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INCIDENCE AND SEVERITY OF SYMPTOMS ASSIGNED TO *FRAXINUS EXCELSIOR* BACTERIAL DISEASE IN THE LEFT-BANK FOREST STEPPE OF UKRAINE

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Abstract

The aim of the research was to estimate the spread of bacterial disease on European ash (*Fraxinus excelsior* L.), ocularly estimated by lens-shaped blisters, swellings and cracs of the bark in Pridonetsky and Vorskla-Pselsky forest typological sector of the Left-bank Forest-Steppe zone of Ukraine. Studies were carried out in the year 2018 at 112 sample plots in Trostyanetske Forest Enterprise of Sumy region (Vorskla-Pselsky forest typological sector), Chugujevo-Babchanske and Skrypayivske Forest Enterprises of Kharkiv region (Pridonetsky forest typological sector). In spite of the fact that both the incidence and severity of ash bacterial disease symptoms are significantly higher for particular forestries and pooled data from Sumy region, the health condition index (HCI) of ash trees is greater for forestries of Kharkiv region. Therefore, ash stands in the inspected forestries of Sumy region are weakened, and in those of Kharkiv region are severely weakened. Ash tree mortality was rather low in them. The highest ash mortality was registered in Krasnyanske forestry (12.1 %) of Trostyanetsky forest enterprise. No significant correlation was proved between parameters of ash bacterial disease and altitude. Significant correlation was proved between the incidence and severity of ash bacterial disease symptoms, health condition index and mortality, diameter at breast height (DBH) and disease severity. However, only the correlation of incidence and severity of ash bacterial disease symptoms is high for Kharkiv region (r_p=0.74, r_s=0.95) and moderate (r_p=0.54) or high (r_s=0.73) for Sumy region. Obtained distinction in ash disease incidence and severity in different forest typological sectors data may be explained by climate features. Higher precipitation and moisture indices in Vorskla-Pselsky forest typological sector is favorable for ash bacterial disease. However, higher temperature together with lower humidity in Pridonetsky forest typological sector are unfavorable to ash and increases its susceptibility to other damage causes, particularly frost and insects.

Key words: bacterial canker, European ash, health condition, mortality.

Introduction

Health condition of European ash (*Fraxinus excelsior* L.) in Europe have been worsening since recent years (Kowalski 2006, Goberville et al. 2016), which is connected with climate change, unfavorable for forest trees (Shvidenko et al. 2017), and with a spread of injurious organisms. Many causes of European ash decline have been observed, particularly insect pests (Davydenko and Meshkova 2017, Meshkova and Borysova 2017) and pathogens (Goychuk and Kulbanska 2014, Matsiakh and Kramarets 2014, Langer 2017), including invasive fungus *Hymenoscyphus fraxineus* (Kowalski 2006, Pautasso et al. 2013, McKinney et al. 2014), which is present also in Ukraine (Davydenko et al. 2013).

Bacterial disease of European ash, or bacterial canker, or so-called 'tuberculosis' of ash reveals clearly visible symptoms so called 'Eschenrindenrosen' = ash bark roses (Tubeuf 1936). These are smooth layers of suberized tissue surrounding a wound penetrating to the wood. The causal agent of these disease symptoms is the bacterium *Pseudomonas syringae* subsp. *savastanoi* pv. *fraxini* (Kulbanska 2015a). The disease is spread in the natural range of European ash, including the most part of Europe (Janse 1981a, 1982), particularly Ukraine (Goychuk and Kulbanska 2014, Kulbanska 2015a). According to many researchers, infection of trees, occurs through bark wounds and cracks, damage by frost, hail, game, and human activity (Janse 1981b, Kulbanska 2015b).

The data on the disease and its correlation with certain climatic or forest conditions are fragmentary (Kulbanska 2015a, Meshkova et al. 2018). Studies are usually focused on the pathogen itself and the peculiarities of pathological changes in tree plants under the influence of pathogen or on the resistance of different *Fraxinus* species to infection (Kulbanska 2014).

However, comparative assessment of spread and development of ash bacterial disease in different regions has not been carried out yet.

The aim of the presented study was to estimate the spread of bacterial disease of European ash in Pridonetsky and Vorskla-Pselsky forest typological sector of the Left-bank Forest-Steppe zone of Ukraine.

