

Article

Seasonality in Apple Leaf Molybdenum Contents During the Growing Season and Stages of Greatest Need in This Nutrient

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Abstract: Molybdenum (Mo) is an important nutrient participating in nitrogen, sulfur, iron, and copper metabolism, as well as a cofactor in the biosynthesis of auxin and abscisic acid. Apple leaf Mo seasonality and periods of apple tree demand remain unclear due to insufficient data. This study aimed to identify peak leaf Mo levels throughout the growing season to distinguish when apple trees require the most Mo. To analyze seasonal patterns, we determined the leaf Mo contents 11–12 times per season on untreated and Mo-treated apple trees over four seasons in 2020–2023. Foliar fertilizing stimulated a significant increase in leaf Mo status. The Mo contents in untreated tree leaves were significantly lower than in trees after foliar fertilizing. However, in fertilized trees, nutrient contents were low after ten-day periods of heavy precipitation. High leaf Mo levels coincided with periods of intense apple shoot and fruit growth (average Mo level of 0.305 mg kg^{−1} in untreated trees and 0.386 mg kg^{−1} in fertilized ones) and apple fruit development and ripening (average Mo level of 0.754 mg kg^{−1} in untreated tree leaves and 1.069 mg kg^{−1} in fertilized ones). Mo application via foliar fertilizing in July's heat is essential for apple tree fruit growth and development to counter negative weather effects. Foliar fertilizing significantly increased tree yields in 2020, 2022, and 2023, resulting in substantially higher overall productivity (169.7 kg/tree^{−1} without vs. 215.6 kg/tree^{−1} with fertilizer).



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1. Introduction

Leaf tissue analysis is widely used to assess the nutritional status of apple trees [1–3]. The nutrient accumulation curves of apple trees are good indicators of element requirements at each stage of plant development. There are several studies on the seasonality of nutrient uptake by apple plants and seasonal changes in nutrient contents in leaves. Thus, in Washington (USA), the accumulated amounts of N, P, K, Ca, and Mg were estimated during the vegetation cycle of six apple orchards [4]. In North Carolina (USA), the seasonal accumulation of N, P, K, Ca, and Mg was assessed in the apple cultivar group “Delicious” [5]. Leaf K, Ca, and Mg accumulations were assessed in eight “Delicious” and eight “Virginia Gold” apple cultivars in Virginia (USA) [6]. In Poland, the seasonality of N, P, K, Ca, and Mg accumulation was assessed in 40 orchards over three consecutive seasons [7]. A very interesting study on the seasonal dynamics of both macro- (N, P, K, Ca, and Mg)

and micronutrients (B, Cu, Fe, Mn, and Zn) was conducted in Brazil [8]. There is a lot of information in the literature on the seasonality of the primary nutrients, but papers related to the need for microelements by apple plants and the seasonality of their contents are presented to a much lesser extent. Nevertheless, a lack of micronutrients can also have a depressing effect on plants [9].

However, among the microelements, nutrients with varying degrees of study for apple trees can also be distinguished. One of the least studied nutrients is molybdenum (Mo); it is a very important nutrient. The use of Mo fertilizers, for example, can significantly stimulate nitrogen absorption by plants [10].

The functions of Mo in plants are very important: several enzymes are known in which Mo is a cofactor: it is a part of the active site of the key enzyme that regulates nitrogen metabolism (nitrate reductase) [11], it is a part of the active sites of enzymes that are involved in sulfur metabolism, and the synthesis of auxins and abscisic acid occurs with the participation of the enzyme with a Mo cofactor aldehyde oxidase [12–15]. Mo metabolism is also closely related to the metabolism of sulfur, iron, and copper [16].

Studying the characteristics of seasonal changes in some microelements remains a major problem. There is quite a lot of conflicting information about the content of some nutrients in the literature, usually related to various regions of apple cultivation with different soil and climatic conditions [3,8,17]. Leaf nutrient content varies significantly during vegetation. Simultaneously, numerous factors, mainly agrotechnological (fertilizers, pesticides, growth regulators, cultivars, rootstocks, etc.), along with weather and soil conditions, can significantly affect leaf micronutrient content and seasonality [18–21].

Information on apple leaf Mo content is quite contradictory. For example, some sources report that, at the end of July–beginning of August, the Mo content of apple leaves was at the level of 0.1 mg kg^{-1} (dry matter) d.m. [3,17], whereas under the influence of foliar fertilizing, its content in the leaves during this period increased to 0.2 mg kg^{-1} d.m. [22]. There is also insufficient information in the literature on Mo seasonality in leaves and the stages of highest demand for this nutrient. For example, in the research paper “Seasonal Variation of Plant Mineral Nutrition in Fruit Trees” [23], it is reported that the Mo seasonal changes in apple leaves have been studied, but no information has been provided on the results. There is also a report by Kanguuehi [24], where, during the analysis of apple tree leaves within the framework of one experiment, the Mo content in different replicates differed by 10 times. Since the apple leaf Mo content is relatively low among other microelements, the influence of various external and internal factors can significantly change its concentration.

We have conducted a series of experiments to test micronutrient fertilizers in foliar fertilizer programs in tank mixtures with plant protection products over the past few years. In this study, we tried to identify the Mo seasonality both under the influence of foliar fertilizer treatments and without extra nutrient supply. The goal of this study was to determine the peaks in the leaf Mo content of the ‘Bogatyr’ apple cultivar during the growing season to clarify the phenological stages of the greatest demand for this micronutrient and its relationship with weather to improve apple tree foliar fertilizing programs.

2. Materials and Methods

Our study was conducted during the 2020, 2021, 2022, and 2023 growing seasons. The experiments were carried out in a commercial orchard of the ‘Bogatyr’ apple cultivar, grafted on the B118 rootstock, planted in 2002, and with a planting pattern of $6 \text{ m} \times 4 \text{ m}$ (416 trees/ha) in the JSC “Dubovoye” ($52^{\circ}36'59.8'' \text{ N}$ $40^{\circ}17'03.4'' \text{ E}$). The orchard was planted in 2002; upon planting, composted cow manure was applied at a rate of 60 t ha^{-1} ,

and the soil was deep-plowed (45 cm). The orchard was drip-irrigated. The drip tapes were placed on the ground. The soil between the rows was sown with a bean–cereal mixture and was kept under grass cover. Chemical fertilizers were broadly applied, at a low rate, once every 4–5 years (the last time was in spring 2021). Complete fertilizer “ammophoska” ($(\text{NH}_4)_2\text{SO}_4 + (\text{NH}_4)_2\text{HPO}_4 + \text{K}_2\text{SO}_4$) was used; the application rate was $\text{N}_{12}\text{P}_{15}\text{K}_{15}\text{S}_{14}\text{Mg}_{0.5}\text{Ca}_{0.5}$. The soil was typical chernozem on carbonate loam. The orchard soil had the following characteristics in 2020 before the experiment began (Table 1). The soil’s primary nutrient levels were high. The soil’s acidity can also be assessed as quite favorable for apple tree cultivation.

Table 1. Soil profile—primary nutrient contents and acidity levels (before the experiment).

Soil Layers, cm	N Hydrolyzable mg kg ^{−1}	P Bioavailable mg kg ^{−1}	K Exchangeable mg kg ^{−1}	Ca Exchangeable mg kg ^{−1}	pH _{kCl}
0–20	154.3	169.4	143.2	3973	6.18
21–40	132.0	157.1	122.5	3801	6.48
41–60	102.8	94.5	98.8	3776	6.09
61–80	72.6	81.4	104.0	3868	6.26

The annual foliar fertilizing programs are in Table 2, and Table 3 contains the information about the applied products. The working solution consumption was 1000 L ha^{−1}. The pH of the working solution ranged from 4.8 to 8.2 pH units, depending on the combination of fertilizers and plant protection products. Weather conditions during the treatments are shown in Table 4.

Table 2. Foliar fertilizing programs.

