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MORPHOLOGICAL AND STRUCTURAL MODIFICATION INDUCED BY AIR POLLUTANTS IN *ACER CAMPESTRE* L. LEAVES

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Abstract: The study estimated modification in morphology and structure of *Acer campestre* L. leaf blades under two diverse levels of industrial air contamination. The samples have been collected from the same trees growing on the identical place but in two separated with long time periods: 1990, 1991 – first period and 2004 – second; that are characterized with great difference of pollutant levels, especially for SO_2 at least twice in amount through heavy polluted period. The structure and developing of winter buds and leaves lamina have been monitoring through severe contamination to reveal any adaptation responses. Increasing of palisade tissue and decreasing of spongy parenchyma have been detected, during high levels of industrial contamination, but not in the period with relatively low pollution levels, that correlated with increasing the palisade ratio. Augmented palisade ratio stated for an adaptive response of tolerant plants toward the stress factor.

Keywords: leaf blade, industrial pollution, polluted air, sulphur dioxide, *Acer campestre*.

Introduction

The pollution of atmosphere with toxic gases, from industries, transportation, vehicle traffic and other anthropogenic activities cause many negative effects on plant, animal and human populations (Joshi and Swami, 2009; Dhariwal, 2012). Atmospheric pollution contains high concentration of particulate matter (PM_{10}, $PM_{2.5}$), sulphur (SOx) and nitrogen (NOx) oxides etc.; when their amounts exceeded the tolerance limit of plants, harmfully affect them (Thambavani and Maheswari, 2012).

The studies of tree species in terms of air pollution and their categorization as sensitive and tolerant is an important work with great practical significance. Since the sensitive species can serve as bioindicators (Kurteva and Stambolieva, 2007; Randhi and Reddy, 2013), the tolerant species can be used for construction of green belts and clearing the air from pollutants (Vyankatesh and Arjun, 2013; Kumar et al., 2013; Deepika and Haritash, 2016). Green plants are well known for their abilities to reduce air and noise pollution, but it is important that plants used for the development of urban forest be tolerant to air pollutants (Cavanagh et al., 2009; Pandey et al., 2015). Recently has been renewed interest in the use of vegetation to improve air quality, tolerant to the air pollution tree species are used for construction a diverse specific green filters around rivers banks, roads and railways, parks, gardens, playgrounds, cemeteries, roadside etc., for phytoremediation of environment thought localization and disposition of dust and toxic emissions (Freiman et al., 2006; Nowak et al., 2006; Bealey et al., 2007; Calatayud et al., 2007; Jim and Chen, 2007; McDonald et al., 2007; Escobedo et al., 2008; Kumar et al., 2013; Pandey et al., 2015; Deepika and Haritash, 2016).

Tolerance of plant towards air pollutants is specific to a site and depends on the type and level of pollution (Noor et al., 2014). In the last decades many anatomical,

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morphological, physiological, biochemical and other studies have been done and are still needed in order to classify tree species to realize more efficient utilization and optimization of urban and industrial environments (Pourkhabbaz et al., 2010; Thambavani and Maheswari, 2012; Lohe et al., 2015; Pandey et al., 2015; Deepika and Haritash, 2016; Uka et al., 2017).

In the last decades, human exposure to sulphur dioxide (SO_2), carbon monoxide and lead concentrations in the air decreased strongly due to the effectiveness of European air quality policies. Rendering data from 1990 to 2012 in the 28 EU countries, the level of all air harmful emissions of pollutants are decreased (EEA, 2015; Fig. 1).

