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Leaf contents of nitrogen and phenolic compounds and their bearing with the herbivore damage to *Tibouchina pulchra* Cogn. (Melastomataceae), under the influence of air pollutants from industries of Cubatão, São Paulo[1]

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ABSTRACT - (Leaf contents of nitrogen and phenolic compounds and their bearing with herbivore damage to *Tibouchina pulchra* Cogn. (Melastomataceae), under the influence of air pollutants from industries of Cubatão, São Paulo). The Atlantic Forest on the slopes of Serra do Mar around Cubatão (São Paulo, Brazil) has been affected by massive emissions of pollutants from the local growing industrial complex. The effects of air pollution on the amounts of leaf nitrogen, total soluble phenols and total tannins of *Tibouchina pulchra* Cogn., a common species in the area of Cubatão, were investigated, as well as the possible influence of the altered parameters on the leaf area damaged by herbivores. Fully expanded leaves were collected at two sites: the valley of Pilões river (VP), characterized by a vegetation virtually not affected by air pollution and taken as a reference; and valley of Mogi river (VM), close to the core region of the industrial complex, and severely affected by air pollution. No differences were observed for any parameters between samples collected in the summer and winter in both sites. On the other hand, compared to VP, individuals growing in VM presented higher amounts of nitrogen and lower amounts of total soluble phenols and total tannins, as well as higher percentages of galls per leaf and higher leaf area lost to herbivores. Regression analysis revealed that the increase in leaf area lost to herbivores can be explained by the increase of the content of nitrogen and decrease in the contents of total soluble phenols and total tannins. Although significant, the coefficients of explanation found were low for all analyses, suggesting that other biotic or abiotic factors are likely to influence leaf attack by herbivores.

RESUMO - (Conteúdo foliar de nitrogênio e compostos fenólicos e sua relação com o ataque de herbívoros em *Tibouchina pulchra* Cogn. (Melastomataceae) sob a influência da poluição atmosférica emitida em Cubatão, São Paulo). O crescente processo de industrialização na região de Cubatão (São Paulo, Brasil) e as conseqüentes emissões de poluentes vêm há tempos afetando a vegetação da Serra do Mar ao seu redor, um remanescente de Mata Atlântica. Os efeitos da poluição aérea nos conteúdos foliares de nitrogênio, fenóis totais e taninos totais em indivíduos de *Tibouchina pulchra* Cogn., espécie predominante na região, foram avaliados, assim como a possível influência dessas alterações na intensidade de herbivoria foliar. Folhas completamente expandidas foram coletadas em duas áreas amostrais: Vale do Rio Pilões (VP), com pouca influência da poluição aérea de Cubatão e considerada como área de referência; e Vale do Rio Mogi (VM), área próxima ao centro do complexo industrial, sendo fortemente afetada pela poluição. Para todos os parâmetros, não foi observada diferença significativa entre os valores obtidos no verão e no inverno em ambas as áreas amostrais. Entretanto, comparados ao VP, os indivíduos do VM apresentaram maiores conteúdos de nitrogênio e menores de fenóis e taninos totais, assim como maiores porcentagens de galhas por folha e de área foliar perdida por herbivoria. As análises de regressão mostraram que o aumento na porcentagem de área foliar perdida por herbivoria pode ser explicada pelo aumento no conteúdo de nitrogênio e diminuição das concentrações de fenóis e taninos. Embora significativos, os coeficientes de explicação encontrados são baixos, indicando uma possível influência no ataque de herbívoros de outros fatores, bióticos ou abióticos, não avaliados neste estudo.

Key words - Air pollution, herbivory, *Tibouchina pulchra* Cogn., Cubatão, nitrogen, phenolics

Introduction

Over the last decades, the quality of the air has substantially changed due to emissions of pollutants into the atmosphere as consequence of increasing human activities. A drastic example of environmental disturbance can be seen around Cubatão (State of São Paulo, Southeast Brazil), one of the most important industrial complexes of South America, housing 23 industries, including fertilizer, chemical and petrochemical plants and steel works. According to CETESB (1998), an official organization responsible for monitoring and controlling pollutant emissions, the estimated emissions of pollutants in the region of Cubatão in 1994 were close to 32,000 ton.year^{-1} of particulate matter, 17,000 ton.year^{-1} of sulfur dioxide, 17,000 ton.year^{-1} of nitrogen oxides, 4,000 ton.year^{-1} of volatile hydrocarbons, 70 ton.year^{-1} of gaseous fluorides and 70 ton.year^{-1} of ammonia. In addition to these pollutants, Jaeschke (1997) pointed out ozone and peroyacetyl nitrate (PAN) as important pollutants in Cubatão, especially in areas at higher altitudes.