Mouse-Ear Stage: Green Leaf Tips 10 mm Above the Bud Scales	Pink Bud Stage: Flower Petals Elongating; Sepals Slightly Open; Petals Just Visible	Beginning of Flowering: About 10% of Flowers Open	Flowers Fading: Majority of Petals Fallen	Fruit Size up to 20 mm	Fruit Diameter up to 40 mm	Fruit About Half Final Size	Fruit About 60% Final Size	Fruit About 70% Final Size	Fruit About 80% Final Size	Beginning of * Ripening: First Appearance of Cultivar-Specific Color
10	57	61	67	72	74	75	76	77	78	81 **
2020										
	Biostim Growth	Biostim Growth		Ultramag Chelate	Ultramag Potassium	Ultramag Potassium	Ultramag Potassium	Ultramag ***	Ultramag Calcium	Without any **** treatments
	1.5 L ha ⁻¹	1.5 L ha ⁻¹		Zn-15 1.0 kg ha ⁻¹	3.0 l ha ⁻¹	3.0 L ha ⁻¹	3.0 L ha ⁻¹	Calcium	3.0 L ha ⁻¹ /	
	Ultramag Boron	Ultramag Boron			Ultramag Calcium	Ultramag Calcium	Ultramag Calcium	3.0 L ha ⁻¹ /	0.015 g L ⁻¹	
	1.0 L ha ⁻¹	1.0 L ha ⁻¹			3.0 L ha ⁻¹ / 0.015 g L ⁻¹	3.0 L ha ⁻¹ / 0.015 g L ⁻¹	3.0 L ha ⁻¹ / 0.015 g L ⁻¹	0.015 g L ⁻¹		
	Ultramag Chelate				Biostim Universal					
	Zn-15 1.0 kg ha ⁻¹				1.5 L ha ⁻¹					
10.04	29.04	07.05	22.05	07.06	17.06	27.06	06.07	16.07	27.07	
2021										
	Ultramag Boron	Ultramag Boron		MicroFid Zinc	Ultramag Potassium	Ultramag Potassium	Ultramag Calcium	MicroFid Zinc	Ultramag Calcium	
	1.0 L ha ⁻¹	1.0 L ha ⁻¹		1.0 L ha ⁻¹	3.0 L ha ⁻¹	3.0 L ha ⁻¹	3.0 L ha ⁻¹ / 0.015 g L ⁻¹	1.0 L ha ⁻¹	3.0 L ha ⁻¹ /	
	MicroFid Zinc				Ultramag Calcium	Ultramag Calcium	Igida 1.5 L ha ⁻¹		0.015 g L ⁻¹	
	1.0 L ha ⁻¹				3.0 L ha ⁻¹ / 0.015 g L ⁻¹	3.0 L ha ⁻¹ / 0.015 g L ⁻¹				
					Igida 1.5 L ha ⁻¹	MicroFid Boron				
					1.0 L ha ⁻¹					
25.04	06.05	14.05	22.05	01.06	09.06	18.06	08.07	15.07	30.07	13.08
2022										
	Biostim Growth	Biostim Growth	Ultramag Calcium	Ultramag Calcium	Ultramag Calcium	Ultramag Calcium	Ultramag Calcium	Ultramag	Ultramag Calcium	SC2020
	1.0 L ha ⁻¹	1.5 L ha ⁻¹	3.0 L ha ⁻¹	3.0 L ha ⁻¹ / 0.015 g L ⁻¹	3.0 L ha ⁻¹ / 0.015 g L ⁻¹	3.0 L ha ⁻¹ / 0.015 g L ⁻¹	3.0 L ha ⁻¹ / 0.015 g L ⁻¹	Calcium	3.0 L ha ⁻¹ /	1.5 L ha ⁻¹
	Ultramag Boron	Ultramag Boron	/0.015 g L ⁻¹	AKh-576-23	Ultramag Potassium	Ultramag Potassium		3.0 L ha ⁻¹ /	0.015 g L ⁻¹	
	1.0 L ha ⁻¹	1.0 L ha ⁻¹	AKh-576-23	2.0 L ha ⁻¹ / 0.6 g L ⁻¹	3.0 L ha ⁻¹	3.0 L ha ⁻¹		0.015 g L ⁻¹		
	Ultramag Calcium		2.0 L ha ⁻¹ / 0.6 g L ⁻¹	Ultramag Potassium	Biostim Growth	AKh-577-23				
	3.0 L ha ⁻¹ / 0.015 g L ⁻¹			3.0 L ha ⁻¹	1.0 L ha ⁻¹	2.0 L ha ⁻¹ / 0.5 g L ⁻¹				
	AKh-576-23				AKh-576-23					
	2.0 L ha ⁻¹ / 0.6 g L ⁻¹				2.0 L ha ⁻¹ / 0.6 g L ⁻¹					
22.04	03.05	16.05	29.05	13.06	24.06	04.07	16.07	24.07	05.08	15.08
2023										

Table 2. Cont.

Mouse-Ear Stage: Green Leaf Tips 10 mm Above the Bud Scales	Pink Bud Stage: Flower Petals Elongating; Sepals Slightly Open; Petals Just Visible	Beginning of Flowering: About 10% of Flowers Open	Flowers Fading: Majority of Petals Fallen	Fruit Size up to 20 mm	Fruit Diameter up to 40 mm	Fruit About Half Final Size	Fruit About 60% Final Size	Fruit About 70% Final Size	Fruit About 80% Final Size	Beginning of * Ripening: First Appearance of Cultivar-Specific Color
10	57	61	67	72	74	75	76	77	78	81 **
Ultramag Super	Ultramag Phosphorus	Biostim Universal	Ultramag	AKh-576-23	Biostim Universal	AKh-577-23	AKh-576-23	Ultramag	SC2020	SC2020
Zinc-700	Super	2.0 L ha ^{−1}	Calcium Active	2.0 L ha ^{−1} / 0.6 g L ^{−1}	3.0 L ha ^{−1}	2.0 L ha ^{−1} / 0.5 g L ^{−1}	1.0 L ha ^{−1} / 0.6 g L ^{−1}	Calcium	L ha ^{−1}	L ha ^{−1}
1.0 L ha ^{−1}	1.5 L ha ^{−1}	Ultramag Boron	3.0 L ha ^{−1}	Ultramag Calcium	Ultramag Calcium	Ultramag Calcium	Ultramag Calcium	3.0 L ha ^{−1} /		
Ultramag Helat	Ultramag Boron	1.0 L ha ^{−1}		3.0 L ha ^{−1} / 0.015 g L ^{−1}	3.0 L ha ^{−1} / 0.015 g L ^{−1}	3.0 L ha ^{−1} / 0.015 g L ^{−1}	3.0 L ha ^{−1} / 0.015 g L ^{−1}	0.015 g L ^{−1}		
Fe-13	1.0 L ha ^{−1}	Ultramag Super		Ultramag Potassium		Ultramag Potassium				
1.0 kg ha ^{−1}	AKh-576-23	Sulfur-900		3.0 L ha ^{−1}		3.0 L ha ^{−1}				
	1.5 L ha ^{−1} / 0.6 g L ^{−1}	1.0 L ha ^{−1}								
26.04	08.05	20.05	27.05	07.06.	16.06	29.06	06.07	19.07	31.07	15.08

* The 1st row of the table contains the names of the phenological phases of the apple tree when sprayings were performed. ** The 2nd row of the table contains the number of the phenological phase according to the BBCH Monograph [25]. *** Mo-containing agrochemicals are highlighted in bold; a darker gray fill means more Mo content in the agrochemical; after the slash is the Mo concentration (in g per L); at the bottom of the table cells are the dates of treatments; empty cells with a date mean that foliar fertilizers were not included in the tank mixture. **** Table rows 4, 6, 8, and 10 show agrochemical names and application rates (per hectare) for foliar fertilizing treatments in the corresponding growth stages.

Table 3. The contents of nutrients in agrochemicals for foliar fertilizing, % a.i. (active ingredients).

