PhD THESIS

The influence of some crop systems on the growth, fructification and fruit quality of the *Aronia melanocarpa* species in the pedoclimatic conditions of FRDS Bistrița

(SUMMARY OF THE PhD THESIS)

PhD student **Anca Maria Chiorean**

Scientific coordinator **Prof. univ. dr. Mirela Irina Cordea**

INTRODUCTION

Fruit cultivation, a hey part of agricultural sciences, boasts a long history and has significantly evolved with scientific advancements. Horticulture, a broad discipline, is deeply intertwined with the development of human civilizations, with archaeological evidence pointing to plant cultivation as far back as 10,000 years ago. Currently, horticulture emphasizes sustainable practices and smart technologies, and research focuses on enhancing plant cultivation, improving production quality, and ensuring their adaptability to climate change.

The cultivation of fruit shrubs, an essential component of fruit growing, has ancient origins, with evidence dating back to the Daco-Roman period. Their ability to adapt to diverse growing environments and the wide array of available species make fruit shrubs an appealing crop for producers. The fruits of these shrubs are a valuable source of vitamins and minerals, as well as antioxidants that play a vital role in regulating metabolic processes and maintaining health, ensuring optimal hydration, providing energy, and promoting satiety. Recently introduced species in Romanian cultivation, such as aronia, lonicera, and goji, have rapidly gained popularity due to the complex composition of their fruits, their remarkable nutritional properties, and their distinctive taste.

PHD THESIS STRUCTURE

The thesis entitled: ***The influence of some cropping systems on the growth, fructification and fruit quality of the Aronia melanocarpa species in the pedoclimatic conditions at SCDP Bistrița*****,** presents a comparative study carried out over three years at the Bistrița Fruit Growing Research and Development Station (SCDP Bistrița), evaluating four aronia cultivars ('Nero', 'Melrom', 'Viking' and 'Galicjanka') in two cultivation systems (ecologic and conventional), with an emphasis on growth parameters, productive potential and biochemical composition of the fruits growth in local pedoclimatic conditions.

The thesis contain 143 pages structured în nine chapters, with 71 figures and 22 tables. The thesis was divided in two parts, the first part includes the bibliographical documentation of the research, with one chapter with 12 figures which extends over 24 pages (20% of the total work). The second part is represented by the personal contribution which extends over 96 pages, comprises eight chapters and contains the research from the experimental years 2022-2024, 21 tables and 59 figures. The bibliography is made up of local and foreign scientific works, totaling 168 sources, of which 160 citations from publications and 8 web citations.

CURRENT STATE OF KNOWLEDGE

Chapter 1. General aspects of the species *Aronia melanocarpa* presents a brief retrospective of the national and global situation regarding the cultivation of fruit shrubs as well as current trends in the horticultural field. Also, detailed aspects of aronia are presented, including the origin, cultivation area, food, economic and therapeutic importance, biological and cultural particularities, as well as diseases and pests of the species.

PERSONAL CONTRIBUTION

Chapter 2. Research objectives presents the three main objectives that laid the foundations for the research carried out.

Chapter 3. Pedoclimatic characterization of the experimental area – this chapter presents the pedoclimatic conditions of the area in which the research was carried out, with a focus on the 2022-2024 study period.

Chapter 4. Study 1. Determination of the supply of plants and soil in nutrients and assessment of the phytosanitary status of the plantation - this chapter analyzes the nutritional status of the soil and aronia plants, materialized through soil and leaf analyses. This assessment aimed to identify the specific nutritional needs of the crop, in order to optimize the applied nutrition plan. The phytosanitary status of the chokeberry plantation during the experiment was maintained at an optimal level despite the annual presence of the hairy bug. The phytosanitary treatment had a predominantly preventive role, as no fungal or bacterial infections were identified.

Chapter 5. Study 2. **Determination regarding the phenology, growth and productive potential parameters of chokeberry varieties** presents a detailed description of the varieties, experimental results on phenology, aspects of the organization of the experiment and agrotechnical practices applied in the two cultivation systems (ecologic and conventional). Results on growth parameters (height, bush volume) and production indicators (productions/plant) are also presented. including statistical interpretation of the analyzed parameters,

Chapter 6. Study 3 – Determination regarding the identification and quantification of biochemical compounds in chokeberry fruits - this part of the study focused on the comparative analysis of the biochemical composition of the fruits of the 'Nero', 'Melrom' and 'Viking' varieties harvested in 2022, with the secondary objective of determining the influence of the crop systems on bioactive compounds. The 'Galicjanka' variety was omitted due to the lack of fruiting in the first year. The approach involved the identification and quantification of biochemical compounds (ascorbic acids, carbohydrates, polyphenols) by advanced chemical methods, in order to evaluate how the different cultivation systems could modify the composition of the fruits. This approach allowed for a detailed characterization of the biochemical profile

of chokeberry fruits, providing valuable insights into their nutritional properties and health implications.