Materials and Methods

Study was carried out in 2018 in two groups of forest stands in the fresh ash and lime oak stands of the Left-bank Forest-Steppe zone of Ukraine. The territory of Trostyanetske Forest Enterprise belongs to Vorskla-Pselsky forest typological sector, and the territory of Chugujevo-Babchanske Forest Enterprise and Skrypayivske Training and Experimental Forest Enterprise belongs to Pridonetsky forest typological sector (Ostapenko and Vorobyev 2014, Nazarenko and Pasternak 2016).

The parameters by which the territory of the certain forest enterprise belongs to a particular forest-typological sector were evaluated from long-term data (2005–2018) of air temperature and precipitation for two meteorological stations: Zmiyiv (49°41” N, 36°21” E) in Kharkiv region and Trostyanets (50°48” N, 34°96” E) in Sumy region (rp5.ru 2019).

The following climatic parameters were calculated:

T-index (T) is the sum of the average monthly air temperatures over the months with temperatures above 0 °C. Moisture index (W) according to Vorobyev (Ostapenko and Vorobyev 2014) is evaluated by formula (1):

$$W = (R/T) - 0.0286 \times T, \qquad (1)$$

where: R is precipitation for months with positive air temperature.

Continentality index A is the difference between mean monthly temperature of January and July (Ostapenko and Vorobyev 2014). Additionally, Selyaninov hydrothermal index (HTI, Selyaninov 1937) was calculated for 2005–2018 by the formula (2):

$$HTI = 10 \times \frac{\sum P}{\sum t}, \qquad (2)$$

where: ΣP is precipitation for a period with mean month air temperature over 10 °C, mm; Σt is the sum of daily air temperature for the same period, °C (Table 1).

Table 1. Climatic parameters for investigated group of points (2005–2018).

Meteorological station	*T*, °C	*R*, mm	*W*	*A*, °C	HTI mm/°C
Zmiyiv (49°41” N, 36°21” E), Kharkiv region	105.9	383	0.59	27.5	0.97
Trostyanets (50°48” N, 34°96” E), Sumy region	103.0	421	1.14	27.0	1.10

Survey of stands partially timbered with European ash was carried out in four forestries (Neskuchanske, Lytovske, Makivske and Krasnyanske forestries) of Trostyanetske Forest Enterprise of Sumy region and in two forest enterprises of Kharkiv region: Chugujevo-Babchanske Forest Enterprise (Kochetokske forestry) and Skrypayivske Training and Experimental Forest Enterprise (Skrypayivske and Mokhnachanske forestries) (Table 2). Sample plots were located along the profiles, directed perpendicular to the river terraces (Nazarenko and Pasternak 2016). The altitude of sample plots location was 143–182 m a.s.l. in Sumy region and 107–184 m a.s.l. in Kharkiv region (see Table 2).

Table 2. Location of inspected forest stands.

Forest Enterprises	Forestry	Latitude, N	Longitude, E	Altitude, m a.s.l.	Number of plots	Number of ash trees
Sumy region (Vorskla-Pselsky forest typological sector)						
Trostyanetske	Krasnyanske	50°48"	34°77"	182	19	513
Trostyanetske	Lytovske	50°36"	34°86"	143	26	409
Trostyanetske	Makivske	50°53"	34°97"	165	8	34
Trostyanetske	Neskuchanske	50°46"	34°90"	171	20	573
Kharkiv region (Pridonetsky forest typological sector)						
Chugujevo-Babchanske	Kochetokske	49°52"	36°44"	151	10	433
Skrypayivske	Skrypayivske	49°70"	36°53"	107	12	490
Skrypayivske	Mokhnachanske	49°74"	36°51"	184	28	690
Total	–	–	–	–	112	3142

Broadleaved tree species (*Quercus robur* L., *F. excelsior* L., *Betula pendula* Roth, *Alnus glutinosa* (L.) Gaertn, *Acer platanoides* L., *Ulmus laevis* Pall, etc.) dominated in both groups of stands.

Survey covered 3142 trees of European ash (see Table 2) at the age of 5–110 with 0.6–0.8 relative density of stocking.

2–4 subplots of 10×10 m were inspected in each forest plot. The number of plots depended on ash proportion in the stand. The aim was to assess at least 25 ash trees per plot, if possible. Only in inspected stands of Makivske forestry were rather few ash trees.