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The city of Cubatão is located in the Santos Basin, between the city of Santos and the elevations of the Serra do Mar (23°45'-23°55' S and 46°18'- 46°27' W), a coastal mountainous region which rises up to 800 m above sea level. The inadequate topographic location of the industrial complex, between the ocean and the steep slopes of the Serra do Mar, combined with unfavorable meteorological conditions (sea-land winds are predominant during daytime), hinders the dispersion of pollutants. The consequences of years of increasing amounts of pollutants acting on the vegetation are the extensive areas of visibly degraded forest on the Serra do Mar around Cubatão (Gutberlet 1996, Pompéia 1997).

Plants submitted to pollution stress may be sensitive to some of the pollutants, which results in changes in their morphology, physiology, biochemistry or growth rate. Vascular plants synthesize a vast array of secondary metabolites, like alkaloids, terpenes, cyanogenic glycosides, phenolics etc. Such compounds are not directly involved in the fundamental cell metabolic processes and are not universal in their occurrence (McNaughton 1983). It has recently been demonstrated that air pollution may induce qualitative and quantitative changes relative to many compounds of the secondary metabolism (Zobel 1996). Secondary plant metabolites may have important roles in the defense of the plants against herbivores and pathogens, since they alter the nutritional value, palatability and digestibility of the plant material (Coley et al. 1985, Bennett & Wallsgrove 1994). Hence one of the indirect effects of pollution may be alteration on the resistance of the plants to herbivory, caused by qualitative and/or quantitative changes in the composition of secondary metabolites. Katoh et al. (1989a) reported a decrease in the levels of tannins in *Cryptomeria japonica* growing near a steam power station, which resulted in increased feeding rate by *Dasychira abietis argentata*. Hautala & Holopaingn (1995) showed that under certain concentrations of fluorides the levels of alkaloids of *Hordeum vulgare* increase and may affect insect herbivory. Certain pollutants, like sulfur dioxide and ozone may cause an increase in the leaf nitrogen levels (Rowland et al. 1987a, Jones & Coleman 1991), which may favor herbivores. On the other hand, the latter can affect the numbers, kinds and relative abundance of plant species in a community, since they select among parts of individual plants, individuals, species and patches, thus influencing plant growth and mortality rates (Huntly 1991). Therefore herbivory can be viewed as an extra stressing factor pressing plants under the influence of pollution and reducing even further their chances of survival.

Such complex ecological interactions involving pollutants, plants and herbivores are likely to be occurring in the Atlantic Forest around Cubatão. It is reasonable to expect that under the stress caused by air pollutants the levels of secondary metabolites should decrease. At the same time, the levels of nitrogen may increase, both by partial blockage of the primary metabolism and the consequent inhibition of the diversion of proteins to other metabolic paths, and by the accumulation of nitrogenous pollutants inside the leaf tissues. Both trends, the increase of foliar nitrogen and the reduction of the levels of secondary metabolites, have the potential to enhance herbivore damage. In order to test these hypotheses, we planned to analyze the foliar contents of nitrogen, total soluble phenols and total tannins of *Tibouchina pulchra* Cogn. (Melastomataceae), a native, pollution-resistant and frequent species in the region of Cubatão, and to verify the existence of any relation between alterations on those parameters and the degree of foliar damage by herbivory. Taking into account that the study was planned to be carried out in the field, seasonal variations of metabolites were expected. For this reason, collections and measurements were carried out in the summer and winter over two consecutive years.

Material and methods

The distribution of industries, air circulation and mass transport delimit areas with distinct influences of air pollution in the region of Cubatão (Klumpp et al. 1994). Two sampling sites were selected – VM and VP (figure 1). Both are located at similar altitudes and are virtually under the same meteorological conditions, differing, however, as to the influence of the air pollution (Klumpp et al. 1994, Pompéia 1997).

