EFFECTS OF BORON FERTILIZATION ON SUNFLOWER WHEN GROWN IN LOW BORON SANDY SOIL

Khalid Dhassi¹*, Saad Drissi², Kacem Makroum¹, Fatimzahra Nasreddine³, Fouad Amlal¹, and Abdelhadi Aït Houssa⁴

¹Faculty of Sciences, Chouaïb Doukkali University, El-Jadida, Morocco
²National Agricultural school of Meknes, Department of Agronomy and Plant Breeding (ENAM), Meknes, Morocco
³Department of Agronomy, Hassan II Institute of Agronomy and Veterinary Sciences (IAV Hassan II), Rabat, Morocco
⁴Agricultural Training and Research Center, Providence Verte company, Morocco Submitted: 2018-11-17 Accepted: 2018-12-22

ABSTRACT

Agronomists evaluate soil boron fertility with the threshold of around 0.5 mg kg⁻¹(hot water extraction). The nonappearance of boron deficiency on sunflower, when grown on low boron sandy soil, was investigated to test the validity of this boron guideline fertility. The soil boron content was around 0.19mg kg⁻¹. Pot experiments were conducted during 2015 season and repeated during 2016 season. Six boron levels were applied to soil: (0, 0.2, 0.5, 1, 5, and 10mg kg⁻¹) in 2015 and (0, 2, 5, 10, 15, and 20mg kg⁻¹) in 2016. Boron was applied as Solubor-C (Na₂B₈O₁₃.4H₂O, 21% of boron). The results revealed that soil boron supply higher than or equal to 1mg kg⁻¹ resulted in visible leaf damage. Also, a significant decrease in kernels weight was recorded at high boron levels (15 and 20 mg kg⁻¹). The kernels boron content did not increase with a successive increase in dose of boron supply. It was sufficient, around 16mg kg⁻¹, for all boron tested amounts. Also, plant content on other nutrients was not affected by boron application. The soil residual boron enrichment was around 0.3 mg kg⁻¹ for each boron application of 2 mg kg⁻¹.

Keywords: Sunflower, Boron, Sandy Soil

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the very sensitive species to boron deficiency stress (Souza et al., 2004). Symptoms of this deficiency appear on sunflower youngest leaves which show a bronze color and a malformation (Blamey et

* Corresponding Author : Email: dhassikhalid@gmail.com al., 1997). Boron deficiency is common in sandy soils (Neto et al., 2009; Souza et al., 2012). It affects the fecundation rate (Chitralekha & Nirmala, 2000). Boron bio-availability evaluation is based on soil and foliar analysis (Alloway, 2008). Using the hot water extraction method, the soil boron content around 0.5 mg kg⁻¹ is reported as a sufficient soil threshold for crop production (Rashid & Ryan, 2008). For plant mineral content, the boron content threshold for sunflower kernels is around 15 mg kg⁻¹. However, this threshold is around 34 mg kg⁻¹ for the last leaf of sunflower at flowering (Blamey et al., 1978).

On the other side, plant boron content interval between boron deficiency and boron excess in plants is so close (Ruiz et al., 2016). Phytotoxicity symptoms in sunflower are described by the apex and leaves damage. They are reported in soils with boron content higher than 1 mg kg⁻¹ (Eaton, 1940).

Taking into account these boron fertility guidelines and its adequacy to interpret several soils, the sandy soil of Loukkos area (Northwestern Morocco) can be considered as boron deficient due to its low native content on this micronutrient (0.19 mg kg⁻¹). However, no boron deficiency symptoms have been noticed on sunflower grown in this soil. This confused response of sunflower may be attributed to a possible plant boron hidden deficiency as reported by Zabiole et al. (2010).

Therefore, the objective of this study was to understand the behavior of sunflower

to boron application on sandy soil for two successive growing seasons. Such a study will help to establish adequate reasoning of boron supply for sunflower grown on a sandy soil located in a Mediterranean area.