Agrochemicals	N	P ₂ O ₅	K ₂ O	CaO	MgO	SO ₃	B	Cu	Fe	Mn	Mo	Na	Si	Zn	Amino Acids of Plant Origin
Biostim Growth	4.0	10.0			2.0	1.0	0.1		0.4	0.2				0.2	4.0
Biostim Universal	6.0		1.3			5.0									10.0
Igida	4.0		3.0	7.0	4.7				27.0						10.0
MicroFid Zinc	1.0		0.7		0.3	10.5	0.05	0.05		0.02		4.5	3.6	11	
SC2020				10.0											
Ultramag Boron							11.0								
Ultramag Calcium	10.0			17.0	0.8		0.05	0.02			0.001			0.02	
Ultramag Calcium Active				10.0											
Ultramag Chelate Fe-13									13.0						
Ultramag Phosphorus Super	6.4	35.0			4.0									2.5	
Ultramag Potassium	2.6		22.0												
Ultramag Super Sulfur-900	5.0					70.0									
Ultramag Super Zinc-700	1.5													40.0	
AKh-576-23	4.6				4.0		0.5	0.05	1.0	4.0	0.05			3.0	
AKh-577-23	2.0			10.0	5.0				0.7	2.0	0.04				

Table 4. Air temperature (°C) and air humidity (%) at the time of application.

Mouse-Ear Stage: Green Leaf Tips 10 mm Above the Bud Scales	Pink Bud Stage: Flower Petals Elongating; Sepals Slightly Open; Petals Just Visible	Beginning of Flowering: About 10% of Flowers Open	Flowers Fading: Majority of Petals Fallen	Fruit Size up to 20 mm	Fruit Diameter up to 40 mm	Fruit About Half Final Size	Fruit About 60% Final Size	Fruit About 70% Final Size	Fruit About 80% Final Size	Beginning of Ripening: First Appearance of Cultivar-Specific Color
10	57	61	67	72	74	75	76	77	78	81
2020										
10.04. 9.1 °C 72%	29.04. 14.5 °C 51%	07.05. 16.9 °C 76%	22.05. 10.5 °C 63%	07.06. 19.1 °C 82%	17.06. 22.3 °C 49%	27.06. 19.9 °C 71%	06.07. 23.1 °C 80%	16.07. 15.8 °C 77%	27.07. 21.4 °C 49%	Without any treatments
2021										
25.04. 8.9 °C 78%	06.05. 15.0 °C 47%	14.05. 20.4 °C 64%	22.05. 17.3 °C 56%	01.06. 12.7 °C 83%	09.06. 18.1 °C 86%	18.06. 20.7 °C 52%	08.07. 23.4 °C 51%	15.07. 24.1 °C 54%	30.07. 21.9 °C 77%	13.08 20.0 °C 79%
2022										
22.04. 9.1 °C 90%	03.05. 13.9 °C 48%	16.05. 10.8 °C 79%	29.05. 14.3 °C 66%	13.06. 19.1 °C 67%	24.06. 20.8 °C 64%	04.07. 22.7 °C 59%	16.07. 20.7 °C 71%	24.07. 23.1 °C 78%	05.08. 24.6 °C 64%	15.08. 23.1 °C 62%
2023										
26.04. 12.4 °C 85%	08.05. 9.9 °C 44%	20.05. 15.2 °C 62%	27.05. 20.8 °C 68%	07.06. 17.6 °C 57%	16.06. 19.9 °C 62%	29.06. 16.1 °C 94%	06.07. 22.4 °C 85%	19.07. 18.1 °C 72%	31.07. 20.5 °C 68%	15.08. 22.4 °C 74%

The trees were trained as a sparse-tiered crown, usually pruned every three years. The last pruning was in 2022 (including skeletal branch removal).

There were 60 trees in each treatment. The repetition of the experience was fourfold. Each repetition consisted of 15 trees. Leaves were sampled for analysis from two plots: 1. trees were completely without foliar fertilizer treatments (Control (Cont)); 2. foliar fertilizer program (Foliar fertilizing (Ff)).

Leaf samples were taken from the middle of one-year-old shoots from the middle height of the tree canopy. We collected leaf samples on the day of spraying, approximately 1 h before foliar treatment. We usually took the leaves for analysis between 8:30 a.m. and 9:00 a.m. We sampled not-damaged typical leaves, without petioles (cut off with shears). We took one leaf from each side of the tree. During the season, samples were taken 11–12 times, depending on the number of sprayings. For each treatment, a sample of 60 leaves was collected from 15 apple trees across various points within the experimental plot. The leaves were selected without petioles. Before analysis, the leaves were wiped with a napkin moistened with distilled water. Then, leaves were dried at a temperature of 105 °C in a drying cabinet SHS-80-01 (SKTB SPU, Smolensk, Russia) until a constant weight was reached. Next, the IKA MF 10 laboratory mill (IKA-Werke GmbH, Staufen im Breisgau, Germany) was used to grind the leaves. The crushed leaves were stored in metal boxes, and immediately before the analysis, they were dried again to a constant weight, allowing a deviation of ± 0.005 g. The sample weight for the analysis was 1.0 g d.m. Three samples for the analysis were prepared from each of the fifteen tree plots (twelve samples from the treatment). The plant material was digested for analysis using a wet route method with HNO_3 on a WX-6000 microwave system (PreeKem, Shanghai, China). Leaf Mo was determined by atomic absorption spectroscopy (MGA-1000, Saint Petersburg, Lumex, Russia) [26–28]. The wavelength for spectrometry was 313.26 nm, the limit of detection was 0.03 mcg l^{-1} , and the limit of quantification was 0.35 mcg l^{-1} .

The experimental data obtained during the study were processed using the analysis of variance method at p value < 0.05 (level of significance: 5% (0.05)), and the Least Significant Difference (LSD) was calculated using the Fisher method [29]; the data distribution was normal, and the number of unrelated samplings was two. The LSDs that were higher than the computed LSD value were considered to be significant. Statistical data treatment was carried out using Excel 2019 and the AgCXStat add-on [30]. We also calculated Pearson correlation coefficients and regression equations with determination coefficients.

3. Results

In 2020, the total precipitation during the observation period (April–September) was 187.6 mm (with a norm of 350 mm (on average in 1971–2021)), and in January–March of this year, there was 77.6 mm of rainfall. At the same time, there was only 14.4 mm of precipitation in March (in the first half of the month, there was 13.8 mm). April was also dry—with only 21 mm of precipitation—which is much less than the multi-year average. The total rainfall in May was 77.2 mm, which partially compensated for the drought in April (Figure 1b). Subsequently, the summer was dry, and the sum of active temperatures (the sum of average daily temperatures above $+10$ °C, SAT) during the observation period (April–September) was 2791.4 °C.

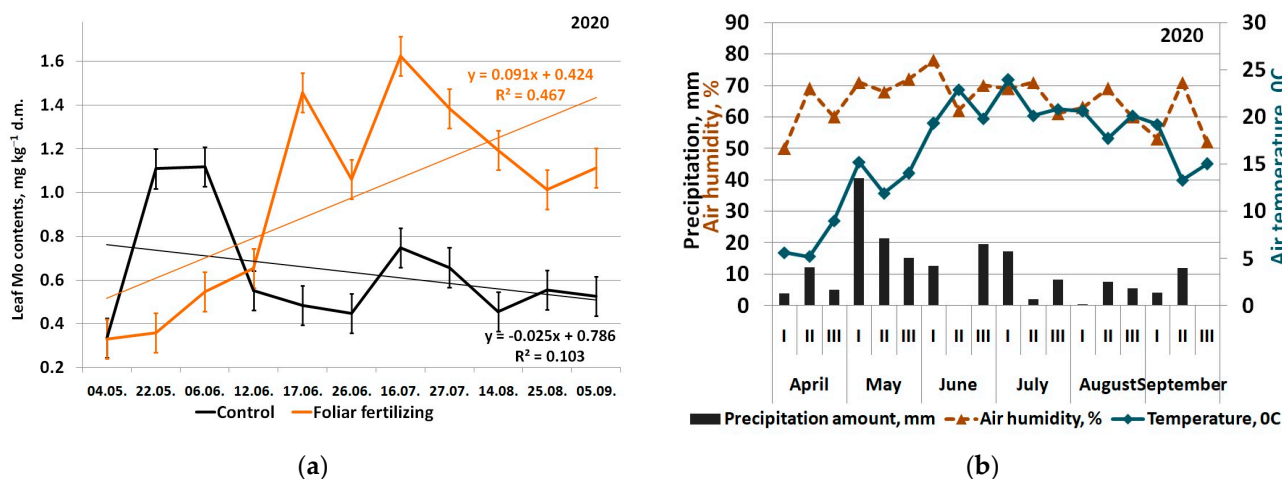


Figure 1. Seasonal changes in apple leaf Mo contents (a) average ten-day precipitation, air temperature, and air humidity (b) in the 2020 season.