The VM site is located at the entrance of the valley of the Mogi river, an area downwind the industries of Cubatão and showing severe damage on its vegetation. VM has altitude of 140 m, mean annual temperature 22°C, mean humidity 84% and mean annual precipitation 2500 mm (Domingos 1998). It is situated close to the core of the industrial complex and under the influence of pollutants from fertilizer industries, steel works and chemical products industries. Particulate matter, fluorides and compounds of nitrogen and sulfur (Klumpp et al. 1994, Jaeschke 1997) heavily polluted the area. According to data from the monitoring station located in the area, the mean concentrations of nitrogen oxides, sulfur dioxide and ozone in 1991-1995 were, respectively, 26.2 $\mu g.m^{-3}$, 30.2 $\mu g.m^{-3}$ and 29.4 $\mu g.m^{-3}$ (Domingos 1998). The local Atlantic Forest has a secondary formation with decreased number of species (44), genera (53) and families (30) of vascular plants, with the

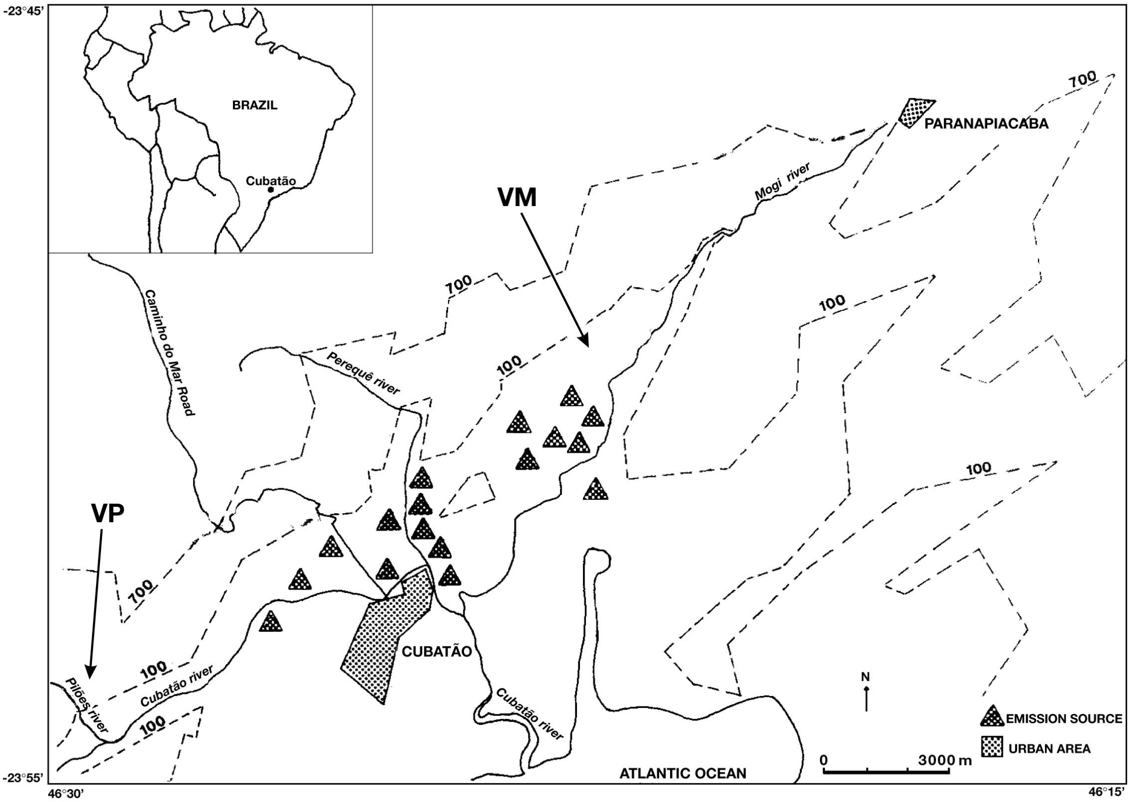

Figure 1. Map of the research area showing the localization of the two sites used for sampling in relation to the industrial complex of Cubatão (adapted from Klumpp et al. 1996).

predominance of Melastomataceae and Meliaceae. Among those species, *Tibouchina pulchra* stands out as to the number of specimens (Pompéia 1997).