MATERIALS AND METHODS Experimental soil

Pot experiment on sunflower was conducted during 2015 and repeated during 2016 growing season. The studied soil was collected from the Loukkos area (34.96 ° N lat., -6.21 ° W long and 60 m alt., North-West Morocco). The soil is sandy (89% sand, 5.85% clay and 4.6% silt) with a boron content around 0.19 mg kg⁻¹ (Hot water extraction). It is not calcareous (0.2% of total CaCO₃) and has a pH of 7.8. The other soil properties are reported in Table 1. The soil was air dried. 30 plastic pots (0.42 m length, 0.25 m width and 0.16 m depth) were filled with 20 kg of soil. A leaching dispositive was installed for each pot to reuse the leachate for irrigation.

Soil properties	
pH (soil: water ratio of 1\5)	7.8
Electrical conductivity (dS m ⁻¹) (soil: water ratio of 1\5)	0.04
Organic matter (%) (Walkley and Black method)	0.74
Total carbonate (%) (Volumetric method)	0.20
Available nutrient content (mg Kg ⁻¹)	
P ₂ O ₅ (Olsen method)	39.5
K ₂ O (Ammonium acetate extraction)	90.5
MgO (Ammonium acetate extraction)	65.5
CaO (Ammonium acetate extraction)	1681
Na ₂ O (Ammonium acetate extraction)	12.5
Manganese (DTPA extraction)	22.7
Iron (DTPA extraction)	22.5
Copper (DTPA extraction)	0.19
Zinc (DTPA extraction)	0.59
Boron (Hot water extraction)	0.19

Table 1. Studied soil characteristics

Crop management and experimental design

Six seeds of sunflower (cv. Leila) were sown in each pot. Only one plant per pot was

kept 7 days after emergence. Sunflower was planted from mid-April to August during 2015 season and from May to August during 2016 season. The minimum and maximum averages temperatures were around 10.5°C and 40.3 °C, respectively, for 2015 season and 19.4°C and 31.9°C, respectively, for 2016 season. The rainfalls were 79 mm and 12 mm, respectively, for 2015 and 2016 seasons. Irrigation was done whenever required.

Six B levels were applied to the soil for each experiment:

-Experiment 1 (2015 season): control (no boron supply), 0.2, 0.5, 1, 5 and 10 mg kg⁻¹ boron were tested. 50% of these levels were applied at sowing and 50% at the miniature floral head. -Experiment 2 (2016 season): control (no boron supply), 2, 5, 10, 15 and 20 mg kg⁻¹. These boron levels were applied at sowing.

For both experiments, Solubor-C $(Na_2B_8O_{13}.4H_2O, 21\% \text{ of boron})$ was used as a source of boron. Each pot received one of the boron tested rates. A completely randomized blocks design with five replications was used.

During the growing seasons (2015 and 2016), sunflower received around 190 kg ha⁻¹ of nitrogen, 105 kg ha⁻¹ of phosphorus (P_2O_5) and 160 kg ha⁻¹ of potassium (K_2O). These major nutrients were applied as ammonitrate, mono ammonium phosphorus and soluble potassium sulfate. Concerning micronutrients, the plants received 1.25 kg ha⁻¹ of copper, 6.4 kg ha⁻¹ of manganese and 4 kg ha⁻¹ of zinc as sulfated forms. During the growing seasons, different fungal diseases were controlled using Azoxystrobin, Mandipropamide, and Mancozeb.

Measurements

In the first growing season (2015), the stomatal conductance was measured at the early flowering stage using a porometer (Leaf Porometer, Model-Sc-1, Decagon Devices). The chlorophyll content index of the young leaves, just before floral button stage, was measured using a chlorophyll meter (CCM-200, Opti-Sciences). Also, the leaf area at harvest was measured using a leaf area meter (Area Meter AM300, ADC BioScientific Ltd).

For both the years, the total aerial dry weight and its allocation into stem, leaves, and kernels were measured at harvest. The kernels yield components (the number of kernels per head, pollination rate using Formula 1 and thousand kernels dry weight) were determined.

Pollination rate= (Number of kernels per head/ Total number of ovules per head)*100 (1)

In order to determine plant nutrient status at harvest for each boron level, the kernels and leaves were mashed separately and mixed. Thereafter, the boron content was determined colorimetrically (UV-Visible, Varian) using the Azomethine H spectrometric method (Capelle, 1961) after dry digestion in a muffle furnace at 520°C and acid digestion (sulfuric acid).

