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MONITORING OF HEALTH STATUS OF *QUERCUS CERRIS* L. IN THE EASTERN BALKAN RANGE AND THE LUDOGORIE (BULGARIA)

MARGARITA GEORGIEVA*, DIMITAR NEDJALKOV, GEORGI GEORGIEV, MARIA MATOVA, GERGANA ZAEMDZHIKOVA, PETAR PETKOV, PLAMEN MIRCHEV

Forest Research Institute – Bulgarian Academy of Sciences, 132 "St. Kliment Ohridski", Sofia, Bulgaria *Corresponding author: margaritageorgiev@gmail.com

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Abstract: The objective of the present study was to examine the health status of *Quercus* cerris L. stands in the region of the East Balkan Range and the Ludogorie for the period between 2009 and 2018, on the base of forest monitoring conducted in Bulgaria under the 'International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests' (ICP Forests). The health condition of *Q. cerris* trees was surveyed annually in seven permanent sample plots (PSPs) based on the assessment of defoliation and damages caused by biotic and/or abiotic factors. For the entire studied period, it was established that *Q. cerris* stands was in relatively good condition. Decline of single trees due to their natural maturity was observed. In next years, a persistent tendency for deterioration of tree condition was recorded, and at the end of the studied period, the damaged and dead trees exceed the number of healthy ones. The results of ten-year monitoring shown that fungal pathogens caused the most significant impact in this process and the number of affected trees was nine times more than damages caused by insects and abiotic factors. Among the complex of pathogens, the influence of Hypoxylon mediterraneum (De Not.) J.H. Mill. was the most considerable on sample trees. The pathogen was established in 85% of PSPs, and in some of them almost all trees were affected. Dryomyia circinans (Giraud, 1861) and representatives of Geometridae were the most numerous of the insect pests. Formation of frost cracks was often noticed on sample trees in result of low winter temperatures. Illegal felling of trees in PSP is still a huge problem in studied PSPs, as in two of them all assessed trees were completely cut down.

INTRODUCTION

International Co-operative Programme Forests (ICP Forests) was launched on 4.10.1985 in Freiburg, Germany under the Convention on Long-range Transboundary Air Pollution (CLRTAP) of the United Nations Economic Commission for Europe (UN/ECE, 1992) in response to wide public and political concern over extensive forest damage that had been observed in Europe in the beginning of the 1980s. Twenty-nine countries participated in the Programme until 1992. The studies were conducted on 33 459 Permanent sample plots (PSP) and 620 560 trees were evaluated (Rossnev and Pavlova, 1993), and in 2005 the participating countries were already 32 (Rossnev and Pavlova, 2005). At present, 42 countries participate in ICP Forests as well as Canada and the United States of America (ICP Forests, 2018). ICP Forests monitors forest condition in Europe in Level I monitoring based on around 6000 PSPs on a systematic transnational grid of 16 x 16 km throughout Europe and beyond to gain insight into the geographic and temporal variations in forest condition. Bulgaria signed the Convention in 1983, and in 1986, it was involved in the implementation of the ICP Forests, starting the network of PSPs located at intersections in a network of 16 x 16 km from our forest fund. The country is divided into 10 regions (Pavlova et al., 2012), including the site of the current work - Eastern Balkan Range. Initial PSP pledges were reduced in 2010, and by 2018, two teams from the Forest Research Institute and University of Forestry surveyed 160 areas.

The forestland in Bulgaria covers 38.2% of the territory or 4.2 million ha as dominating deciduous forests, and the share of coniferous stands is 29.6% (1.2 million ha) (EAF, 2016). The Turkey oak (*Quercus cerris* L.) occupies drier and moderately rich habitats in the plain and hilly regions, where it forms large forests on an area of 258 400 ha. The species is most widely distributed in the Mizia region – 58.9% (Rossnev *et al.*, 2006b), forms 7% of the forested area and 24.6% of oak forests in the country (Rossnev *et al.*, 2007).