Stand age was not homogenous in most of plots, because ashes of vegetative origin and seed specimens of different age were presented. Therefore, we considered the diameter at breast height (DBH) as more reliable parameter.

Besides DBH several parameters of ash health condition were additionally assessed in each sample plot.

The incidence of bacterial disease related to *Pseudomonas syringae* subsp. *savastanoi* pv. *fraxini* was estimated as proportion of living ash trees with characteristic symptoms (lens-shaped blisters and swellings as well as vertical and lateral cracks, Fig. 1) within the sample plot. Then the average value was calculated for each forestry.

Fig. 1. Lens-shaped blisters and swellings on the ash bark attributive to bacterial disease of ash.

Severity of bacterial disease for each ash tree was estimated by score: 0 – absence of lens-shaped blisters and swellings; 1 – sporadic lens-shaped blisters and swellings as well as vertical and lateral cracks; 2 – multiple lens-shaped blisters and swellings with numerous vertical and lateral cracks (Janse 1981b). Severity of bacterial disease for each sample plot was evaluated as an arithmetic average of all trees scores.

Tree mortality was expressed as a percentage of dead ash trees out of the total ash trees per plot.

Category of tree health condition was evaluated on a range of visual characteristics (crown density and color, the presence and proportion of dead branches in the crown, etc.) according to ‘Sanitary rules in the forests of Ukraine’ (Anonymous 1995). Each tree was referred to one of six categories of health condition (1st – healthy; 2nd – weakened; 3rd – severely weakened; 4th – drying up; 5th – recently died; 6th – died over year ago). Health condition index (HCI) for each plot was calculated as mean of the health condition categories of studied trees.

According to ‘Sanitary rules in the forests of Ukraine’ (Anonymous 1995) the stands with HCI 1.5–2.4 are considered as weakened, with HCI 2.5–3.4 as severely weakened, with HCI 3.5–4.4 as drying up and with HCI above 4.5.

The data for all inspected plots were pooled for each forestry, and then for each region for analysis.

Normality tests, summary statistics, one-way analysis of variance (ANOVA), Tukey HSD test with a significance level of $p<0.05$ were performed. Shapiro-Wilk test has proved the normality of analyzed parameters (ash bacterial disease incidence, severity, health condition index and mortality) when pooling them together for all plots in every forest typological region. At the same time normality was not proved when considering data separately for diameter and health condition index of particular forestries. Therefore, we calculated correlations between analyzed parameters by parametric and non-parametric methods (Pearson's and Spearman's correlation). Microsoft Excel software and statistical software package PAST: Paleontological Statistics Software Package for Education and Data Analysis (Hammer et al. 2001) were used.

Results and Discussion

The data of Table 1 show that the temperature parameter T-index is a bit higher in Zmiyiv (Pridonetsky forest typological sector), and precipitation for vegetation period is higher in Trostyanets (Vorskla-Pselsky forest typological sector). Both *W* and HTI are higher in the neighborhood of Trostyanets meteorological station, than in the neighbourhood of Zmiyiv meteorological station. Such climate features agree with the location of inspected regions. Investigated plots in Vorskla-Pselsky forest typological sector are characterized by greater latitude and lower longitude (Table 2). Therefore, it is characterized by lower temperature and more precipitation (Table 1).

Proportion of ash trees per plot was the lowest in Makikvske forestry (10.6 % in average), respectively about 50 % in other inspected forestries of Sumy region and 30–40 % in inspected forestries of Kharkiv region (Table 3). At the same time, in separate plots of each forestry the proportion of ash trees varied within a rather wide range, for example from 3.1 to 81.8 % in Lytovske forestry.

Table 3. Ash trees parameters in inspected forestries*.