Against the background of such weather conditions, the seasonality of leaf Mo content in 2020 under the influence of the foliar fertilizers strongly differed from the changes in the leaves of trees that were not sprayed with fertilizers (Figure 1a). In the non-sprayed variant, the leaf Mo content increased sharply by mid-May–early June (0.334 to 1.117 mg kg⁻¹, LSD₀₅ = 0.087) but then decreased by mid-June (0.484 mg kg⁻¹), although it remained significantly higher than the initial level (0.334 mg kg⁻¹). A significant increase in the leaf Mo content was also noted in the treatment with foliar fertilizer application during this period, but it was not as sharp compared to the amount of the nutrient in the leaves on 04.05 (0.329 to 0.547 mg kg⁻¹). It is noteworthy that the leaf Mo content increased sharply in the absence of foliar fertilizing, while with fertilizer treatments, the increase in Mo levels was not as high.

The leaf Mo increased significantly in the treatment with foliar fertilizing from mid-June until the end of July (0.652 to 1.623 mg kg⁻¹), although with some variations. There is no doubt that one of the reasons for this is repeated treatments with low-Mo-content agrochemicals. The July peak of increase in leaf Mo is noteworthy in the variant without fertilizer application, although it is not so sharp (0.448 to 0.748 mg kg⁻¹). The nutrient content in the foliar fertilizing treatment increased more than three times compared to the middle of June (0.484 to 1.623 mg kg⁻¹).

Subsequently, the leaf Mo content in both variants decreased, but it remained very high in the treatment with foliar fertilizing (0.545 mg kg⁻¹ in Control; 1.111 in Foliar fertilizing treatment). The leaf Mo content had a slight tendency to decrease in the control without treatments during the whole season, whereas under the influence of foliar fertilizing, there was a clear trend towards an increase in the nutrient content. Moreover, after the completion of the foliar fertilization, some increase in the nutrient content was also noted, even at a very high level of supply in early September. Higher nutrient content was also seen after foliar fertilizing in early September, even at a high level of Mo concentration.

In 2021, the total precipitation for the observation period was 269.6 mm, and for January–March, this was 71.4 mm (Figure 2b). March was quite dry (only 3.2 mm), but in April, there was 50.4 mm of precipitation, which allows us to speak about a relatively satisfactory moisture supply at the beginning of the growing season. Difficulties could have arisen in the second half of the observation period, when there was insufficient precipitation and the average daily air humidity did not exceed 70%, dropping to 30% on certain days. SAT during the observation period was 2996.9 °C. We did not record extremely high or extremely low temperatures at this time. The minimum temperature was −2.1 °C (5 April 2021) before bud break, and the maximum was 35.7 °C (23 June 2021). Average

daily temperatures did not exceed 30 °C and were not lower than 5 °C. That is, despite some unevenness in the distribution of precipitation over the observation period and a certain lack of moisture in the summer, the conditions in 2021 were quite favorable for growing the domestic-bred Bogatyr apple tree cultivar and were generally typical for the Tambov Oblast.

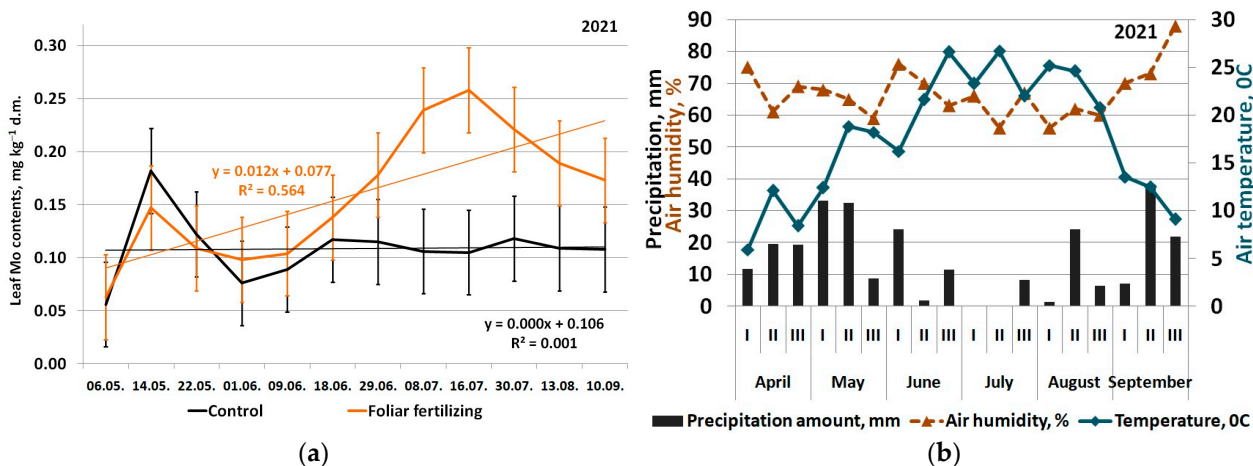


Figure 2. Seasonal changes in apple leaf Mo contents (a) average ten-day precipitation, air temperature, and air humidity (b) in the 2021 season.

Apple leaves had substantially less Mo in 2021 than in 2020, with an average content 6 times lower (Figure 2a). Moreover, this decrease was noted in both experimental variants (0.056–0.122 mg kg⁻¹ in Control and 0.063–0.258 mg kg⁻¹ in Foliar fertilizer treatment; $LSD_{05} = 0.042$). The general nature of the seasonality of leaf Mo content was similar across the two years of research, as evidenced by the trend lines in the figures. The leaf Mo contents in the control were generally at the same level, except for a peak with a sharp increase in mid-May (0.182 mg kg⁻¹ on 14 May 2025). It is noteworthy that a similar peak in the foliar fertilizing treatment in 2020 was later. It is also interesting that in the control without fertilizing in 2021, there was no second peak in leaf Mo contents, but under the influence of fertilizer application, there was one (0.258 mg kg⁻¹ on 16 July 2025). The Mo contents increased from mid-June until mid-July under the influence of fertilizer application (0.104 mg kg⁻¹ to 0.258 mg kg⁻¹), which is an obvious result of the use of an agrochemical with molybdenum, albeit at a low concentration. Subsequently, a decrease in the nutrient content in this treatment was noted.

Despite 41.4 mm of precipitation in the first quarter of 2022, the observation period's total of 339.7 mm matches long-term averages (Figure 3b). While March 2022 (as in previous years) was arid, with just 8.8 mm of precipitation, April's 52.4 mm of precipitation provided a sufficient water supply for the growing season's start. Moreover, there was 48.8 mm of precipitation in the first ten days of the month. The most significant difference between the 2022 growing season and the previous year was the distribution of precipitation. In 2022, the bulk of the precipitation was in the second half of the growing season. Rainfall in July (40 mm in the second and third ten day periods) and September contrasted with August's dryness. With such an uneven distribution of precipitation during the growing season in May, June, and August, there were periods when the average daily air humidity dropped to 30%, which can be characterized as an air drought. It should be noted that the average daily air humidity was at 70% for almost the entire observation period. The SAT for the observation period was 2783.8 °C. We did not record excessively high or extremely low temperatures during the observation period. The minimum temperature was −3.4 °C

(5 April 2022) before bud break, and the maximum was 34.3 °C (11 July 2022). Average daily temperatures did not exceed 25 °C and were not below 5 °C.