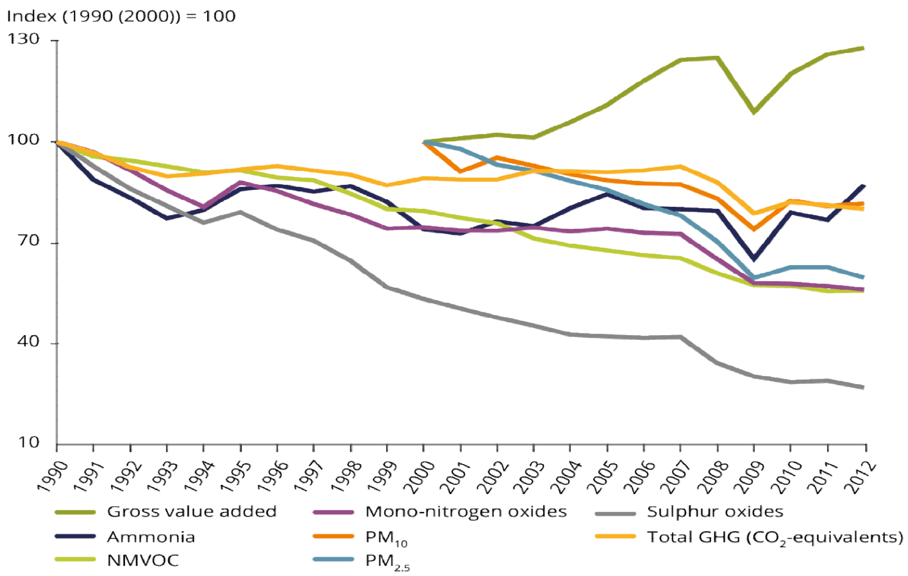


Figure 1. Emissions of air pollutants (EEA, 2015)

Emissions of all air pollutants fell substantially during the period 1990–2004 in the 32 EEA member countries (EEA-32), resulting in improved air quality over the region (EEA, 2007). The investigation estimated modification in structure of *Acer campestre* L. leaf blades under two varied levels of industrial air contamination. The structure of leaf blades and developing of winter buds and leaf laminas have been monitoring to reveal any modifications as adaptation respond.

Materials and methods

Characteristics of the regions

The region of metallurgical plant Kremikovtzi (42º47′N; 23º30′E), the largest metalworking factory situated in Sofia's valley, 17 km northeast from Sofia and is a source of serious environmental pollution, so called "hot spot" producing cast iron and coke from 1963. Accordingly to national data the Sofia's district air basin is heavily polluted with SO_2, NxOx, Pb, As, Zn, Cu etc., as: 8.8% of nitrogen oxides (NOx); 11.1% of non-methane evaporate organic compositions; 29.6% of carbon monoxide (CO); and 39.5% of dust contribute to the total amount of harmful emissions in Bulgaria. It is accepted that heat power stations of "Kremikovtzi" and the metallurgic plant emit 90% of the total pollution amount in Sofia district (Tzekova et al., 2004).

The investigated tree species *Acer campestre* L. (field maple) develops on territory of coke-chemical and agglomeration plants of MP "Kremikovtzi". These industrial units are the main sources of pollution disposing dust, soot and ash. Coke-chemical plant emitted nitrogen oxides, benzenes, phenols, ammonia, hydrogen cyanide and others. Although, sulphur dioxide is the dominant gaseous waste product and its concentration exceeds the limit values.

The control trees of field maple are growing in the National Park Vitosha (42º30′N; 23º15′E), near to the lift station at Dragalevtzi. That area has been chosen as a control, as a place with relatively clean air and similar environmental conditions, both fields have tempered climate.

Common characteristic of *Acer campestre* L.

Acer campestre L. has a common name field maple (Leinemann and Bendixen, 1999), in North America and Australia it is known as hedge maple, but it can be also called common maple.

Field maple is a widespread species and has a very wide ecological range, although it is more common in mesophilous stands as a component of deciduous temperate forests; the natural distribution covers most of Europe, southwest Asia from Turkey to the Caucasus, and North Africa in the Atlas Mountains (Mitchell, 1974; Rushforth, 1999; Nagy and Ducci, 2004; Calatayud et al., 2007; Zecchin et al., 2016; Woodland Trust, 2017). Field maple never formed separate communities, occurs in wet and dry places in deciduous forests and bushes, bare slopes and along river valleys. It is characterized by wide vertical distribution and participated in the formation of dry oak, flooded river forest and dense coniferous forests, from sea level up to 1600 m and above (Petrov and Palamarev, 1989; Zecchin et al., 2016). Common maple is a really easy going type, grows in different soil composition, with age limit 100 years (Petrov and Palamarev, 1989).

This species is very plastic environmentally and well tolerant to soil drought and air pollution. It is grown as an ornamental tree, with over 30 cultivars (Encyclopedia of Life, 2017), broadly planted in gardens and parks due to tolerance of pollution and rich autumn colours (Woodland Trust, 2017).