The second site (VP) is taken as the reference area. It is located in the valley of Pilões river, Southwest the industrial complex. VP has altitude 150 m, mean annual temperature 23°C, mean humidity 87% and mean annual precipitation of 3000 mm (Domingos 1998). Air pollutants are normally present in low concentrations, stemming mainly from vehicles. Geographic barriers and a location out of the land-to-sea breeze way, avoid air pollutants from the industrial complex to reach VP (Jaeschke 1997). The mean concentrations of nitrogen oxides, sulfur dioxide and ozone in 1991-1995 were, respectively, 7.6 $\mu g.m^{-3}$, 5.4 $\mu g.m^{-3}$ and 29 $\mu g.m^{-3}$ (Domingos 1998). The area show no signs of degradation induced by air pollution. Pompéia (1997) mentions that 77 species, 69 genera and 38 families of vascular plants compose the local forest, with the predominance of Nyctaginaceae and Euphorbiaceae. *T. pulchra* is among the five most important tree species, represented commonly by few, but large individuals.

In order to minimize the influence of the stage of plant development, leaves were sampled from individuals with similar height in both sites. Since plants 2.5 m tall were largely available on the edge of the forest, this height was assumed as a standard. Five different individuals approximately that tall were chosen in both sites in four different months, January and February (summer) and June and July (winter), in 1996 and 1997. In this way, leaves of 40 individuals were sampled in each site.

Fully expanded leaves were collected from the highest and more exposed branches of each individual. Approximately 120 leaves were collected from each individual. Among them, 40 were randomly taken for the estimation of the total leaf area and the leaf area lost to herbivores. This was done using the Leaf Area and Analysis Program and a microcomputer connected to a video camera. The system permits to measure the leaf length, width, total and missing area. The percentage of leaf area lost to herbivores was assumed to be the difference between the leaf total and missing area. The number of galls per unit of leaf area for each individual was obtained from the same 40 leaves.

The approximately 120 leaves of each individual were dried at 60°C, powdered and used for chemical analyses, all of which were carried out in triplicate samples. The nitrogen contents were determined by the Micro-Kjeldahl procedure using apple leaves as reference (NIST 1515) (Bataglia et al. 1983). Total soluble phenols were analyzed by the Folin-Denis method and total tannins by the colorimetric method of protein precipitation (Waterman & Mole 1994), tannic acid being used as reference in both procedures.

All data raised were statistically treated using ANOVA with three fixed factors, site, season and year. A pairwise multiple comparison procedure (Bonferroni's method) was applied whenever significant differences emerged through ANOVA. Square root transformation was employed whenever the normality and equal variance tests had failed. Regression analyses were used to detect correlation between variables and intensity of herbivory.

Results and Discussion

Statistical analyses revealed no significant differences between values obtained in summer and wintertime. Differences were noted, however, in comparisons between sites and year of collection for all parameters considered. The results, then, are presented as annual means (table 1).

The mean leaf concentrations of nitrogen were higher in leaves of the individuals of *T. pulchra* collected in VM than in VP (table 1). Similar results were observed by other authors: Domingos (1998), in her study of chemical alterations in adult individuals of *T. pulchra*; Pradella (1997), by exposure of young plants of *T. pulchra* to the air of both sites (active biomonitoring); and Domingos et al. (1998), in their analyses of several bioindicator plants and other native tree species exposed to the air of VM and VP. In our study, for both sites the mean values of foliar nitrogen were significantly higher in 1997 than in 1996. Meteorological variation from year to year, influencing the concentrations of pollutants in the air, may account for the differences observed.

According to Mayer & Lopes (1997), the contents of total nitrogen in the soil of VP and VM were respectively 0.23% and 0.13% in the surface layer and 0.03% and 0.02% in 50-70 cm of depth, indicating that the soil in VM is poorer in nitrogen than the soil of the reference site. Based on these results, it might be assumed that the higher concentrations of nitrogen in leaves from VM can not be explained by the higher uptake from the soil.

Table 1. Mean leaf concentrations of nitrogen (mg.g^{-1} dry matter), total soluble phenols and tannins (% dry matter) and mean percentage of leaf area damaged by herbivory and percentage of galls per leaf in individuals of *Tibouchina pulchra* (N = 20) in each year of sampling at two different sites at Serra do Mar: valley of Pilões river (VP) and valley of Mogi river (VM).