Forestry	Proportion of ash trees per plot, % **±SE	DBH, cm ±SE **
Sumy region (Vorskla-Pselsky forest typological sector)		
Krasnyanske	54.4±3.17a (32.1–80.0)	35.8±2.19a (13–54)
Lytovske	46.0±6.01a (3.1–81.8)	34.5±2.77a (3–67)
Makivske	10.6±3.73b (2.0–29.4)	7.6±1.01b (4–13)
Neskuchanske	51.2±3.19a (27.4–83.7)	34.5±2.02a (16–53)
Kharkiv region (Pridonetsky forest typological sector)		
Kochetokske	40±3.3d (20–50)	29,0±1.5c (23–39)
Skrypayivske	39.2±4,17d (10–50)	27,8±3,62c (10,2–43,8)
Mokhnachanske	31.8±3.2d (10–70)	23.1±1.85c (5.8–52.6)

Note: *The data for all inspected plots were pooled for each forestry. **Means followed by the same letter are not significant within column at the 95 % confidence level. Figures in brackets show the minimal and maximal value.

Average DBH of ash trees (and age, respectively) was the lowest in Makivske forestry (7.6 cm) and almost the same (34.5–35.8 cm) in the rest inspected forestries of Sumy region, but significantly lower (23.1–29 cm) in inspected forestries of Kharkiv region (Table 3).

Bacterial disease of ash was revealed in all inspected forestries, except Makivske forestry, and varied within each forestry (Table 4). So, in some areas of Krasnyansky and Lytovske forestries, disease incidence ranged from 0 to 100 %, however, the average values differed more than three times (9.3 and 30.1 %, respectively).

Table 4. Health condition parameters.

Forestry	**Disease incidence, %** ±SE	**Disease severity score ±SE**	**Health condition index ±SE**	**Tree mortality ±SE**
Sumy region (Vorskla-Pselsky forest typological sector)				
Krasnyanske	9.3±5.14 b (0–100)	0.4±0.07 b (0–0.78)	1.9±0.10 a (1.3–2.9)	12.1±2.29 (3.6–19.4)
Lytovske	30.1±6.61 a (0–100)	0.8±0.14 a (0–1.63)	2.2±0.12 a (1.2–3.0)	14.3***
Makivske	0,0 c	0.0 c	1.8±0.36 a (1.0–3.0)	0.0
Neskuchanske	18.6±3.82 ab (0–50)	0.7±0.13 a (0–1.66)	1.9±0.08 a (1.2–2.5)	2.4***
Kharkiv region (Pridonetsky forest typological sector)				
Kochetokske	10.7±1.53 d (1.5–17.5)	0.11±0.017 d (0.01–0.2)	2.7±0.07 b (2.4–3.0)	3.1±0.81 d (0–7,5)
Skrypayivske	8.4±1.58 de (0–15.9)	0.11±0.024 d (0–0.24)	2.6±0.04 b (2.4–2.8)	2.3***
Mokhnachanske	5.0±0.95 e (0–16.1)	0.06±0.011 e (0–0.16)	2.6±0.07 b (2.1–3.3)	2.1±0.95 d (0–22.9)

Note: *Means followed by different letters in each column are significantly different at the 95 % confidence level. **Figures in brackets show minimum and maximum of parameter in each forestry. ***Tree mortality observed only in one plot.

Analysis of pooled data for Sumy and Kharkiv regions show that both average ash tree diameter (31.9 and 25.5 cm) and bacterial disease incidence (16.1 and 7.0 %) are significantly higher in Sumy region (Fig. 2).

Test for equal means proves such significance: F=4.89, p=0.03 for diameter and F=7.75, p=0.04 for bacterial disease incidence.

Bacterial disease severity score was rather low in most of Sumy (0.4–0.8 points) and Kharkiv plots (0.06–0.11 points) (Table 4).

Analysis of pooled data for Sumy and Kharkiv regions (0.64 and 0.08 point respectively) show that average severity score is significantly higher in Sumy region (F=55.4, p<0.001) (Fig. 3).

In spite of the fact that both, the incidence and severity of bacterial disease, are greater for particular forestries and pooled data from Sumy region, the HCI of ash trees is greater for forestries of Kharkiv region (Table 4).