The total nitrogen, potassium and calcium contents were extracted using acid digestion (acetic and sulfuric acids). Then, the concentration reading was done using the continuous flow (Skalar San ++) for nitrogen and the atomic absorption spectrophotometer (Varian AA 240, F.S.) for potassium and calcium.

Copper, Iron, manganese, and zinc were determined using the atomic absorption after chemical digestion (sulfuric, perchloric and nitric acids). At harvest, the oil content of kernels was evaluated by the Soxhlet method (Association of Official Analytical Chemists, 1990) and the protein content was determined using the Formula 2 (Taha at al., 1980):

Kernels protein content (%) = Kernels nitrogen content (%) X 6.25(2)

After harvest, the soil residual boron content was determined colorimetrically (UV-Visible, Varian) using the hot water extraction method (Berger & Truog, 1939). The soil boron enrichment was determined using Formula 3. Soil boron enrichment (mg kg⁻¹) = Soil boron content at harvest (mg kg⁻¹) – Initial soil boron content (mg kg⁻¹)(3)

Statistical analysis

Data of the studied experiments were analyzed by variance and regression analyses ($p \le 0.05$). Also, the difference between boron treatments ($P \le 0.05$ level) was evaluated by the Student–Newman–Keuls test. All analyses were conducted using SPSS software (Version 20).

RESULTS

Boron deficiency and its phytotoxicity symptoms

For both experiments, no symptoms of boron deficiency were noticed in sunflower plants for all boron treatments during all the growing season. Such observation is justified by the absence of a significant difference in the chlorophyll content index of the young leaves, measured just before flower bud stage, between different boron treatments (Table 2). Nevertheless, a mottling of the apex and the leaf margins was observed with boron supply higher than or equal to 1 mg kg⁻¹. These phytotoxicity symptoms become more severe at boron supply over than 5 mg kg⁻¹. As a physiological response, stomatal conductance, measured at the early flowering stage, showed a significant decline, around 19.42 %, at high boron supply above 5 mg kg⁻¹ (Table 2).

Biomass production

At harvest, the total aerial dry weight did not show any positive response to boron supply. However, we recorded a significant decrease of around 32% at high boron treatments (15 mg kg⁻¹ and 20 mg kg⁻¹) compared to the control (Table 3). Similarly, the leaf and head dry weights showed significant decreases of around 68% and 29%, respectively, compared to the control at high boron applications (15 mg kg⁻¹ and 20 mg kg⁻¹). However, the stem dry weight did not have any specific response to boron supply (Table 3).

Similarly to the aerial biomass response, the kernels weight and the kernels number per plant did not show any positive response to boron supply. Nonetheless, at high boron levels (15 mg kg⁻¹ and 20 mg kg⁻¹), significant decreases around 27% on the kernels weight and 44% on the number of kernels per head were recorded (Figure 1a and Figure 1b). Concerning the thousand kernels dry weight, it was increased by 32% at high boron supply (15 mg kg⁻¹ and 20 mg kg⁻¹) (Figure 1c). Furthermore, no significant response of boron application on fecundation rate (around 95%) was noticed (Figure 1d).

Mineral, protein and oil contents of kernels

For both experiments, the kernel boron content at harvest did not significantly increase even at high boron rates (15 mg kg⁻¹ and 20 mg kg⁻¹). This content was around 16 mg kg⁻¹ (Table 4). However, boron foliar content at harvest, determined in 2015 season, showed a significant increase. The minimum boron content was recorded in control plants (52 mg kg⁻¹), while the maximum content around 1054 mg kg⁻¹ was for boron supply of 10 mg kg⁻¹ (DATA is not be showed).

The kernels contents on nitrogen, zinc, and copper did not reveal a significant difference between boron treatments. Moreover, phosphorus, potassium, calcium, and manganese revealed a significant difference that seems not related to boron supplementation (Table 4).

Boron rates (mg kg ⁻¹)	Content index of chlorophyll *	Stomatal conductance** (mmol m ⁻² s ⁻¹)
((111161111 8)
0	28.9ª ± 4.8	134.3 ^{ab} ± 17.0
0.2	25.8ª ± 3.2	138.0 ^{ab} ± 24.1
0.5	28.3ª ± 2.8	149.6ª ± 19.1
1	27.5ª ± 2.6	150.1°±17.1
5	27.4ª ± 4.9	138.5 ^{ab} ± 9.3
10	27.0ª ± 4.5	114.5 ^b ± 5.8

Table 2. Effects of soil boron supply on chlorophyll content index of young leaves and on stomatal conductance of sunflower (2015 season)

Remarks: Values are means and standard deviation.