A study on the health status of *Q. cerris* forests was conducted in the period 2004-2005 in the region of North-east Bulgaria, where the most damaged forests were situated (Rossnev *et al.*, 2006b). In some stands, the relative share of dead trees ranged between 55 and 80%, and wilting trees were on an area of 13 140 ha. The factors contributing to the worse of health status were the long period of drought, decreasing of growth and vitality of trees, severe development of pathogens *Hypoxylon mediterraneum*, *Diplodia mutila* and *Inonotus nidus-pici*, gradual attacks by insect pests (Rossnev et al., 2006b).

The aim of the study was to analyze the variability in the health status of *Quercus cerris* stands in the region of the East Balkan Range and the Ludogorie by both historical data and the results obtained on the base of annual monitoring conducted in Bulgaria under the ICP Forests.

MATERIALS AND METHODS

In the period 2009-2018, the health status of *Quercus cerris* stands in the region of the East Balkan Range and the Ludogorie was examined on the base of annual forest monitoring conducted in Bulgaria under the 'International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests' (ICP 'Forests'). The implemented activities of the project are related to the construction of a network for inventory and large-scale monitoring of forest ecosystems, assessment of the crown condition of sample trees, analysis of damages, caused by biotic and abiotic factors, assessment of floristic composition and soil quality (Level I). The main groups of causal agents were divided as caused by insects, fungi, abiotic factors and direct action of man (legal or illegal cuts, mechanical damages, etc.) (Eichhorn *et al.*, 2016).

During the ten-year period, *Q. cerris* trees (between 210 and 251) were surveyed annually in seven PSPs (Table 1) based on the assessment of tree crown defoliation rate and the degree of damages, caused by biotic and/or abiotic factors. The main characteristics of forest stands were defined by the State Forest or Hunting Enterprises forest management plans.

Site	Region	Forestry	GPS Coordinate		Altitude,	Slope	Age to	Canopy	Habitat
			N	E	m	Slope	2018	Callopy	code
Varbitsa pass	Eastern Balkan	Sadovo	42°52'55"	26°39'09"	620	26	80	0.8	91GO*
Podvis	Eastern Balkan	Karnobat	42°48'43"	26°52'28"	295	0	57	0.7	91MO**
Forest nursery	Eastern Balkan	Aitos	42°45'35"	27°18'26"	353	5	72	0.8	91MO
Ravna gora	Black sea coast	Staro Orjahovo	43°02'03"	27°40'13"	59	0	72	0.8	91MO
Avren	The Avren Plateau	Varna	43°08'45"	27°41'40"	257	0	68	0.7	91MO
Devnja	Southern slopes of the Dobrudzha Plateau	Suvorovo	43°12'59"	27°40'13"	161	0	74	0.7	91MO
Sveshtari	Ludogorie	Seslav	43°42'43"	26°48'27"	295	0	54	0.8	91MO

Table 1. Main characteristics of the sample plots

The assessment of tree condition in the transnational Level I network was conducted on 40 sample trees in each PSP according to European-wide, harmonized methods described in the ICP Forests Manual by Eichhorn *et al.* (2016). Defoliation surveys were conducted in combination with detail assessment of damages caused by biotic or abiotic factors. The rate of defoliation (loss of leaves in the assessable crown compared to a local reference tree in the field) was estimated in 5% steps, ranging from 0% (no defoliation) to 100% (dead tree). Defoliation values were grouped in five classes: '0' – no defoliation (leaf loss up to 10%); '1' – slight defoliation (leaf loss >10-25%); '2' – moderate (leaf loss >25-60%); '3' – severe (leaf loss >60<100%); '4' – dead (100% leaf loss).

The general health status in different PSPs was measured as a weighted arithmetic mean obtained by the formula:

Weighted arithmetic mean =
$$\frac{\sum 0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4}{\sum n_0 + n_1 + n_2 + n_3 + n_4}$$

where: $0, 1, \dots 4$ – defoliation class; $n0, n1 \dots n4$ – number of trees in the relevant defoliation class.

Annual results, obtained by the monitoring, are stored in a relational database in Microsoft Access organized into tables including defoliation and damage cause data that are processed statistically by MS Excel 2013 and One-way ANOVA of Statistica 12.