Acer campestre L. is given as a resistant to industrial emissions, relatively sensitive to high O_3 levels (Calatayud et al., 2007; Benham et al., 2010), tolerant to SO_2, intermediate to hydrogen fluoride (U.S. Department of Agriculture, 1973). It grows slowly that is why has limited use, mainly as a drought-resistant tree (Petrov and Palamarev, 1989). In Europe, maple species are among the trees showing more typical ozone symptoms in the field (Innes et al., 2001), and plants of this genus have a high potential to be used as bioindicators for that kind of contamination.

Plant material and methods

The study examined the modification of anatomical leaf blades structure of *Acer campestre* L. under industrial pollution. The material was collected at the same time of the growing season, the end of July, from the south side of the crown at 160-200 cm high, randomly. The trees were of a similar age, approximately 15 years old samples, sun exposure and with uniform height and growth form.

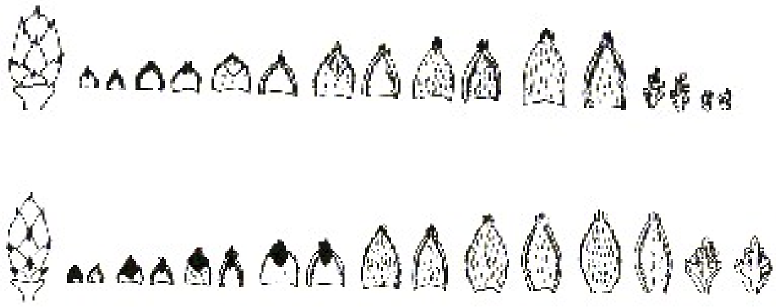
The middle parts of the leaf blades were cut and fixed in 90% ethanol (90 cm^3), ice acetic acid (5 cm^3) and formalin (5 cm^3). The cross sections were prepared manually and observed without coloration with light microscopy to examine the anatomical characteristics of the leaf blades. The measurements were repeated 30 times per one parameter. All measurements were carried out with Carl Zeiss light microscopy for routine research, with the objective lens 40 and eyepiece 8X, and documented with Primotech, photographs X 200.

Cell size and thickness of the layers were assessed statistically with t-test. The influence of the pollution on the linear growth of the leaf blades, length and width, was evaluated with ANOVA (two way).

Results and discussions

Winter buds

Winter buds of *Acer campestre* L. from the polluted area have bigger length with a mean 12.6 mm (σ=0.04), while in control the average value of the length was 9 mm (σ=0.06), $p<0.05$ significance. The coating scales in control plants are 6 pairs arranged like a tiles, with increasing size from outside to inwards. First four pairs of scales are hard and with dark brown colour, hairy on edge and tip, below them are two pairs cataphylls which are green in colour, densely shaggy outside and inside and cover 4 rudimentary leaves (2 larger and two smaller). In plants growing in the contaminated area, the coating scales are 7 pairs larger in size. Inchoate leaves are usually only two larger in size (Fig. 2).



(a)

(b)

Figure 2. Wintering buds of *Acer campestre* L.: (a) Vitosha; (b) Kremikovzi

Registered changes that were observed in the subjected to pollution plants are expressed as greater bud size and coating scales, covering less in number but larger rudimentary leaves in comparison to the control. Thereby during leafing, which in contaminated region start 10-15 days earlier than in the control plants, are ensured faster growth, development and normal feeding of assimilation organs. This can be accepted as adaptive response toward adverse influence of sulphur emissions on unformed rudimentary leaf blades.

Leaf blades

Reductions of emissions up to 50% for SO_2, lead and CO are reported since the mid of 1990s (EEA, 2007). During the investigation periods, the concentration of sulphur dioxide in the observed area falls down, approximately 2-3 times, up to 0.5 mg/m^3 and that can be undoubtedly mentioned from the obtained results (Tables 1; 2; 3).

In the both periods with different levels of contamination, the leaf surfaces of the control samples were better developed than the treated once. In the performed morphological analysis during the period with heavy air contamination, reducing of leaf surfaces in plants subjected to anthropogenic stress has been registered. The mean of leaf surfaces was 15.6 cm^2 for the field maples growing in polluted region, while in the controls the surface of the lamina was twice bigger 44.53 cm^2. In observed trees, growing in Kremikovtzi, the leaf laminas were normally with dry tops of units and large necrotic spots around the veins of the lobes, injured 17.8% of the total leaf area.