Means within the same column with a common letter do not differ significantly (Student–Newman– Keuls test, $P \le 0.05$ level)

* The chlorophyll content index was measured on young leaves just before flower bud stage

** The stomatal conductance was measured at the early flowering stage

Table 3. Effects of soil boron supply on total aerial dry weights and its allocation into head, leaves, and stem (g plant⁻¹) for 2015 and 2016 seasons

Boron rates (mg kg ⁻¹)	Aerial biomass dry weight	Head dry weight	Stem dry weight	Leaves dry weight
		2015		
0	135.9ª± 2.0	85.2ª ± 2.7	32.5ª ± 3.1	18.2ª ± 2.6
0.2	140.1ª± 1.5	81.2 ^ª ± 4.2	36.9ª ± 1.2	22.0ª ± 3.5
0.5	138.7ª± 8.8	85.1ª ± 5.0	32.5ª ± 3.1	$21.2^{a} \pm 4.0$
1	145.7ª± 4.8	88.8 ^a ± 3.7	34.6ª± 1.6	$22.4^{a} \pm 0.4$
5	142.2ª± 6.3	87.0 ^a ± 2.3	32.7ª ± 3.3	22.5ª ± 3.2
10	135.5ª± 8.8	81.2 ^ª ± 6.4	$34.4^{a} \pm 4.6$	19.9ª ± 1.4
		2016		
0	185.6ª± 8.8	114.9 ^ª ± 2.0	47.2ª ± 1.6	23.4ª ± 8.9
2	185.3ª± 10.9	112.8ª ± 3.7	45.9ª ± 1.9	26.6ª ± 9.7
5	180.9ª± 5.7	110.8ª ± 3.0	49.0 ^ª ± 4.5	20.9 ^{ab} ± 3.9
10	179.2ª± 10.1	111.6ª ± 1.5	49.0 ^ª ± 2.3	18.4 ^{ab} ± 8.8
15	145.6 ^{ab} ± 9.7	91.3ª ± 2.3	41.1ª ± 6.5	13.2 ^{ab} ± 5.3
20	126.1 ^b ± 17.6	79.2 ^b ± 4.6	39.3ª ± 11.1	07.5 ^b ± 3.2

Remarks: values are means and standard deviation for each growing season, means within the same column with a common letter do not differ significantly (Student–Newman–Keuls test, P≤ 0.05 level).



Figure 1. Effects of soil boron supply on kernels dry weight (a), number of kernels per head (b), thousand kernels dry weight (c), and pollination rate (d). Vertical bars denote standard deviation (n=5)

Table 4. Effects of soil boron supply	on nutrient contents of	of sunflower	kernels at harvest	for 2015 and
2016 seasons				