RESULTS

All assessed *Quercus cerris* PSPs were situated in the low-laying forest vegetation zone, with a significant variety of altitude - 59 m a.s.l. (Ravna gora) to 620 m a.s.l. (Varbitsa pass) (Table 1). Only PSP Varbitsa pass is situated on a sloped terrain. All others are on a horizontal plane that is important condition for good water supply, associated with the soil drainage as a natural process by which water moves across, through and out of the soil as a result of the force of gravity. All investigated stands had a high degree of canopy cover – up to 0.7-0.8. Dominant number of sample trees were coppice-originated forest, aged from 54 to 80.

Trends in defoliation rate was calculated on the base of weighted arithmetic mean. During the years, the weighted arithmetic mean defoliation for all stands varied between 0.45 and 1.73 (Table 2). There has been no significant trend of defoliation increase due to biotic or abiotic factors and stands were in relatively good condition. It was established that *Q. cerris* stands were in relatively good condition for the entire studied period (Fig. 1). For the first time, decline of single trees was observed in the initial stage of the period (3.3% in 2012), under consideration, that it is normal natural falling of mature trees. In next years, a persistent tendency for deterioration of tree condition was recorded, and at the end of the studied period, the damaged and dead trees exceeded the number of healthy ones (weighted arithmetic mean -0.88 to 1.28.

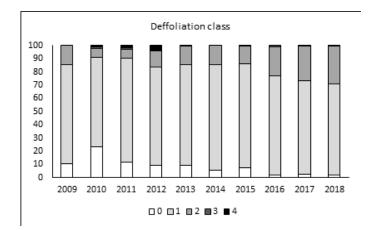


Fig. 1. Change in Quercus cerris health status in Eastern Balkan range and the Ludogorie in the period 2009-2018

According to the defined value of weighted arithmetic mean for ten-year period, it was established that PSP Forest nursery (Aitos) was in the worst health status with a value between 0.95 (2010) and 1.48 (2018). PSP Varbitsa pass showed the best health condition with a value of weighted arithmetic mean 0.45 (2012) to 1.27 (2018). In that sample plot there were no trees of 2nd-3rd degree until 2014, and the last years that were 18.2%. The trend was similar in the other above commented PSP Forest nursery: 2nd-3rd degree trees were 12.5% at the beginning to reach 47.5% over the past year (Table 2). In all PSPs the value of weighted arithmetic mean increased, with a significant rise from 0.45 to 1.27 in PSP Varbitsa pass that is a stand with the oldest trees and from 0.65 to 1.50 in PSP Podvis. Insignificant fluctuations were noticed in PSP Sveshtari with the youngest (54-year old) trees.

In the PSP Devnja in 2015 all trees were cut (illegal logging), so there are no data for this year (Table 2). Illegal felling of trees is still a huge problem in the studied PSPs. In 2015, all assessed trees were completely cut down in PSP Devnja, in Sveshtari 2.5-27.5% of the trees were cut for the period 2011-2018, and in Avren - 40% in 2013.