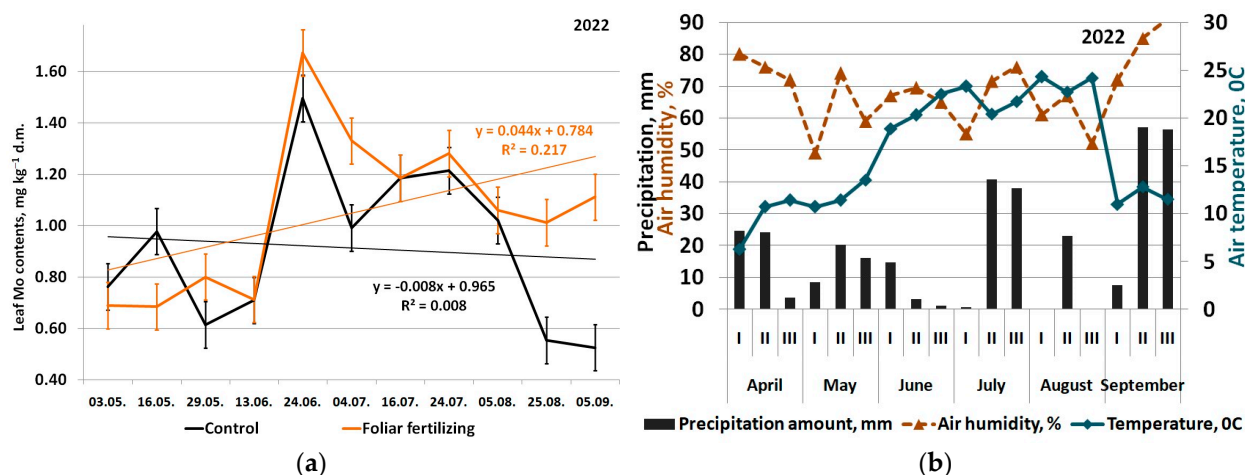


Figure 3. Seasonal changes in apple leaf Mo contents (a) average ten-day precipitation, air temperature, and air humidity (b) in the 2022 season.

The nature of the seasonal changes in leaf Mo contents during the observation period in 2022 was similar both in the control and in the foliar fertilizing treatment, despite the control and foliar treatments diverging in May. We observed a peak in the control peak in nutrients of 16.05.22 (0.977 mg kg⁻¹), which then fell sharply (to 0.614 mg kg⁻¹ on 29 May 2022), a pattern not seen in the foliar treatment. However, until early August, nutrient content changes remained largely similar, despite significant quantitative differences. For example, there was a sharp increase from mid-June with a peak in nutrient contents at the end of June (1.495 mg kg⁻¹ in Control; 1.672 mg kg⁻¹ in Foliar fertilizer treatment on 24 June 2022; LSD₀₅ = 0.113). The nutrient contents in both treatments increased by 2–3 times. Notably, the Mo-containing agrochemical's foliar application on 3 May 2022 and subsequent treatments on 29 May 2022 and 13 June 2022 showed no visible impact on leaf nutrient content versus the control. A significant increase in nutrient concentration was noted in both treatments by 24 June 2022. In the foliar fertilizing treatment, a significantly higher leaf Mo content was noted after August 5, when a considerable decrease in the nutrient concentration occurred in the control (0.554 mg kg⁻¹ in Control; 1.012 in Foliar fertilizing; 25 August 2022). The nutrient concentration in the foliar fertilizing variant was almost two times higher than in the control until the end of the observation period, when the leaf Mo contents decreased in both treatments (1.111 vs. 0.525 mg kg⁻¹). Despite some differences in the nature of the nutrient seasonality in the leaves compared to the previous years, the general trend of changes during the season was the same: in the control, the leaf Mo content slowly decreased during the observation period, while in the foliar fertilizing treatment, it increased.

The total precipitation for the observation period was only 151.8 mm in 2023 (for January–March, this was only 58.6 mm). The year 2023 was distinguished by dry weather conditions, not only when compared with multi-year averages but also when related to other years of study. Insufficient soil moisture was a problem throughout the growing season, starting with a lack of winter reserves despite March's relatively high precipitation (42.6 mm). Then, in April, there was only 19.2 mm of rainfall, which allows us to speak of a significant lack of moisture at the beginning of the growing season (Figure 4b).

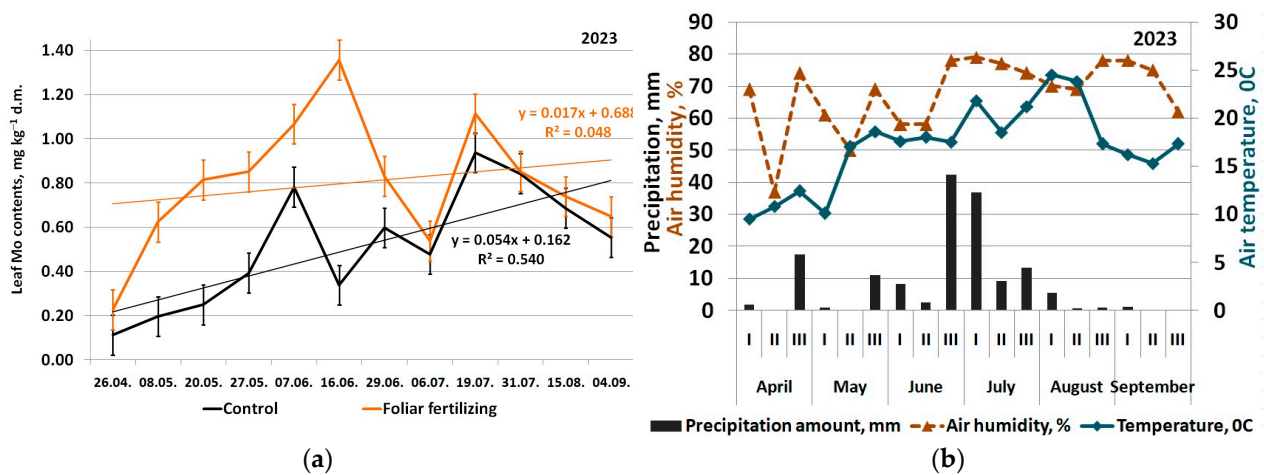


Figure 4. Seasonal changes in apple leaf Mo contents (a) average ten-day precipitation, air temperature, and air humidity (b) in the 2023 season.

We observed a dry period from the beginning of May to the end of June, when there was 22.8 mm of precipitation over 50 days. After a short “wet respite” (the third ten-day period of June and the first one of July—79.2 mm precipitation in total), we again observed dry weather (for the entire remaining period until the end of September, there was only 30.6 mm of precipitation).

There were periods when the average daily air humidity dropped below 50%, which can be characterized as an air drought, in April, May, and August 2023. The SAT for the observation period was 3023.9 °C. During the observation period in 2023, we did not record extremely high or severely low temperatures. The minimum temperature was just below 0 degrees Celsius, −0.7 °C (15 April 2023), at the beginning of the growing season, and the maximum was 33.9 °C (18 August 2023). Average daily temperatures did not exceed 25 °C in August, when the maximum daily temperatures were recorded, and did not fall significantly below 10 °C during the entire observation period. Thus, the driest weather with the largest SAT over the years of the experiment was noted in 2023, which was not entirely favorable for apple trees.

In 2023, the seasonality of the leaf Mo contents in the foliar fertilizing treatment was generally similar to the changes in the previous year (Figure 4b). In 2023, leaf Mo in apple trees increased from the season’s start, reaching a peak by mid-June (0.113 to 0.782 mg kg⁻¹ in the Control; 0.228 to 1.357 mg kg⁻¹ in the Foliar fertilizer treatment; $LSD_{05} = 0.099$), decreasing somewhat and then peaking again in late July in both experimental variants (0.938 mg kg⁻¹ Control; 1.357 mg kg⁻¹ Foliar fertilizer). Foliar fertilizer application significantly increased Mo content early in the growing season, although peak levels occurred at the same time.