During the period with less levels of SO_2 contamination the alterations in the length of lamina between test and control plants were insignificant, the length in control leaves were 6.61 cm (σ=0.74), in the test 6.64 cm (σ=0.83). The width of leaf surfaces kept significant amendments, with average in control 7.83 cm (σ=0.98), while test laminas have width 7.25 cm (σ=1.13), with $p<0.05$. Many authors confirmed reduction of leaf blade surfaces in plants growing under high contaminated air conditions (Sodnik et al., 1987; Gupta and Ghouse, 1988; Jahan and Iqbal, 1992; Kurteva and Stambolieva, 2007; Pourkhabbaz et al., 2010; Ivănescu and Toma, 2007a).

In the areas with chronic aggressions from atmosphere pollutants, the leaf blade responses of deciduous trees are various and unexpected depending from a great number of factors: genetic, pedo-climatic, natural habitat, degree of acclimatization, distance and position from the source of pollution, age, physiological state, etc. The usual observation as a general response under gaseous and solid noxious industrial substances are: deposits of foreign substances - lime and cement dust, carbon black, soot - on the surface of the leaves; episodes of partial or total defoliation; partial or total withering phenomena; smaller average length and small average surface of the leaves: chlorosis, necroses and leaf bums of various sizes etc. (Ivănescu and Toma, 2007a).

Modification of anatomical parameters

Leaf blades of *Acer campestre* L. are characterized by a well-developed cuticle layer, in control plants the mean is 4.45 µm (σ=0.6), in the experimental 4.75 µm (σ=0.5; Table 3). Air pollution caused deposition of thicker adaxial wax cuticle layer (Tables 1; 2; 3). The thick cuticle is a xeromorphic sign and in plants subjected to gas impact is considered as a mechanism for protection (Ilykun, 1970; Bonte et al., 1976; Ninova, 1970). Perhaps such a protective barrier against the direct influence of toxic gases assist troubled transpiration of leaves in industrial polluted environment (Caput et al., 1978).

Under industrially contaminated environment in *Acer campestre* decreased the size of adaxial epidermis cells. In May the mean for control was 13.33 µm (σ=0.71), while in treated plants was 10.54 µm (σ=0.4). In July, was measured for control 14.62 µm (σ=0.37), and 11.41 µm (σ=0.35) mean for plants in industrial area. The same trend has been kept for the full period of leave development (Tables 1; 2).

Table 1. Difference of means (μm), between polluted and control plants of *Acer campestre* L., during heavy polluted period (1990)

Anatomical measurements (μm)	May	June	July	October
Thickness of the leave lamina (A)	34.83***	1.57	2.17	-5.9
Thickness of the adaxial cuticle (a)	0.29	0.07	1.63*	1.71*
Thickness of the adaxial epidermis (b)	-2.79***	-0.59	-3.21***	-1.25***
Thickness of the palisade mesophyll (c)	25.75***	4.77***	6.33***	-3.09***
Thickness of the spongy mesophyll (d)	11.57***	0.65	-4.33***	-2.92***
Thickness of the abaxial epidermis (e)	-0.04	-3***	2.25***	0.33
Thickness of the abaxial cuticle (f)	0.05	-0.3	-0.5	-0.71
Thickness of mesophyl (M)	37.32***	5.42***	2	-6.01***
Coefficient of palisadness	8%***	2%	4%***	0%

**p<0.05; **p<0.01; ***p<0.001*

Table 2. Difference of means of anatomical parameters (μm), between polluted and control samples of *Acer campestre* L., during heavy polluted period (1991)

Anatomical measurements (μm)	May	June	July	October
Thickness of the leave lamina (A)	40.87***	8.73	8.65	-6.04
Thickness of the adaxial cuticle (a)	0.71	0.54	0.18	0.58
Thickness of the adaxial epidermis (b)	-2.14***	-0.58	-075	-0.08
Thickness of the palisade mesophyll (c)	18.44***	9.91***	14.96***	-1.18
Thickness of the spongy mesophyll (d)	25.57***	-8.84***	-6.06***	-3.25***
Thickness of the abaxial epidermis (e)	-1.75***	-1.25***	2***	0.16
Thickness of the abaxial cuticle (f)	0.33	-0.05	-1.58*	-2.25***
Thickness of mesophyl (M)	44.03***	10.07***	8.9***	-4.43***
Coefficient of palisadness	-1%	13%***	9%***	1%