Site		Parameters	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
	Weighted arithmetic mean		0.64	0.55	0.55	0.45	0.55	0.55	1.09	1.18	1.00	1.27
Varbitsa pass	2	0+1	100	100	100	100	100	100	81.8	81.8	81.8	81.8
arbit nass	*%	2+3	0.0	0.0	0.0	0.0	0.0	0.0	18.2	18.2	18.2	18.2
>	0	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Weightee	l arithmetic mean	0.68	0.65	0.78	0.90	0.95	1.05	1.35	1.48	1.50	1.43
vis		0+1	100	100	100	100	95.0	95.0	65.0	52.5	50.0	57.5
Podvis	%	2+3	0.0	0.0	0.0	0.0	5.0	5.0	35.0	47.5	50.0	42.5
I	•	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Weighted arithmetic mean		1.08	0.95	1.03	1.20	1.33	1.23	1.25	1.45	1.35	1.48
est	3	0+1	82.5	87.5	87.5	77.5	67.5	77.5	72.5	57.5	65.0	52.5
Forest	%	2+3	17.5	12.5	12.5	22.5	32.5	22.5	27.5	42.5	35.0	47.5
		4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Weighted arithmetic mean		1.20	0.75	0.95	0.78	0.83	1.05	0.93	1.25	1.23	1.25
na		0+1	0	97.5	97.5	97.5	92.5	90.0	97.5	75.0	77.5	75.0
Ravna oora	%	2+3	20.0	2.5	2.5	0.0	7.5	10.0	2.5	25.0	22.5	25.0
I	-	4	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0
	Weightee	l arithmetic mean	1.25	0.85	0.98	1.25	1.08	1.13	0.95	1.13	1.4	1.33
en		0+1	72.5	95.0	95.0	77.5	91.7	87.5	97.5	87.5	60.0	67.5
Avren	%	2+3	27.5	5.0	5.0	22.5	8.3	12.5	2.5	12.5	40.0	32.5
'		4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Weighted arithmetic mean		1.10	1.28	1.35	1.73	1.13	0.81	-	0.98	0.98	0.95
nja		0+1	82.5	72.5	77.5	65.0	83.4	93.7	-	95.0	97.5	97.5
Devnja	%	2+3	17.5	22.5	15.0	17.5	16.6	6.3	-	5.0	2.5	2.5
		4	0.0	5.0	7.5	0.0	0.0	0.0	-	0.0	0.0	0.0
·=	Weighted arithmetic mean		1.08	0.90	1.24	1.19	1.20	1.28	0.85	1.00	1.03	1.24
ıtar		0+1	90.0	92.5	81.8	80.6	80.0	70.0	100	100	93.1	73.5
Sveshtari	%	2+3	10.0	7.5	15.2	19.4	20.0	30.0	0.0	0.0	6.9	26.5
Ś	*01	4	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 2. Health status of Quercus cerris trees in PSPs during the period 2009-2018

*Share of trees by damage degrees, %

The results of the 10-year monitoring data show that fungal pathogens caused the most significant impact on the process of drying (Fig. 2). The number of trees, affected by pathogens was nine times more than damages, caused by insects and abiotic factors. From the abiotic factors, the most significant were the frost cracks in the result of low winter temperatures.

Currently, it was indicated that insect pests had a limited influence on the drying process during the studied period, but they are the major potential threat for *Q. cerris* stands. These pests have a 7-8 year gradation and can defoliate forests significantly during their calamity period, thus causing the most severe damages.

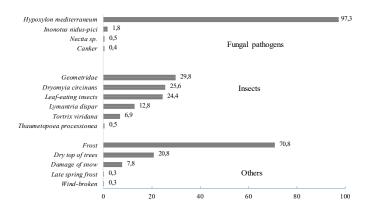


Fig. 2. Relative share of damage causes

The main groups of causal agents on *Q. cerris* trees were fungal pathogens followed by insects, and abiotic factors (Table 3). Fungal pathogens were the predominant cause of damages and responsible for up to 52.5% of all recorded damage symptoms. Damages caused by insect and abiotic agents were the second major group responsible in 2018 for 33.9% of all damage symptoms. Within this agent group, defoliators were the most common damaging agents, and among abiotic agents – more symptoms were attributed to frost cracks, snow and drought.

	Number of	Health status distribution, %						
Year	Trees	Health	Damages of fungal	Damages of insects				
	11000	trees pathogens		and abiotic factors				
2009	251	58.2	19.5	22.3				
2010	251	49.8	45.4	4.8				
2011	244	40.2	53.3	6.5				
2012	242	38.8	52.5	8.7				
2013	225	47.6	46.2	6.2				
2014	227	47.2	45.8	7.0				
2015	210	41.4	49.1	9.5				
2016	242	45.5	43.8	10.7				
2017	240	32.5	42.1	25.4				
2018	245	24.5	41.6	33.9				

Table 3. Distribution of trees by health status

Among the complex of pathogens, the influence of fungal pathogen *Hypoxylon mediterraneum* (De Not.) J.H. Mill. was the most considerable on studied sample trees (Fig. 2). The pathogen was established in 85.0% of PSPs, and in some of them almost all of the trees were affected with the most serious consequences for their health status.