For plots from Sumy region, the value of HCI is the least (1.8) for inspected plots in Makivske forestry with an absence of bacterial disease of ash. The value of health condition index is 1.9 for inspected plots in Krasnyanske and Neskuchanske forestries with disease incidence 9.3 and 18.6 % and is the highest for inspected plots in Lytovske forestry with maximal disease incidence (30.1 %). Significant difference of health condition index of

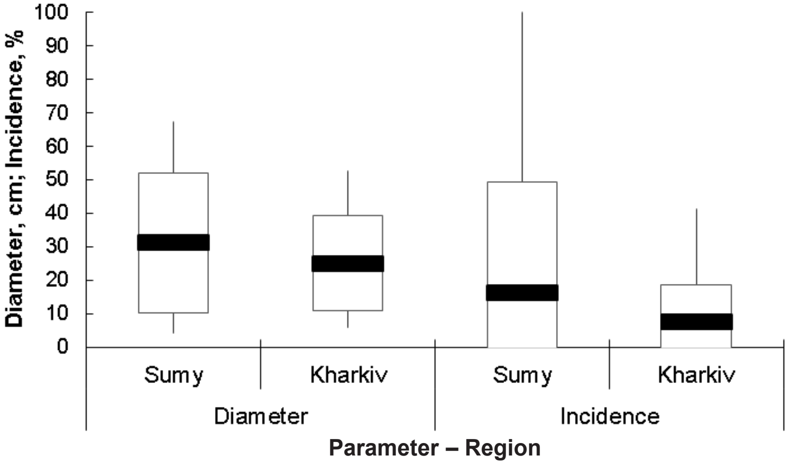

Fig. 2. Incidence of ash bacterial disease and mean tree diameter pooled by regions.

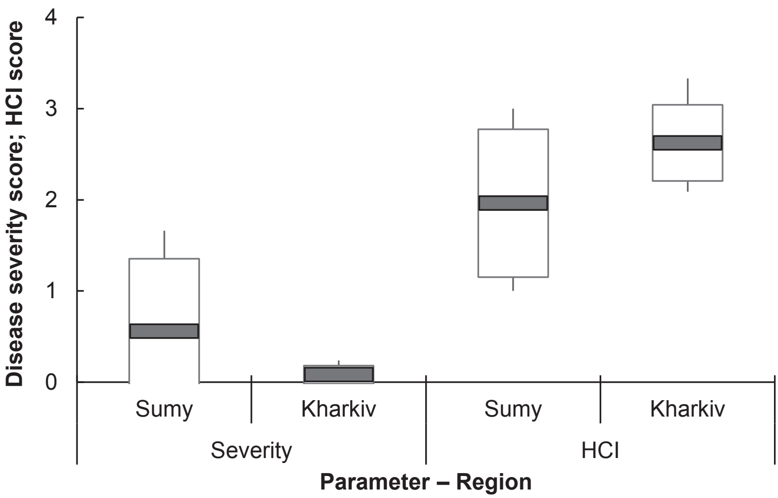

Fig. 3. Severity of ash bacterial disease in inspected forestries and HCI pooled by regions.

stands was proved between forestries of Sumy and Kharkiv region. For pooled data HCI is 2 and 2.6 points for Sumy and Kharkiv region respectively (F=73.4, p<0.001).

Therefore, according to 'Sanitary rules in the forests of Ukraine' (Anonymous 1995) ash stands in the inspected forestries of Sumy region are weakened, and in the inspected forestries of Kharkiv region are severely weakened.

Ash tree mortality was rather rare among inspected plots (Table 4). No mortality of ash was observed at all in inspected plots of Makivske forestry and only on one of the plots in Lytovske Neskuchanske and Skrypayivske forestries. The highest ash mortality was registered in Krasnyanske forestry (12.1 %) of Trostyanetsky forest enterprise.

No significant correlation was proved between parameters of ash bacterial disease and altitude (p>0.1). Significant correlation using both Pearson's and Spearman's tests was proved between bacterial disease incidence and severity, HCI and mortality, as well as DBH and disease severity (Table 5). However, only the correlation between bacterial disease incidence and severity is high for Kharkiv region (r_p=0.74, r_s=0.95) and moderate (r_p=0.54) or high (r_s=0.73) for Sumy region. The other correlation index values are slight or moderate.

Table 5. Correlation between diameter, health condition, mortality of ash trees, and bacterial disease incidence and severity.