Although the number of Mo treatments in 2023 matched the previous year’s, leaf Mo contents were 1.3 times lower.

The seasonal changes in leaf Mo contents in the control generally repeated the variations in the foliar fertilizing treatment, with peaks in June and July. The leaf Mo contents had a clear tendency (Figure 4) to increase during the observation period in the control treatment in 2023 (in contrast to the 2020–2022 seasons (Figures 1–3)). We suggest conducting further observations of the seasonal dynamics of the nutrient content in the next few years.

Figure 5 shows the average values of Mo contents in apple leaves over 4 years of research on the phenological stages of plant vegetation. In the initial phases, the Mo content practically does not differ according to the research variants. Attention is drawn to the fact that in the control, the Mo content had three peaks, and the first one was in phase 61, the “Beginning of flowering” (about 10% of flowers open). In the foliar fertilizing treatment,

an increase in the Mo content was also noted in this period, but there was no pronounced peak, although the first Mo application was at phase 57, the “Pink bud stage”.

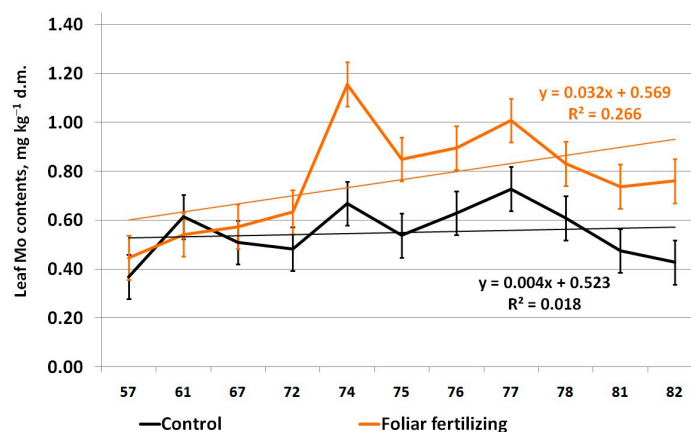


Figure 5. Average Mo contents over 4 years of research on plant development stages.

Foliar fertilizing impact is more noticeable in this variant as the season progresses, with a substantially increased Mo level. However, the most important thing is the coincidence of peaks at stage 74, “Fruit size up 40 mm”, and stage 77, “Fruit about 60% of final size”. Consequently, the average data for the study period confirm the seasonal peaks identified in our above-mentioned analysis. Peaks were most pronounced when applying foliar fertilizers, but they were not so clear in the control. Even in this variant, the increase was significant: from stage 72 of “Fruit size up to 20 mm” to stage 74, the Mo concentration increased from 0.433 to 0.669 mg kg^{−1} (by 1.5 times). The second peak in the control was an increase from 0.538 mg kg^{−1} in stage 75, “Fruit above half final size”, to 0.729 mg kg^{−1} in stage 77, “Fruit about 70% final size”.

The Mo content in the leaves has shown great variability over the years of research (Table 5). The seasonal changes in Mo levels in apple leaves were typical of individual years. Despite this similarity, in the control (no Mo agrochemicals applied), the Mo concentrations were between 0.056 mg kg^{−1} (four-year minimum in 2021) and 1.495 mg kg^{−1} (four-year maximum in 2022).

Table 5. Minimum, maximum, and average values of apple leaf Mo contents, in kg mg^{−1}.

Value	2020		2021		2022		2013	
	Cont	Ff	Cont	Ff	Cont	Ff	Cont	Ff
Minimum	0.334	0.329	0.056	0.063	0.525	0.684	0.113	0.228
Maximum	1.117	1.623	0.182	0.258	1.495	1.672	0.938	1.357
Average	0.635	0.975	0.109	0.160	0.913	1.049	0.514	0.805
LSD ₀₅	0.087		0.042		0.113		0.099	

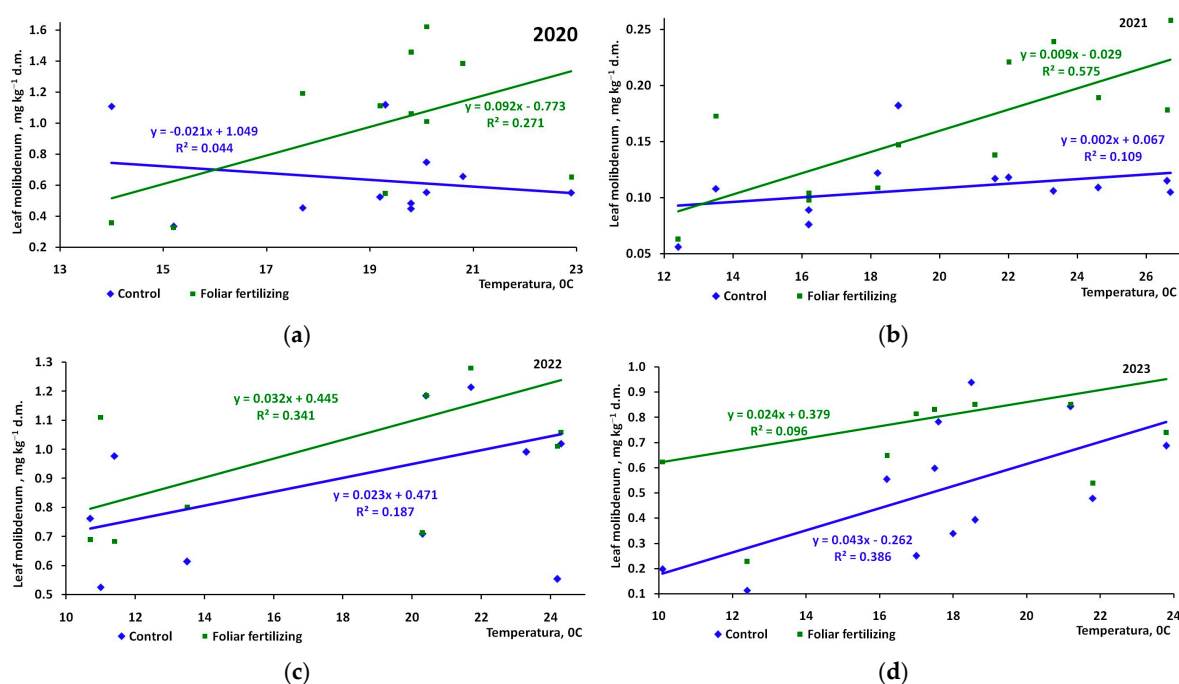
Under the influence of foliar fertilizing, the concentration of Mo in apple leaves varied even more—from 0.063 to 1.672 mg kg^{−1}. As a result, there were significant changes in the average values for each year. The different variations make it harder to understand Mo’s seasonal changes and the determination of the right fertilizer application rate. We think that the weather played a significant role in the inconsistent Mo levels found in the leaves.

The leaf Mo content generally increased with rising temperatures (Table 6, Figure 6). The strength of this relationship depended on the treatments and varied across separate years of study. Foliar fertilizer application boosted this trend, unlike in the untreated control, where the relationship was negligible in 2020 and 2021. A noteworthy observation is that the highest correlation between temperature and leaf Mo (in the foliar fertilizer treatment) was found in 2020–2021 (maximum correlation), unlike the control’s minimal correlation.

Table 6. Correlation coefficients between the apple leaf Mo contents and weather conditions *.

Treatments	2020	2021	2022	2023
Average daily air temperature for a ten-day period				
Control	−0.211	0.330	0.480	0.622
Foliar fertilizer	0.521	0.758	0.474	0.310
Average daily air humidity for a ten-day period				
Control	0.43	−0.365	0.335	0.363
Foliar fertilizer	−0.27	−0.522	0.136	−0.375
Total precipitation over a ten-day period				
Control	−0.248	−0.083	0.249	0.091
Foliar fertilizer	−0.445	−0.661	−0.065	−0.257

* Correlations that represent a more or less clear trend over the research period are highlighted in bold.