Boron rate	Nitrogen	Phosphorus	Potassium	Calcium	Boron	Manganese	Zinc	Copper
(mg kg⁻¹)	(%)	(%)	(%)	(%)	(mg kg ⁻¹)	(mg kg⁻¹)	(mg kg⁻¹)	(mg kg⁻¹)
				2015				
0	2.8ª±0.1	0.6 ^{ab} ±0.1	0.9ª±0.1	0.1 ^b ±0.1	11.3ª±0.8	26.4ª±2.1	28.8ª±3.6	8.2ª±0.6
0.2	2.8ª±0.2	$0.6^{ab}\pm0.1$	0.8 ^{ab} ±0.1	0.3ª±0.1	15.3ª±3.4	19.8 ^b ±1.9	26.6ª±2.1	8.2 ^a ±1.0
0.5	2.8ª±0.1	$0.6^{ab}\pm0.1$	0.8 ^{ab} ±0.1	$0.2^{ab}\pm0.0$	14.1ª±1.0	20.2 ^b ±0.8	27.4ª±1.8	8.5ª±1.1
1	2.7ª±0.1	0.6 ^b ±0.1	0.9 ^{ab} ±0.1	0.1 ^b ±0.0	13.1ª±4.1	25.6ª±0.5	30.0ª±6.3	7.5ª±0.5
5	2.7ª±0.2	0.6 ^{ab} ±0.0	0.8 ^b ±0.1	0.2ª±0.1	15.2ª±4.3	21.8 ^b ±0.6	28.5ª±2.3	9.3ª±1.2
10	2.6ª±0.0	0.7ª±0.1	0.9 ^{ab} ±0.0	0.1 ^b ±0.1	13.1ª±2.0	27.6ª±1.9	27.0ª±0.9	8.3ª±0.8
				2016				
0	2.0ª±0.1	0.8 ^{bc} ±0.1	0.8ª±0.1	0.2 ^{abc} ±0.0	18.4ª±2.8	21.9 ^b ±1.2	49.1ª±4.1	12.6ª±1.6
2	2.0ª±0.2	0.6 ^d ±0.0	0.7 ^b ±0.1	0.2 ^a ±0.0	13.9ª±4.3	20.4 ^b ±0.7	48.7ª±6.8	12.3ª±1.4
5	2.1ª±0.1	0.7 ^d ±0.0	0.7 ^b ±0.0	$0.2^{ab}\pm0.0$	17.1ª±3.8	21.8 ^b ±0.6	48.1ª±4.1	12.8ª±0.6
10	2.0ª±0.1	0.8 ^b ±0.1	0.7 ^{ab} ±0.1	$0.2^{bc}\pm0.0$	16.1ª±4.4	23.4 ^b ±2.6	50.3ª±3.0	12.6ª±1.4
15	2.3ª±0.2	0.7 ^{cd} ±0.0	0.7 ^b ±0.1	$0.2^{\text{abc}}\pm 0.1$	16.0ª±3.4	25.4 ^b ±3.6	47.9ª±1.9	12.5ª±1.7
20	2.5ª±0.7	0.9ª±0.1	0.8ª±0.1	0.2 ^c ±0.1	17.1ª±11.2	30.5 ^ª ±6.7	47.8ª±2.8	12.7ª±1.0

Remarks: values are means and standard deviation. For each growing season, means within the same column with a common letter do not differ significantly (Student–Newman–Keuls test, P≤ 0.05 level)

Boron rates (mg kg ⁻¹)	Protein content (%)	Oil content (%)
	2015	
0	17.2ª ± 0.7	44.4 ^b ± 2.0
0.2	17.7ª ± 1.5	46.1 ^{ab} ± 2.0
0.5	17.6ª ± 0.7	51.2 ^ª ± 0.7
1	17.0 ^ª ± 0.9	44.6 ^b ± 5.1
5	16.7ª ± 1.1	50.4ª± 0.8
10	16.2ª ± 0.2	46.8 ^{ab} ± 3.5
	2016	
0	12.4ª ± 0.7	52.2ª± 2.2
2	12.7ª ± 1.1	53.5ª± 2.1
5	13.2ª ± 0.6	53.8ª± 1.0
10	$13.0^{a} \pm 1.0$	51.2ª± 1.4
15	14.2ª ± 1.3	51.9ª± 1.5
20	15.4 ^a ± 4.7	51.6ª± 3.7

Table 5. Effects of soil boron supply on proteinand oil contents of sunflower kernelsfor 2015 and 2016 seasons

Remarks: values are means and standard deviation. For each growing season, means within the same column with a common letter do not differ significantly (Student– Newman–Keuls test, P≤ 0.05 level).

Table 6.Boron soil enrichment (boron
content at harvest - initial boron
content) under different boron
supplies for 2015 and 2016 seasons

Boron rates	Boron soil enrichment		
(mg kg⁻¹)	(mg kg ⁻¹)		
	2015		
0	0.0 ^b ± 0.0		
0.2	0.0 ^b ± 0.1		
0.5	$0.0^{b} \pm 0.1$		
1	$0.1^{b} \pm 0.1$		
5	$0.7^{a} \pm 0.1$		
10	$0.9^{a} \pm 0.4$		
	2016		
0	0.1 ^d ± 0.2		
2	0.3 ^d ± 0.2		
5	0.8 ^c ± 0.3		
10	$1.6^{b} \pm 0.4$		
15	3.1ª±0.5		
20	3.5ª ± 0.4		

Remarks: values are means and standard deviation. For each growing season, means within the same column with a common letter do not differ significantly (Student– Newman–Keuls test, P≤ 0.05 level).