Low level of precipitation were evidenced in the studied regions during the 2000-2001 and 2013-2014 periods, that greatly increased the susceptibility of Q. *cerris* trees to the stress-induced fungus *H. mediterraneum*. Symptoms of infected trees included crown dieback, yellowing of leaves, bark cankers, abundant tannin exudation, reduced growth and tree mortality. Damages of *H. mediterraneum* were observed most frequently in PSP Ravna gora (between 42.5% in 2009 to 92.5% in 2018). For the entire studied period, the lowest number of infected trees (up to 20%) was found in PSP Forest nursery.

Besides the high virulent capacity of H. mediterraneum, the economic significance on the infected stands was also determined by the pathogenesis of the disease that most often ends with the dead of diseased trees. The disease was not found only in PSP Varbitsa pass (Table 4). In PSP Ravna gora almost all trees were severely attacked. In PSPs Avren and Sveshtari the health status was getting worse in 2009 and in two years respectively 82.5% and 77.4% of the trees were damaged by the pathogen. The reduction in the number of affected trees in PSP Sveshtari in next years is due to the requirement of the European methodology of ICP Forests. The cut sample trees had to be replaced by new ones.

		Percentage of damaged trees								
Years	Podvis	Forest nursery	Ravna gora	Avren	Devnja	Sveshtari				
2009	-	17.5	42.5	-	15.0	45.0				
2010	17.5	17.5	72.5	60.0	37.5	75.0				
2011	22.5	20.0	80.0	82.5	50.0	78.8				
2012	22.5	20.0	80.0	82.5	45.0	77.4				
2013	22.5	20.0	75.0	35.0	36.7	60.0				
2014	27.5	20.0	90.0	45.0	31.2	60.0				
2015	27.5	20.0	92.5	50.0	-	61.5				
2016	30.0	20.0	92.5	55.0	15.0	61.3				
2017	30.0	20.0	92.5	57.5	15.0	41.4				
2018	30.0	20.0	92.5	67.5	15.0	23.5				

 Table 4. Percentage of trees damaged by Hypoxylon mediterraneum in permanent sample plots (2009-2018)

Out of the insect pests, *Dryomyia circinans* (Giraud, 1861) (Diptera: Cecidomyiidae) and species of Geometridae were the most numerous (Fig. 2). *Dryomyia circinans* was established in four PSPs (Ravna gora, Sveshtari, Podvis and Avren), causing damages on all studied sample trees, worsening the aesthetic qualities of the infested trees and causing decrease of growth and physiological weakness. Damages, caused by Geometridae were established only in PSP Podvis.

Lymantria dispar (Linnaeus, 1758) (Lepidoptera: Erebidae) is the third main causal agent among the established insect pests. During the last decade, the attacks of the species were at low level due to the successful introduction of the highly effective species specific pathogen *Entomophaga maimaiga* Humber, Shimazu &

Soper (Entomophthorales: Entomophthoraceae). After the introduction, only 23 000 and 88 000 ha in the country was infested by the pest for the period 2001-2009 and 2010-2017, respectively (Georgiev, 2018).

DISCUSSION

The trends in deterioration of *Quercus cerris* in the region of the Eastern Balkan range and the Ludogorie started in 1990s (Rossnev *et al.*, 2006a), and this process is most pronounced in poor habitats and droughts. Of the biotic causes, the most serious factor is the fungal pathogen *Hypoxylon mediterraneum*.

During the complex research on the health status of Q. cerris stands, conducted in the period 2003-2004 (Rossnev et al 2006b), it was concluded that stands distributed in North-east Bulgaria was in the worst conditions. As a major biotic cause, the pathogen H. mediterraneum was established. The authors found that trees with seed origin and young coppiced forests were less affected than the mature coppice trees. These conclusions are fully covered by the findings of the present study that the most severe damages were caused by *H. mediterraneum* and cracks caused by the frost among the abiotic factors.