**Figure 6.** The relationships between foliar Mo and air temperature recorded in the different treatments in various years of the study ((a) 2020, (b) 2021, (c) 2022, (d) 2023).

The correlation between the studied factors in the control increased, but in the foliar fertilizing treatment, on the contrary, it decreased later in 2022 and 2023. The highest value of the correlation coefficient between these parameters in the control was noted in the driest season of 2023.

No stable correlation between air humidity and leaf Mo content was observed throughout the study period. Compared to the control, foliar fertilizing treatments produced contradictory results in specific years (2020 and 2023—see Table 4 and Figure 7). Higher humidity following treatments lowered Mo levels, while in the control, a slight increase in nutrient contents was shown. We observed no persistent relationship between these factors over the study period.

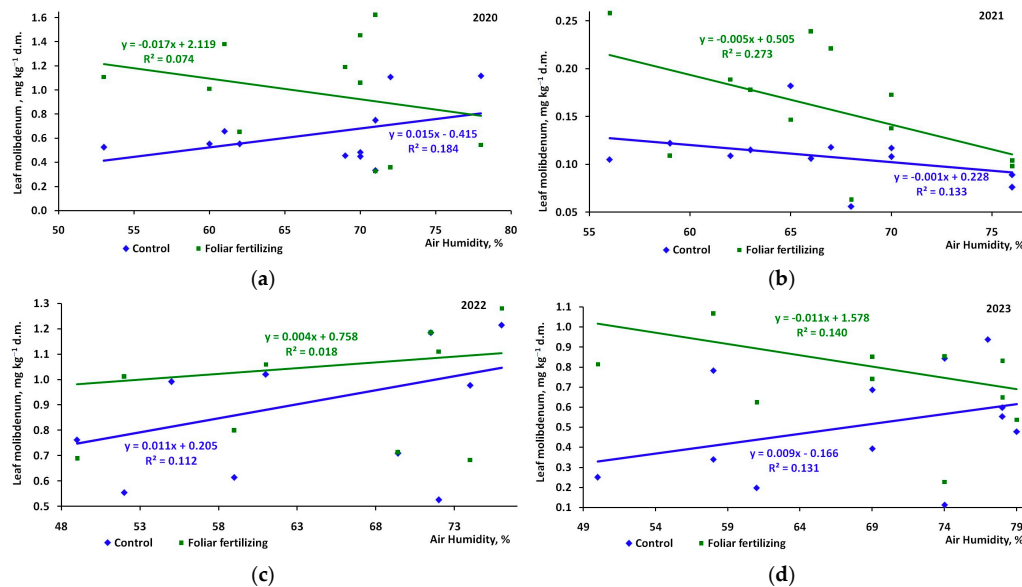


Figure 7. The relationships between foliar Mo and air humidity recorded in the different treatments in various years of the study ((a) 2020, (b) 2021, (c) 2022, (d) 2023).

The foliar fertilizing application noticeably changed how ten days of precipitation affected leaf Mo levels. (Table 4, Figure 8). This relationship was quite noticeable only in 2020 and 2021. In 2022, despite an overall trend, leaf Mo levels in foliar-fertilized plants were unrelated to precipitation. There was a weak correlation between precipitation and leaf Mo status in 2023; however, it is noteworthy that with increasing precipitation, the leaf Mo contents decreased. Leaf Mo status exhibited a slight inverse relationship with precipitation in 2023; as precipitation rose, leaf Mo levels fell. No strong relationship between precipitation and leaf Mo content was observed in any of the study years in the control. At the same time, the nature of the weak relationships in separate years of the research was contradictory—both positive and negative. Available data show no clear link between precipitation and leaf Mo in the unfertilized control.

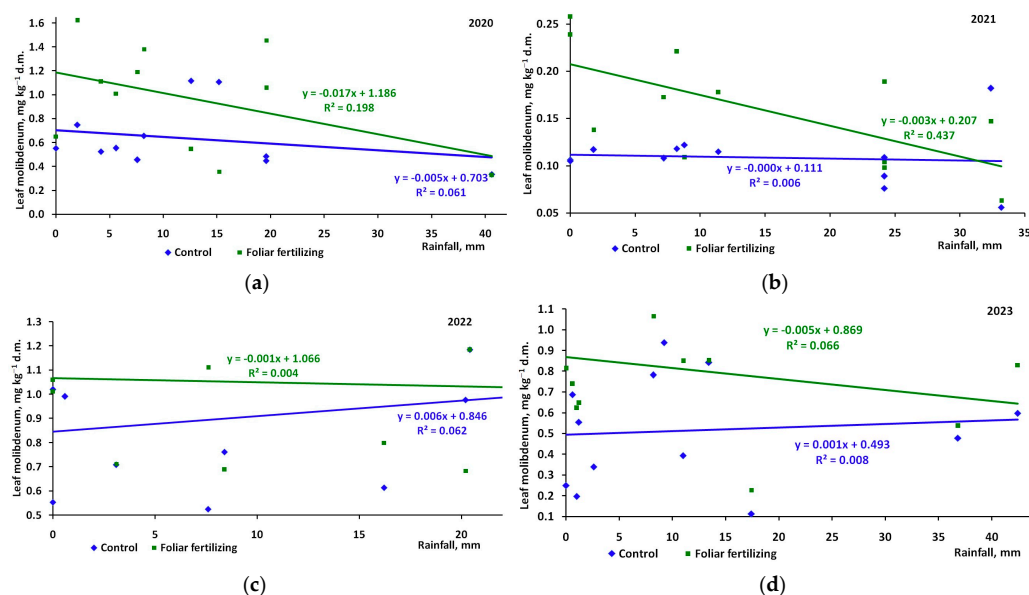


Figure 8. The relationships between foliar Mo and 10-day precipitation amount recorded in the different treatments in various years of the study ((a) 2020, (b) 2021, (c) 2022, (d) 2023).

When we used foliar fertilizing, we can see that there is a negative relationship between the amount of precipitation and the leaf Mo content. This relationship was negative in almost all years of research, but it was more or less considerable only in 2020, 2021, and 2023.

The direct effect of Mo treatments on the yield of apple trees is quite difficult to isolate because we have introduced a complete program of foliar fertilizing (see Table 2). It is noteworthy that yields between the experimental variants practically did not differ in 2021, and in 2020, 2022, and 2023, we observed a significant increase in productivity under the influence of foliar fertilizing (Figure 9). We used agrochemicals with a complex of micronutrients (which had a high Mo concentration, see Table 3)) for foliar sprayings in 2022 and 2023. In other words, this can only indirectly confirm the role of Mo in increasing apple yield.

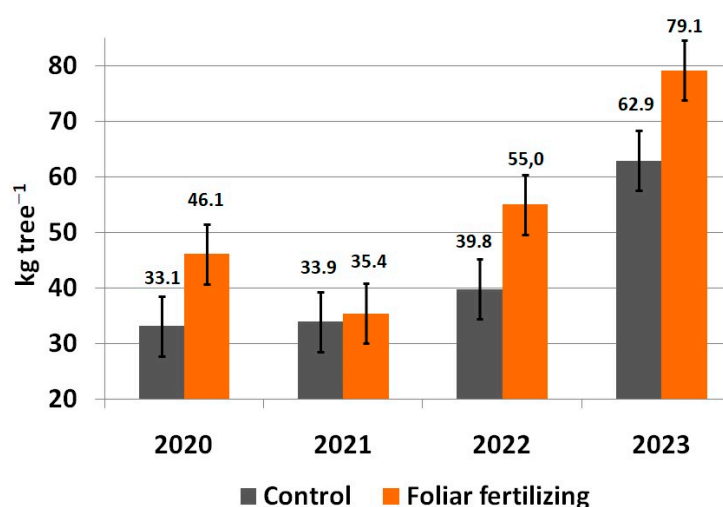


Figure 9. The yield of cultivar Bogatyr/B 118 apple trees under the influence of foliar fertilizing.

4. Discussion

Apple leaf Mo content varied greatly over the four years of study. We observed these differences both within separate growing seasons of the study and between certain years of the research.