Chapter 7. Study 4. Determination of total anthocyanin content (TAC) and antioxidant activity (AAOX) of the identified anthocyanin compounds in aronia fruits – the study aims were to quantify the total anthocyanin content (TAC) in aronia fruits and the specific antioxidant capacity of anthocyanins extracted using various analytical approaches for a better understanding of the profile of bioactive compounds and the impact of different cultivation systems on the quality of these compounds. This distinct analytical approach (differential pH method) from the one used previously is intended to contribute to a more detailed characterization of the biochemical profile of aronia berries, and to the identification of optimal strategies for maximazing their bioactive potential.

Chapter 8. Conclusions and recommendatios– this chapter synthesizes all the results obtained after three years of research, presenting the conclusions and recommendations derived from these data.

Chapter 9. **Originality and innovative contributions of the thesis** – this chapter details the significant contributions that the research brings to the horticultural field, with an emphasis on practical applicability for chokeberry growers.

RESEARCH AIM AND SCOPES

The present thesis aim was the comparative analysis of the performance of some aronia cultivars in two cultivation systems in order to determine their suitability to the pedoclimatic conditions at SCDP Bistrița.To achieve the purpose of this doctoral thesis, the following objectives were established:

- Determining the nutrients status of plants and soil and evaluating the phytosanitary condition of the aronia plantation
- Determinations regarding the phenology, growth parameters and productive potential of the studied aronia cultivars
- Determination of the chemical composition of aronia and the antioxidant activity of the athocyanin compounds

PARTICULARITIES OF THE EXPERIMENTAL ENVIRONMENT

The experiments were carried out at the Bistrița Fruit Research and Development Station (FRDS Bistrita), located in Northern Transylvania, on the Bistrița hills, at an altitude of 358 m and having the geographical coordinates 47°09'57"N 24°29'47"E. The meteorological data from the period 2022-2024, collected at the FRDS Bistrita, highlights a clear trend of increasing average annual temperatures. Compared to the multiannual average of 10.3°C, calculated by the NMA, the years 2022 and 2024 recorded significantly higher values, of 11.21°C, and 2023 was at 10.6°C.

This increase, with record highs reached in 2024, has a direct impact on fruit growing in the area. Although the total amount of precipitation has remained relatively constant, its uneven distribution, with periods of severe drought, especially in 2024, combined with rising temperatures, highlights the vulnerability of the area to climate change. The year 2024 was marked by a pronounced drought, with high temperatures and low precipitation, particularly affecting the vegetation period and fruit development

Study 1. Determination of plant and soil nutritional status and assessment of the phytosanitary conditiom of the aronia plantation

1. Purpose of the study

This study aim was to assess the general nutritional status of the soil and plants and the phytosanitary status, in order to improve the nutrition and phytoprotection plan. For this purpose, foliar analyses and soil analyses were carried out, thus allowing the identification of the nutritional needs of the crop, as well as observations on the attack of diseases and pests and their intensity.

2. Materials and methods

In 2022 year, the level of soil nutrients was assessed by analyzing the pedological profile at Pedological and Agrochemical studies Office (OSPA) Cluj-Napoca and in 2023, the nutritional status of the plants was investigated by foliar diagnosis performed at Research Institute for Soil and Agrochemistry (ICPA) Bucharest. For soil analysis, manual samples were taken from two different points of the experimental plot, from depths of 0-20 cm and 20-40 cm.

For foliar diagnosis, leaf samples from the varieties 'Nero', 'Melrom', 'Viking' and 'Galicjanka' were collected in September 2023. These were dried and ground at FRDS Bistriţa, then analyzed at ICPA Bucharest using specific methods for determining the concentration of nitrogen (Kjendahl), phosphorus (colorimetry), potassium and calcium (flamephotometry), magnesium (flame atomic absorption spectrophotometry) and microelements (copper, iron, manganese, zinc by atomic absorption spectrophotometry). The phytosanitary condition of the plantation was monitored through observations made at intervals of 3-5 days.