Parameters	Year	VP	VM
Nitrogen	1996	18.04 ± 3.60 b B	22.99 ± 3.21 a B
	1997	21.03 ± 3.01 b A	27.29 ± 2.18 a A
Phenol	1996	6.16 ± 1.98 a A	4.72 ± 1.95 b A
	1997	3.13 ± 0.75 a B	2.52 ± 0.75 b B
Tannin	1996	1.02 ± 0.46 a A	0.46 ± 0.39 b A
	1997	0.60 ± 0.43 a A	0.54 ± 0.37 b A
Damaged area	1996	1.04 ± 0.66 b A	3.24 ± 2.26 a A
	1997	1.25 ± 1.09 b A	3.68 ± 2.52 a A
Galls	1996	4.50 ± 3.87 b A	11.75 ± 4.87 a A
	1997	4.13 ± 5.02 b A	6.75 ± 4.14 a A

Significant differences ($p<0.05$) among sites and years of sampling are indicated by different letters. For each parameter, small letters compare values from both sites of sampling, in each year and capital letters compare values from both years of sampling, in each site.

The increased leaf nitrogen may be due to higher concentrations of atmospheric nitrogen oxides in VM, which might represent an additional nitrogen supply to the plant. Atmospheric nitrogen oxides react with the extracellular water, forming nitrate and nitrite ions, which are assimilated by the nitrogen metabolism (Rowland et al. 1987b, Lea et al. 1996). However, some pollutants, like sulfur dioxide and ozone, or other stress factors can cause increases in the leaf nitrogen levels. It is thus difficult to relate increased levels of leaf nitrogen with either absorption of nitrogen oxides from the air or metabolic disturbances caused by pollution stress. The possibility of a combination of both factors also cannot be ruled out.

Lower mean concentrations of total soluble phenols and total tannins were found in leaves collected in VM than in VP (table 1). Contrary to the observed trends in nitrogen values, the contents of phenols and tannins were lower in 1997 than in 1996. Again meteorological conditions may be pointed out as the likely explanation for these differences.

The smaller concentrations of total soluble phenols and tannins, observed in the more polluted area, may be due to the effect of air pollution on photosynthesis and carbohydrate metabolism, causing a decrease in carbon gain and decreasing the rate of synthesis of secondary metabolites derived from the shikimic acid pathway (Jones & Coleman 1991). Katoh et al. (1989b) showed decreased concentrations of soluble phenols in *Cryptomeria japonica* exposed to ozone, which was correlated with lower levels of glucose and shikimic acid. Kainulainen et al. (1995) observed a decrease of glucose, frutose and soluble phenols in needles of *Pinus sylvestris* and *Picea abies* when exposed to SO_2. The lower photosynthetic capacity of the young plants of *T. pulchra* exposed to the air of VM, found by Pradella (1997) and Moraes (unpublished data), may indicate a possible correlation between alterations on photosynthesis, carbohydrate metabolism and decreased concentrations of total soluble phenols and tannins. In addition, Domingos (1998) showed that the increase of nitrogen is followed by a decrease of cations, causing nutritional disharmonies in plants of *T. pulchra* exposed to the heavily contaminated air of VM, a situation that can induce changes in the growth patterns of the plants.

The leaf area analyses showed that leaf areas lost to herbivores and percentages of galls are higher in plants of *T. pulchra* growing in VM than in VP (table 1). Probably the absolute values of leaf herbivory measured are under-estimates, since leaves completely removed or consumed by herbivores were not taken into consideration. No significant differences for these parameters were detected between years of sampling.