**p<0.05; **p<0.01; ***p<0.001*

Modification of some structural parameters are considered as biomarkers when plants are developed under pollutants action. In deciduous plants cyto-histological changes due to the influence of noxious contaminants are extremely phenotypised, important and irreversible by comparison with those which are observed in gymnosperms (Ivănescu and Toma, 2007b). In *Acer campestre* L. in heavy period increasing the thickness of palisade parenchyma has been detected (Tables 1; 2). For control plants in May it is 39.08 μm (σ=1.56) and for experimental plants 64.83 μm (σ=1.66; Table 1). The trend is maintained in June and July, palisade parenchyma for control 51.31μm (σ=1.58) and 56.08 μm (σ=1.46) for plants from polluted environment, and in July of the same year 46 μm (σ=1.26) for control and 52.33 μm (σ=1.82), with reliability r >0.999. The second year with identical contamination, palisade parenchyma is amended in the same direction of increasing its size in polluted plants, with degree of confidence r>0.999. The results are: May control 39.41 μm (σ=1.13) vs 57.85 μm (σ=1.06); June control 43 μm (σ=1.37) vs 61.91 μm (σ=1.58); July palisade mesophyll in control plants is 51.95 μm (σ=1.21) versus 66.91 μm (σ=1.95) for polluted one.

Palisade tissue in field maple is represented by one row of highly elongated cells and no significant changes in the mean values have been found between the test and the control plants under the influence of relatively low level of air contamination (Table 3).

Table 3. Differences of anatomical parameters between means of polluted and control samples of *Acer campestre* L. (μm) (2004)

Anatomical measurements (μm)	Difference of means (July)
Thickness of the upper cuticle (a)	0,3*
Thickness of the upper epidermis (b)	2,35**
Thickness of the palisade mesophyll (c)	0,33
Thickness of the spongy mesophyll (d)	- 3,25
Thickness of the lower epidermis (e)	- 1,22***
Thickness of the lower cuticle (f)	- 0.2

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

The thickness of spongy mesophyll reduced in the experimental plants, for both periods with high levels of contamination (Tables 1; 2), for July the size of spongy parenchyma in control is 54.58 μm (σ= 1.90) and in samples 50.25 μm (σ= 1.63); reaching its up in June (Table 2), for control 52 μm (σ= 1.69) and for test plants 43.16 μm (σ= 1.84); with r>0.999. Spongy parenchyma is more sensitive to gas exchange than palisade parenchyma, and therefore it is more susceptible to damage from gaseous pollutants (Dineva, 2006). The vulnerability is confirmed by Tra et al. (2016), which are explained by decreasing chlorophyll content and anatomical changes in the spongy parenchyma such as reduced intercellular spaces and a thinner layer of spongy parenchyma (Carreras et al., 1996).

On the end of vegetation period is detected reducing the thickness of mesophyll, maybe caused by aging processes, mean for the control 95 μm (σ= 0.72) and 88.99 μm (σ= 0.92) for polluted plans (Table 1). In second year average for control 99.08 μm (σ= 1.04) and 94.65 μm (σ= 0.62) for poisoned trees (Table 2), with reliability r> 0.999.

The ratio between palisade and spongy tissue in the experimental plants of field maple tends to increase, in most cases due to the enlargement of palisade tissue, which is correlated with higher palisade ratio. The larger coefficient of palisadness is a sign of a better resistance to the type of pollution (Nikolaevskiy, 1979). The total thickness of mesophyll in industrial plants from the contaminated area is directed towards increasing. The differences are most indicative in May (Table 2), the thickness of mesophyll in control is 76.57μm (σ= 1.02) and for polluted plants 120.60 μm (σ= 1); May (Table 1) for control 90.99 μm (σ= 0.85), and for plants from industrial zone 128.31 μm (σ= 1.02), reliability r> 0.999. The trend continued in all growth season, but during less contaminated period these modifications were not significant (Table 3; Figs. 3; 4).