3. Experimental results regarding the nutritional status indicated that the soil in the experimental plot, has in general a good level of ecologic matter, nitrogen and potassium, within the recommended optimal limits, typical for this soil class, with slight phosphorus deficiencies.

Regarding the results of foliar analyses, generally acceptable levels of nutrients for aronia cultivation were identified, with a slight deficiency of nitrogen and potassium in the leaves, while the content of other macroelements (phosphorus, calcium, magnesium) and microelements (zinc, copper, manganese, iron) were within the recommended optimal limits. The results of soil and foliar analyses showed that, at the time of sampling, the plants were supplied with essential nutrients in optimal quantities to ensure stable and quality production. However, to prevent possible deficiencies or excesses of nutrients in the future, it is necessary to periodically

monitor the nutritional status of plants and adapt fertilization to the evolution of the crop.

Study 2. Determinations regarding the phenology, growth parameters and productive potential of aronia cultivars

1. Aim of the study

The aim of the study is the comparative evaluation of growth parameters, phenology and productive potential of four aronia cultivars in two cultivation systems (organic and conventional).

2. Materials and methods used in the study

The experiment was designed as a bifactorial model, with two main factors: Factor A represented by four chokeberry varieties ('Nero', 'Melrom', 'Viking' and 'Galicjanka'), and Factor B, consisting of two cultivation systems (conventional, and ecologic) and untreated variant. From the combination of these factors resulted 12 experimental variants. To avoid interference of phytosanitary treatments, an "isolator" plant was used between the variants.

3. Results on the phenological characterization of aronia cultivars in the experimental years 2022-2024

The aronia cultivars ('Nero', 'Melrom', 'Viking' and 'Galicjanka') had a similar phenological behavior in the study years, climatic variations did not significantly influence the development of the phenophases.

The results reveal that all four cultivars developed a simultaneous phenological evolution in 2022- 2023 experimental years, under the influence of relatively constant climatic conditions. The 'Galicjanka' cultivar had an atypical development in 2022, possibly due to transplant shock, recovered later with low yield. The entire vegetative development period did last between 187 and 192 days. The 2024 year was atypical from a phenological point of view due to the high temperatures in spring time and during vegetation period. Physiological precesses were accelerated due to climate warming, thus, a shortening of the fruit development and ripening period by approximately three weeks. These results highlight the need for a long term observations and investigation.

4. Results on growth parameters and productive potential of the four aronia cultivars in the experimental years 2022-2024

The 'Nero' and 'Melrom' cultivars proved to be excellently adapted to the local pedoclimatic conditions, recording constant growth throughout the study, regardless of the cultivation systems (conventional or ecologic). The 'Viking' cultivar showed promising, with good adaptation and constant height growth, uniforming its development over time in both cultivation system. The 'Galicjanka' cultivar, although with a slower initial growth, demonstrated a good recovery capacity a positive evolution in height and increasing yields. The study confirms that both conventional and ecologic cultivation methods are viable for the studied cultivars.

The dynamics of the aerial part volume of aronia plants highlights the importance of an individualized approach for cultivation, adapted of each cultivar. The

'Nero' and 'Galicjanka' cultivars developed a larger plant volume in the ecologic system (V2) and 'Melrom' responded favorably to the conventional system (V1). Compared to the untreated variant (V3), the differences were significant. Environmental factors and varietal characteristics influenced growth more than the type of treatment. These results are suggesting that there is no optimal cultivation system regarding plant volume.

Regarding the productive potential, to 'Nero' and 'Melrom' cultivars, was manifested by early fruiting and increasing yields, ranging between 1kg-2kg/plant. The cultivation systems did not significantly impact this parameter, suggesting that similar yields can be achieved for this cultivars regardless the cultivation system adopted. An increase in the average production/plant was observed for 'Viking' cultivar over two experimental years, with a notable variation between 0.110 kg - 0.495 kg/plant. In contrast, the 'Galicjanka' performed below expectations in the conditions of Bistrița, with low production (0,052 kg – 0,220 kg/plant) and a slow adaptation to the local environment. The productive potential of the cultivars was not fully explored in 2024, when an extreme weather event (hail) affected production, significantly reducing the final harvest.

Despite some extreme events, aronia demonstrated its remarkable robustness and resistance even in difficult conditions. These results demonstrate that there is no universal method for cultivating aronia, but that success depends on adapting practices to each cultivar. Environmental factors and genetic characteristics had a greater impact on growth and productivity than the type of treatment.