The average leaf Mo contents over four study seasons for the control option ranged from 0.109 (2021) to 0.913 mg kg⁻¹ d.m. (2022), and for the foliar fertilizer treatment, this ranged from 0.160 (2021) to 1.048 mg kg⁻¹ (2022). That is, the leaf Mo status was highly variable across the years of study. Many authors report high variability of leaf Mo contents. In particular, Sharma [31] reported that leaf Mo contents in apple trees varied between 0.44 and 0.85 mg kg⁻¹. Other scientific papers mentioned above in the Introduction Section report alternative values for leaf Mo content, which also differ considerably from one another.

Mo takes part in the biosynthesis of various phytohormones and thus participates in the passage of the plant's hormonal cycle, which ensures the growth and productivity of plants, including apple trees. Thus, Mo, as part of the molybdenum cofactor (Moco), plays an important role in the synthesis of auxin [32]. In the study of Zhang et al. [33], it is well shown (using soybean as an example) how a good supply of Mo enhances plant growth by stimulating auxin synthesis. Moreover, some authors have reported on samples with different Mo content, which was associated with the different presence of Mo transporters GmMOT1.1 and GmMOT1.2, which stimulated the absorption and Mo transport in plants. At the same time, Moco is also part of aldehyde oxidase, which catalyzes the last stage of abscisic acid biosynthesis [21].

Thus, Mo participates in the plant hormonal cycle in two different stages. The first stage is the synthesis of auxins (a group of phytohormones controlling growth); the peak

of vegetative growth activity in apple trees occurs during the period after flowering (in the Tambov Oblast, this is the end of May–June). The second stage is during the ripening period of the fruits, when there is a peak in the activity of abscisic acid (August–September; this may vary in different cultivars). We observed peaks in leaf Mo contents in our study during these periods in 2020, 2021, and 2023, although with some seasonal variation. In 2022, the June peak in leaf Mo contents was most clearly expressed; however, by the end of July–beginning of August, the leaf nutrient status was also relatively high. The leaf Mo contents further remained at this level under the influence of foliar fertilizing, whereas in the control, it sharply decreased, possibly under the impact of dry weather. So, we do not believe that the first or the second peak in the Mo content is caused only by the influence of Mo application. We suggest that the peaks are a result of increased plant requirements for phytohormone synthesis and movement during seasonal development (auxin for initial shoot and fruit growth, and then abscisic acid and auxin for fruit growth and maturation).

The life activity of plants is strongly influenced by temperature, an essential abiotic factor; heat stress restricts many processes, such as nutrient uptake [34]. Because of weather conditions, the demand for Mo may change. The second peak's presence in non-treated plants supports our claim of rising plant Mo needs then.

There is no unambiguous identification of peaks of Mo content in some of the literature sources; in particular, Smith et al. [35] report that in an experiment conducted during one growing season, there was no apparent seasonal pattern for Mo. However, according to Stoller [36], the main peak in auxin content in plants is precisely at the end of seed formation and at the beginning of active vegetative growth. Nanda and Anand's [37] research on *Populus nigra* cuttings revealed high endogenous auxin in June, a consequence of the plants' active meristems at that time of year.

After the flowers are fertilized, seed development begins. Seeds and their endosperm also synthesize auxin. Furthermore, auxin is synthesized in the primary endosperm during embryo development and the secondary endosperm afterward, supporting fruit growth and preventing premature abscission [38]. Auxin synthesis increases sharply at the end of flowering due to fruit development and shoot growth. Reduced seed numbers in fruits cause a decline in auxin, leading to fruit abscission, impacting the growth of the remaining fruits [39]. Auxin in seeds increased to day 90 DAFB (days after full bloom), while fruit cortex auxin peaked at day 45 [40]. A study by Song et al. [41] on Fuji/M9 and Fuji/MM106 plans revealed an increase in auxin, plateauing around the first Mo peak (observed in Russian climatic conditions) on 30 to 55 DAFB, followed by a rapid decrease. These authors, however, found that auxin content increased after this reduction on 130 DAFB; thus, the second Mo peak may involve both abscisic acid and auxin synthesis.

It should also be taken into account that, when absorbed by roots, Mo has antagonistic interactions with sulfates, which undoubtedly complicates absorption from the soil, especially at high temperatures and drought [42]. At the same time, the transport of Mo in the plant is associated with the movement of sulfates. The transfer of Mo is carried out by the MOT1 protein (mitochondrial Mo transporter), which is also a major carrier of sulfates [43] and may also significantly limit the Mo transfer. So, the actual availability of Mo in the soil depends on a number of factors (acidity, organic matter content, and precipitation) [20]. Annual fluctuations in these factors may influence peak development in plants without Mo foliar applications.

The temperature rose above 35 °C on some days in 2020, in July and August, which is not quite normal for our climatic zone. It can be said that the plants experienced some heat stress. It is noteworthy that the second August leaf Mo peak in the control (without fertilizer application) was significantly lower than the June peak in this year, which occurred at a typical temperature for the region. Relatively little is known about

the influence of temperature on plant–nutrient interaction, paradoxically [44]. It has been found that even short-term heat stress can reduce root protein concentrations and nutrient uptake [45], which is primarily due to a decrease in protein activity. Previous studies have explored the influence of various factors, including temperature, on the activity of protein carriers responsible for transporting several nutrients—nitrate [46] and ammonium [47] nitrogen, phosphorus [48], iron [49], boron [50], and also molybdenum [33]. The influence of temperature stress in our study was partly confirmed by further results in 2021, when in the third ten-day period of June and in the second ten-day period of July, temperatures rose to 30–35 °C almost daily, and a second peak in leaf Mo contents in the control without fertilizer application was not observed. Also, the second peak of leaf Mo contents was not clearly expressed against the background of short-term heat stress in July–August 2022. Meanwhile, the comparatively mild July 2023 (maximum temperatures below 30 °C) saw a very noticeable second peak in leaf Mo for both control and foliar fertilizing treatments. At the same time, it is noteworthy that, when using foliar fertilizer, the second peak in Mo content was clearly expressed in 2020, 2021, and 2023, and the nutrient content was significantly higher than in the control variant.

The literature evidence indicates that weather, rather than cultivar characteristics, primarily determines the uptake of microelements such as molybdenum. The phenological phases of plant development determine the maxima and minima of nutrient absorption during the season [51]. Although Mo uptake is metabolically controlled, high temperatures increase MoO_4 fixation in acidic soils, thus potentially affecting long-term fertilizer effectiveness [52]. Certain competition between nutrients (nutrient uptake antagonism) may also influence Mo absorption by plant roots. For example, the elevated levels of iron, manganese, and zinc in the nutrient solution enhanced their accumulation but inhibited Mo absorption in the watercress [53]. This suggests that foliar Mo fertilizer application may be crucial for apple trees under heat stress.

5. Conclusions

We observed two peaks of increase in leaf Mo contents without micronutrient fertilizer application during the 2020, 2022, and 2023 seasons, which occurred over the periods of active shoot growth and fruit ripening. The peaks' reliability is confirmed by four years of research data, averaged and charted by phenological phases to show Mo content in leaves. This indicates that plants have a higher demand for Mo in these phenological stages, which is most likely associated with the synthesis of auxins (first peak) and auxin and abscisic acid (second peak). Thus, based on the results of our research, we can conclude that, during these periods, the need for Mo in apple trees increases; the best way to provide plants with Mo at the moment of greatest demand is foliar fertilizing.

Foliar fertilizing with a micronutrient complex, including Mo-containing agrochemicals, significantly increased the leaf Mo status during the observation period, changing the seasonal trend of its content in apple leaves in 2020, 2021, and 2022. The significant yield increase was noted in 2020, 2022, and 2023. We observed a correlation between air temperature and the leaf Mo contents. The interaction strength varied yearly in both control and foliar fertilizing treatments. In addition, only the foliar treatment demonstrated a consistent, negative correlation between 10-day rainfall and leaf Mo contents. To avoid negative effects, Mo applying to apple trees via foliar fertilizing in July's heat is crucial during the fruit growth and development stages.

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