Concerning the kernels oil content, significant fluctuations were recorded during 2015. This response seems not to be related to boron supply. Indeed, no significant response was recorded during the 2016 season (Table 5). Also, no significant effect of boron on protein kernels content was recorded for both studied seasons. The protein kernels contents were around 17%, and 13%, respectively, for the 2015 season and the 2016 seasons (Table 5).

Soil boron residual content

For both experiments, boron soil supply over than 2 mg kg⁻¹ resulted in significant soil enrichment at harvest. This enrichment had a linear trend (R²= 0.99 for 2015 and R²=0.98 for 2016) where a residual content around 0.32 mg kg⁻¹ was recorded for each boron supply of 2 mg kg⁻¹ (Table 6).

DISCUSSION

Results of the present study show that boron deficiency symptoms did not appear on sunflower even the studied soil has a low boron content (0.19 mg kg⁻¹) (Table 1) compared to the threshold of 0.5 mg kg⁻¹ (Rashid & Ryan, 2008). Moreover, the kernels of control plants had adequate boron content (Table 4) which was higher than the referenced limit of 15 mg kg⁻¹ (Blamey et al., 1978). These sufficient boron levels (16mg kg⁻¹) were in agreement with the agronomical responses recorded during both the growing seasons (2015 and 2016). Indeed, the physiological measured parameters (chlorophyll content index and stomatal conductance) did not reveal any positive response to boron treatments (Table 2). Concerning kernels yield components, the enhancement of the thousand kernels dry weight at high boron levels (15 mg kg⁻¹ and 20 mg kg⁻¹) did not enhance the weight of kernels per plant (Figures 1a,1c). Also, the fecundation rate, reported as sensitive kernel yield component to boron deficiency (Chitralekha &

Nirmala, 2000), was not be affected under no boron supplementation (Figure 1d).

Still, boron did not enhance kernels oil content (Table 5), which is considered as the sunflower production goal. Therefore, we can suggest the no requirement of sunflower to this micronutrient when grown on a sandy soil containing boron level of 0.19mg kg⁻¹ (Table 1). In contrast, Oyinlola (2007) reported a positive effect of this nutrient on sunflower kernels and oil yields when grown on a sandy loam soil with a low boron content (0.17 mg kg⁻¹).

Furthermore, obvious phytotoxic symptoms appeared with boron supply higher than 1 mg kg⁻¹ for both trials. This result is in line with the soil boron toxicity threshold of 1 mg kg⁻¹ reported by Eaton (1940). Also, we can note the close line between boron sufficiency and phytotoxicity as described by Ruiz et al. (2016). This phytotoxicity is approved by the highest boron leaf content around 1000 mg kg⁻¹ recorded at 10 mg kg⁻¹ of boron supply compared to the control (only 50 mg kg⁻¹) (DATA is not be showed). These results are in line with the phytotoxic foliar threshold ranged between 100-700 mg kg⁻¹ reported by Aitken & McCallum (1988). However, no relationship has been recorded between leaves and kernels concerning boron content. This may be attributed to the restricted mobility of boron in the plant (Brown & Shelp, 1997).

The phytotoxic effect resulted in a decline on the aerial dry weight, the kernel numbers and the kernel weight (Table 3). Similarly, Ceyhan et al. (2008) reported a negative effect of boron on some sunflowers genotypes cultivated in a boron-deficient calcareous soil.

The reason for this phytotoxic response may be attributed to photosynthesis process damage affected by high boron concentration (Landi et al., 2012). The mechanism behind this photosynthesis process damage is still unclear (Landi et al., 2012). On the other hand, the residual soil boron content showed a linear enhancement with boron levels (Table 6). The residual boron enrichment was around 0.3 mg kg⁻¹ for each boron supply of 2 mg kg⁻¹. Thus, we can pretend a negative residual effect of boron supply over than 5 mg kg⁻¹ for the next cropping season.

CONCLUSION

The absence of a positive response of sunflower to boron application leads us to suggest that soil boron content of 0.19 mg kg⁻¹ is sufficient for sunflower grown in sandy soil. Thus, the supposition of boron hidden deficiency on sunflower is rejected. In this regard, the sandy soil of the Loukkos area deserves further research to determine new soil boron reference content.

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