/decrease
Caffeic acid	450-460	450-460*	450-460*	450-460*
Ferulic acid	450-460	450-460*	450-460*	450-460*
Cinnamic acid	450-460	450-460*	450-460*	450-460*
Esculetin (Aesculetin)	500-520	450*	450/decrease	450/decrease
Coumaric acid	450	450*	450*	450*

*No changes in the emission intensity. Sources: 26, 28 and unpublished data for azulene and berberine.

acids as well as coumaric acid that after ozonation in all doses, solved in water or ethanol fluoresced in blue with maxima at 450-460 nm [29]. They changed neither maxima position, no the emission intensity during acute or chronic

exposures [28]. Unlike coumaric acid, the emission of esculetin, a coumarin derivative, at 450 nm under chronic O_3 (0.1- 0.2 $\mu\text{L/L}$ doses) decreased, and maximum in the spectra from 500-520 nm shifted to short-wavelength to 450 nm

(Table 2). It should know, that 7-alkoxy coumarins generally have a purple fluorescence, whereas 7-hydroxy and 5,7-dihydroxycoumarins tend to fluoresce in blue [29]. Ozone is capable to change the phenol composition in *Citrus* plant [30] that seen from fluorescence of whole leaves (Table 1).

3.4. Analysis of the Appearance of Dopamine and Histamine as Agents of Stress

It has established that not only animals, but also plant and microbial cells form dopamine and histamine under a variety of stresses [31-34]. Dopamine and histamine, known as neurotransmitters of mammals and found in plants and microorganisms, can accumulate in cells. They also showed allelopathic features [32]. The criterion for histochemical reactions of these biogenic amines is the appearance of blue fluorescence at range 460-480 nm excited by ultraviolet light 360 nm. After the histochemical staining with glyoxylic acid, the emission maximum was at 470-480 nm, while with o-phthalic aldehyde the emission peak was at 460 nm. After chronic or ozone exposure, histochemical reactions for biogenic amines on the leaf

surfaces of woody species were established (Table 3). In all control variants, there were no detectable fluorescent reactions for dopamine, but after the treatment with ozone, one can see the blue emission of the cells around dark glands in samples from *Eucalyptus* and *Citrus*. After similar exposure, one found blue emission related to dopamine exactly in secretory cells (laticifers) of *Nerium*. Unlike results with dopamine the reaction for histamine (also lack in control) appeared after the acute ozone exposure and was marked in glands and stomata in *Buddleja* and in some parts of glands in *Eucalyptus*. However, histamine was absent in *Nerium* both in control and after ozonation. It is known [14] that at high stress concentration of histamine (10^{-3} M or higher) in tissues, one can see yellow emission. In the experiments, we observed similar yellow fluorescence in cells around dark glands of *Citrus* that should mark high concentration of histamine in the sample. Both biogenic amines dopamine and histamine were found in *Citrus* and *Eucalyptus*, while in other species either first or second compound recorded (Table 3).

Table 3. The fluorescence of secretory structures after histochemical staining with glyoxylic acid and o-phthalic aldehyde for determination of dopamine and histamine, relatively, formed after chronic or acute ozone exposures. Excitation 360 nm.

Species	Staining with	
	glyoxylic acid	o-phthalic aldehyde
<i>Buddleja davidii</i>	No blue emission	Increased blue fluorescence in glands and stomata (histamine)
<i>Citrus unshiu</i>	Increased blue fluorescence in cells around dark glands (dopamine)	Yellow fluorescence in cells around dark glands (histamine in high concentrations)
<i>Eucalyptus cinerea</i>	Increased blue fluorescence around dark glands (dopamine)	Blue fluorescent parts of glands (histamine)
<i>Nerium oleander</i> L.	Blue fluorescence of secretory cells (dopamine)	No blue emission

These are qualitative data, which demonstrated the presence of the allelochemicals- biogenic amines-on the surface of leaves of woody plants after the exposure to ozone. Receiving of quantitative fluorescent data from secretory cells may be a separate task with the use of special apparatuses in Future.

By applied histochemical methods, we saw a location of the compounds in some secretory structures and out them. The presence of biogenic amines on the leaf surface with secretory structures shows the possibility to release the compounds via the rain leachates.

Dopamine and histamine are known to regulate the fertilization and stimulate the pollen germination of *Hippeastrum hybridum* as well as the seed germination of *Raphanus sativus* [32-34]. Under ozone treatment in Nature, the amount of dopamine and/or histamine increases in pollen of many allelopathically active species, such *Coryllus avellana* and *Populus balsamifera* [31, 32]

4. Conclusion

Model experiments with allelopathically active woody species have shown that the duration and intensity of ozone exposure influenced the ability of their allelochemicals from rain leaf leachates to act on herbs grown under or near the trees. The test-reactions on tropospheric ozone stress may use in the analysis of the allelopathic relations in urban

conditions. They include spectral characteristics of leaves (the color and autofluorescence of secretory cells, containing allelochemicals with surface secretory structures) changed under ozone treatment. As indicator of the changes also serves the formation of biogenic amines - dopamine and histamine in cells under ozone stress. Future studies as in model and natural conditions may be important for the formation of plantings in urban conditions, basing on characteristics of studied species, to recommend the valuable woody species for parks. Ornamental trees like *Brachychiton*, *Buddleja*, and *Eucalyptus* are widely cultivated in many countries of subtropics and tropics. They are exploited as medicinal and economical cultures. Their rain leachates revealed the presence of growth stimulators that is valuable for the germination of herbal species grown in the tree vicinity.

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