Besides the significant role of droughts that determine the drying process, it was also found that the management of stands – late period of cutting, leaded to an increase in their age and is an essential reason for the worsening of their growth and vitality. The findings in both studies on the role of insects are similar - they have a limited influence on the drying process, but are considered as a major potential hazard in their calamity development. Combining damages caused by the severe droughts and the extend rate of defoliated stands, could increase the drying process that would spread in large areas.

The most severe damages could be caused by *Lymantria dispar* that has the potential to defoliate significant oak stands during its calamity period. In last 60 years, the pest had six completed gradations: 1953-1960, 1961-1969, 1970-1977, 1978-1988, 1989-2000, and 2001-2009. In some years, the attacked areas reached between 492 and 1028 thousand ha (Georgiev *et al.*, 2011). These attacks have significant environmental effect as reducing the recreational functions of forests; social and economic impacts by decreasing the growth of attacked trees and increasing the direct costs for using chemical insecticides to reduce the pest spreading. For example, 152 665.3 ha were treated against L. dispar in 1998, and 50.3% of these areas were situated in North-eastern Bulgaria (Mirchev *et al.*, 2003).

Gall midges (*Dryomyia circinans*) causes no serious problems on *Q. cerris*, only physiological weakness of infested trees. Out of the complex factors deteriorating the health status of monitored stands, the attacks of *Geometridae* mixed with Tortricidae had consequences that are more serious. These pests are the second largest and significant group, affecting deciduous forests in Bulgaria after gipsy moth (*L. dispar*) (Mirchev *et al.*, 2003). 162

Among the established fungal pathogens, the disease caused by Hypoxylon mediterraneum had the most serious consequences for *Q. cerris* health condition. The fungus attacks oak species in the Mediterranean countries, Bulgaria and Hungary (Rossnev *et al.*, 2006b, 2007). It infects most often physiologically weakened trees aged 10 to 40 years (Vannini *et al.*, 1996). During the observation of disease damages, it should be taken into consideration that under favourable climatic conditions and sufficient precipitation, necrosis caused by *H. mediterraneum* get callusing, the wet spots disappear and the symptoms of this disease become more difficult to be recognized. Because of this fact, the annual survey could reduce the degree of damages in the monitored stands.

Current data show that the process of Turkey oak decline in the period 2009-2018 has abated. This trend is characterized by mild fluctuations. Rossnev et al. (2006b) indicated a slight increase of the percentage of drying trees in 2003-2004 in the three climatic zones of the region. In the eastern climate region of the middle plateaus (State Forest Tervel) with a low rate of drying trees - up to 7.5%. In the western climatic region of the middle high plateaus (PSP Isperih) the average rate of drying trees was 9.2%, and in the coastal climatic region that includes PSPs Avren and Devnya the number of drying trees reached 5%. They also reported the mosaic structural features of dried stands, that in some of them the number of damaged trees reached 55%.

The results of the current study explores the complicated interactions between abiotic and biotic factors and their impact on the health status of Q. *cerris* stands in the region of Eastern Balkan range and the Ludogorie. In some stands, compensatory mechanisms were manifested, helping damaged trees to overcome the stress. In PSP Varbitsa pass the oldest trees were in the best health status, although growing on a slopped terrain that would negatively affect the soil moisture. These stand features probably are completely compensated by the highest altitude of the PSP that supply significantly more favourable growth conditions.

In all monitored PSPs, the number of recorded damage symptoms per assessed trees was more than one. Therefore the number of cases analysed varied depending on the number of causes. Multiple effect on studied sample trees was assessed. Fungal pathogens, insect and abiotic causes were the most common damage agent group comprising altogether more than 75% of all assessed trees.

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DECLARATION OF INTEREST

The authors declare no existing conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

M. Georgieva - designed and performed the experiments, collected and analysed the data, wrote the manuscript;

G. Georgiev - designed and performed the experiments, collected and analysed the data, wrote the manuscript;

P. Petkov - designed and performed the experiments, collected data;

D. Nedjalkov - analysed the data;

M. Matova - analysed the data;

G. Zaemdzhikova - collected and analysed the data, wrote the manuscript;

P. Mirchev - designed and performed the experiments, collected and analysed the data, wrote the manuscript;

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