Reducing the intercellular air spaces like xeromorphic plants or plants growing in polluted sites cause limit the water loss and gaseous pollutants uptake which is regarded as important trait that enable the tolerant and more resistant genotypes to avoid or to oppose the adverse effects of gas pollutants (Giacomo et al., 2010). During the period with less air contamination in observed plants the spongy parenchyma was well-developed with preserved air voids (Figs. 3; 4) and no significant changes have been detected (Table 3; Fig. 4) that again confirm the tolerance of *Acer campestre* to air contamination.

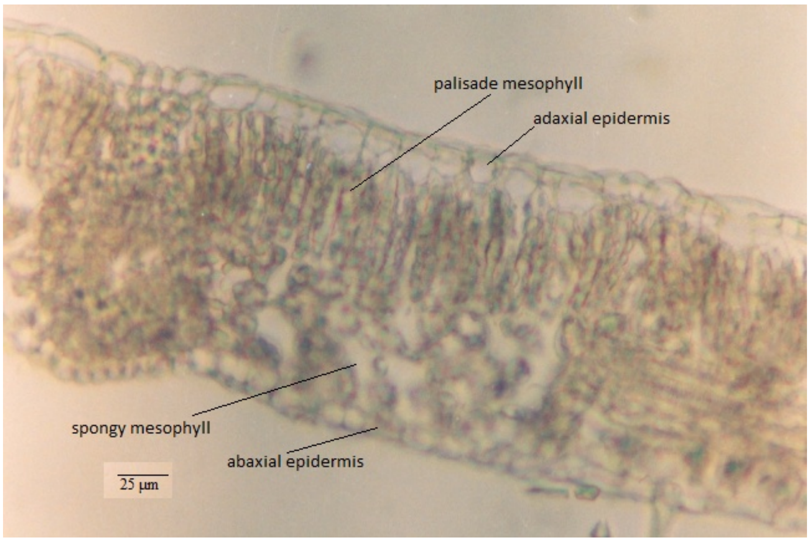


Figure 3. Section through the leaf blade of *Acer campestre* L. – Vitosha – control region

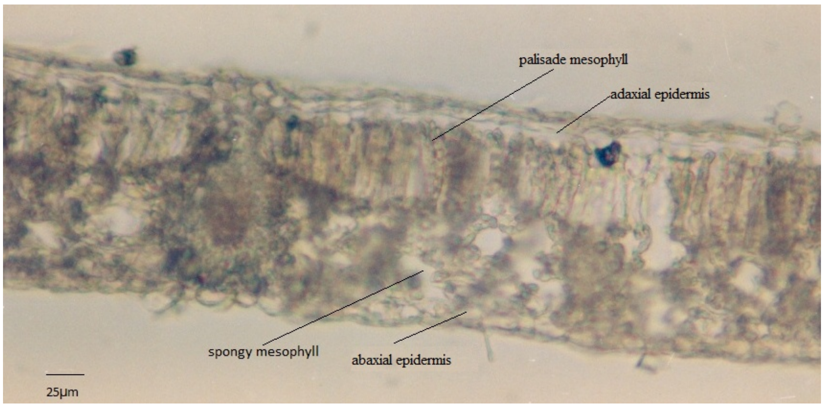


Figure 4. Section through the leaf blade of *Acer campestre* L. – Kremikovtzi – polluted region

Conclusions

The investigation confirmed that field maple well tolerated air and soil pollution. During heavy polluted periods it develops well stated but smaller leaf blades. Winter buds are greater in size, with less in number, with larger rudimentary leaves in comparison to the control. The noticeable differences in lamina structure between heavy and less polluted periods are visible perhaps due to the reduction of harmful emissions in study area. Most of these modifications disappeared when the levels of SO_2 contamination drops down.

Increasing of mesophyll tissues and decreasing of spongy parenchyma correlated with increasing the palisade ratio, during high levels of industrial contamination, but not in the period with relatively slight air pollution. Enlargement of palisade ratio is considering as tolerate and adaptive response of plants toward the stress factor. Almost all statistical and regional climate models predict a rapid spread of *Acer campestre* in the future (Zimmermann et al., 2015).

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