Regions	DBH – Inc	DBH – Sev	DBH – HCI	DBH – Mort	Inc – Sev	Inc – HCI	Inc – Mort	Sev – HCI	Sev – Mort	HCI – Mort
					Pearson r (p in brackets)					
Kharkiv	0.29 (0.04)	**0.39*** (0.01)	0.09 (0.54)	-0.15 (0.30)	**0.74*** (<0.01)	**0.29*** (0.05)	0.17 (0.26)	0.17 (0.24)	0.20 (0.17)	**0.53*** (<0.01)
Sumy	0.06 (0.63)	**0.31*** (0.01)	-0.13 (0.31)	-0.04 (0.76)	**0.54*** (<0.01)	**0.34*** (0.01)	-0.14 (0.29)	0.24 (0.06)	-0.17 (0.19)	**0.28*** (0.03)
					Spearman's r (p in brackets)					
Kharkiv	**0.35*** (0.01)	**0.33*** (0.02)	0.05 (0.72)	-0.12 (0.43)	**0.95*** (<0.01)	0.20 (0.18)	0.23 (0.12)	0.16 (0.28)	0.21 (0.15)	**0.44*** (<0.01)
Sumy	0.19 (0.14)	**0.28*** (0.03)	-0.21 (0.11)	-0.03 (0.79)	**0.73*** (<0.01)	**0.40** (<0.01)	-0.05 (0.70)	0.18 (0.17)	-0.12 (0.33)	**0.25*** (0.05)

Note: Inc – disease incidence; Sev – disease severity; Mort – ash tree mortality; significant correlations (p<0.05) are marked with an asterisk.

Slight correlation between bacterial disease incidence and DBH was proved only for Kharkiv region using Spearman's test (r_s=0.35).

Obtained dissimilarity in ash bacterial disease parameters and health condition in different forest typological sectors data might be explained by climate features (Table 1). Higher precipitation and moisture indices are usually favorable for ash bacterial disease (Kulbanska 2015a). Therefore, this disease was spread and developed in greater degree in Vorskla-Pselsky forest typological sector (Sumy region). At the same time, the water shortage is unfavorable to ash growth and increases its susceptibility to other damage causes, particularly frost, mechanical damage, wood decay fungi and insects (Meshkova and Borysova 2017). Therefore, ash health condition was worse in Pridonetsky forest typological sector (Kharkiv region), characterized by higher temperature together with lower humidity during the vegetation period (Table 1).

The findings suggest that bacterial disease incidence and severity depend on many factors that should be considered in further analysis. Among them, heterogeneity of forest stands by origin, age, ash participation, the relative density of stocking, location in the forest, particularly the distance from roads, clear-cuts as well as additive influence of other damaging agents are of the highest importance.

Conclusions

Research of the spread of bacterial disease of European ash (*Fraxinus excelsior*) in Pridonetsky and Vorskla-Pselsky forest typological sector of the Left-bank Forest-Steppe zone of Ukraine brings to following conclusions:

1. Ash bacterial disease incidence is 16.1 and 7.7 %, and its severity 0.6 and 0.1 points for Sumy and Kharkiv region respectively. Both parameters are significantly higher for particular forestries and pooled data from Vorskla-Pselsky forest typological sector (Sumy region), than for Pridonetsky forest typological sector (Kharkiv region).

2. The health condition index (HCI) of ash trees is higher for forestries of Kharkiv region (2 and 2.6 points for Sumy and Kharkiv region respectively). According to these values, ash stands in the inspected forestries of Sumy region are considered as weakened, and in the inspected forestries of Kharkiv region as severely weakened.

3. Ash tree mortality in inspected plots was rather low (1.6 and 1.8 % for Sumy and Kharkiv region respectively).

4. No significant correlation between parameters of ash bacterial disease and altitude has been observed. Significant correlation was proved between ash bacterial disease incidence and severity, health condition index and mortality, DBH and disease severity. However, only the correlation between ash bacterial disease incidence and severity, was high for Kharkiv region (r_p=0.74, r_s=0.95) and moderate (r_p=0.54) or high (r_s=0.73) for Sumy region.

5. More intensive incidence and severity of ash bacterial disease in Vorskla-Pselsky forest typological sector (Sumy region) can be explained by higher moisture indices (*W* – 0.59 and 1.14, HTI – 0.97 and 1.1 for Kharkiv and Sumy region respectively).

6. Higher health condition index and mortality of ash trees in Pridonetsky forest typological sector can be explained by higher temperature together with lower humidity of vegetation period, which is unfavorable to ash and increases its susceptibility to other damage causes, particularly insects.

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