Morphological, anatomical, physiological and biochemical characteristics of plants are important determinants of herbivore behavior. Contents of nitrogen and water, leaf toughness and thickness, leaf surface components and internal secondary metabolites are some of the important factors that influence herbivore performance (Jones & Coleman 1991). The air pollution may also influence directly the herbivore community, for example affecting the abundance or distribution of

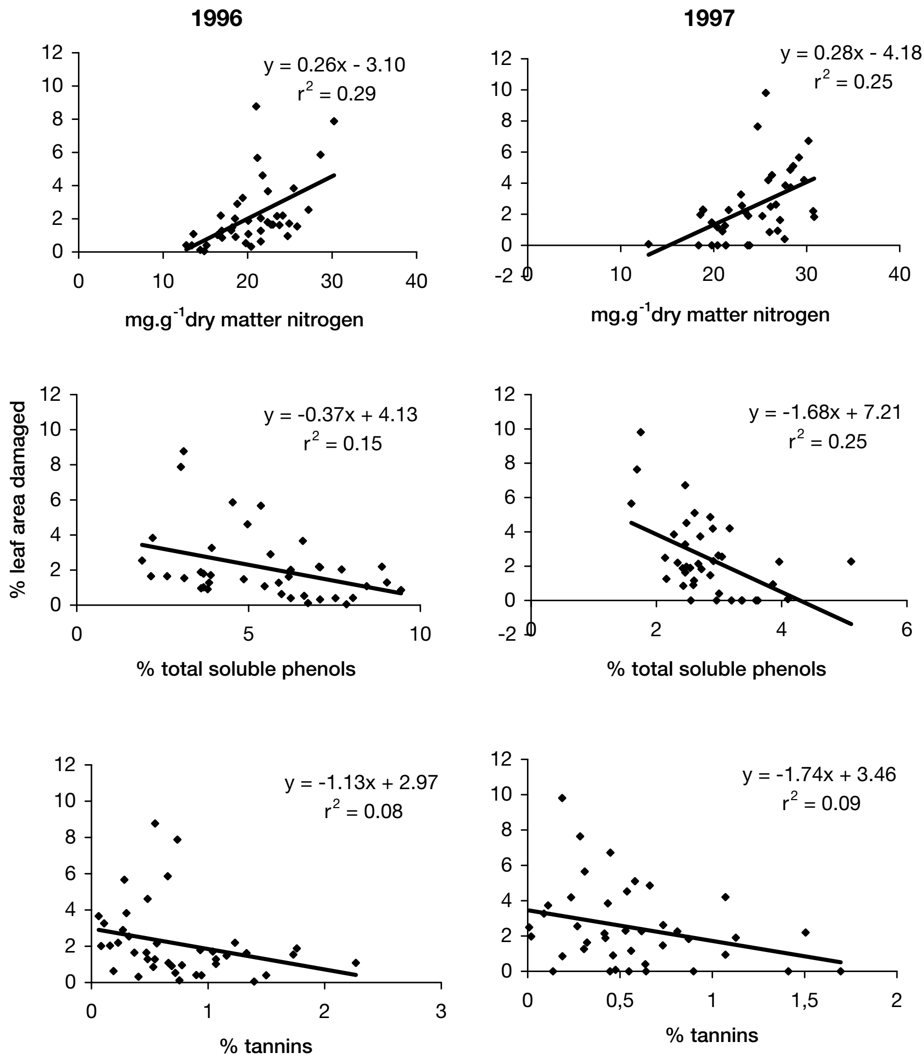

Figure 2. Relationships between the percentage of leaf area damaged by herbivory and the concentrations of nitrogen, total soluble phenols and tannins in leaves of 20 individuals of *Tibouchina pulchra* in each year (1996 and 1997) of sampling at two sites at Serra do Mar. ($p<0.05$).

plant-feeding insects. Indirect effects of air pollution on herbivores (other than metabolic-induced effects), influencing their abundance and distribution, include alterations on the behavior of predators and microclimate changes; by their turn, the latter may induce anatomical or biochemical alterations on the plants (Hughes 1988).

Regression analyses revealed that the increase of the percentage of leaf loss to herbivores can be explained by the increase of the contents of nitrogen and the decrease of the contents of soluble phenols and tannins (figure 2). Although significant, the coefficients of explanation obtained are very low, suggesting that other biotic or abiotic factors also influence the leaf attack by herbivores, as commented by Hughes (1988) and Jones & Coleman (1991).

Our results suggest that, in addition to pollution, plants in VM are submitted to another source of stress, caused by an increase in herbivore attack. The increased nitrogen concentration, improving leaf palatability and nutritional value, and the decreased concentrations of secondary metabolites, which also contribute to improve palatability and weaken the defense capacity of the plant, contribute to favor herbivores and reduce the chances of plant survival.

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