Observations made throughout the study, showed that the phytosanitary condition of the aronia plantation was optimal with no fungal or bacterial infections. This favorable situation can also be attributed to the absence of other aronia plantations in the area, which eliminates the risk of infections, as well as the fact that it is a species that is not susceptible to diseases and pests.

Study 3. Identification and quantification of biochemical compounds in chokeberry fruits

1. Purpose of the study

The purpose of this study was to comparatively analyse the biochemical composition of fruits from the cultivars 'Nero', 'Melrom', 'Viking' in 2022 and to identify how different cultivation systems can influence the bioactive compounds in the fruits. The cultiva 'Galicjanka' was excluded from this part of the study because no fruits were obtained in 2022.

2. Material and methods

The fruits of the three analyzed cultivars ('Nero', 'Melrom', 'Viking') were harvested at maturity and immediately frozen to preserve the integrity of the bioactive compounds. The methods used to identify the biochemical compounds in the fruits were the HPLC method, or high-performance liquid chromatography. This method is an analytical technique used to separate, identify and quantify the components of a liquid mixture. In the study, high-purity reagents were used for the determination of carbohydrates and phenolic compounds. Carbohydrate quantification was performed

using 5-concentration calibration curves, and for phenolic compounds, specific calibration curves (gallic acid, chlorogenic acid, rutin, cyanidin) were used, each with a high correlation coefficient.

3. Results regarding on biochemical compounds identified in chokeberry fruits

The analyses also revealed significant variations in the content of ascorbic acid (AA) and dehydroascorbic acid (DHAA) in chokeberry fruits, which varied significantly depending on the cultivas and cultivation system, aspects with direct implications on the nutritional and antioxidant value. The ecologically (V2) grown 'Nero' cultivar presented the highest levels of these compounds, significantly exceeding the 'Viking' and 'Melrom' cultivars, highlighting it as a valuable source of vitamin C.

Regarding the glucides, the results of the analysis showed that sorbitol was the main component with significant variations between cultivars and a positive influence of the ecologic system on the total content. The high proportion of sorbitol (approximately 60% of total carbohydrates) is a distinctive feature of aronia berries. The sugar content was also variable, with the ecologically grown 'Melrom' cultivar having the highest concentration, suggesting a positive impact of this system on the carbohydrate profile. These findings highlight the nutritional value of chokeberry, including its potential as a source of beneficial carbohydrates, and the presence of sorbitol, a sweetener with a low glycemic index, makes it suitable for people with diabetes.

The composition of anthocyanins in chokeberry, dominated by cyanidin-glucoside, varied significantly between cultivars and cultivation methods. Four anthocyanins were identified, with the predominance of glycosylated forms of cyanidin. Cyanidin-glucoside reached maximum content in the control variants of the cultivars 'Melrom' and 'Nero', indicating efficient accumulation under natural conditions (untreated variant/V3). The cultivar 'Melrom' had higher content of cyanidin-arabinosides and xylosides, especially in the ecologic and control variants. The high content of anthocyanins in the control variants suggest a synthesis independent of external interventions. The 'Viking' cultivar, in ecologic cultivatiom system, had the highest comtent of anthocyanins. Both cultivation methods provide valuable sources of anthocyanins, but the ecological system may influence their specific profile. The content of hydroxycinnamic acids, antioxidant and anti-inflammatory compounds, varied significantly between chokeberry cultivars. The 'Viking' cultivar presented the lowest, while 'Melrom' and 'Nero' had high and similar content, suggesting the influence of genotype in the synthesis of these compounds. The ecologic variant of the 'Nero' cultivar was distinguished by maximum content of caffeic acid-glucoside and 5-feruloylquinic acid, and the ecologic variant of the 'Melrom' variety had a higher concentration of 3-feruloylquinic acid.

The flavonols identified in the control variant/V3 of the 'Nero' cultivar are distinguished by high content, similar to the ecologic variant of the 'Melrom' cultivar. From this point of view, 'Viking' presents a distinct profile, with high amounts of cyanidin and quercitin, but low amounts of quercitin-rutinoside.

The study of the concentration of total phenolic compounds in aronia extracts revealed the distinct biochemical profile of the three analyzed cultivars. It was found that anthocyanins (43%) and hydroxycinnamic acid (40%) are the predominant phenolic compounds, while flavonols and hydroxybenzoic acid are minor (below 15%). Interestingly, the ecologic variant of the 'Melrom' cultivar accumulated the highest amount of phenolic compounds, in the 'Nero' cultivar, polyphenol levels were relatively constant between variants, and in 'Viking', the ecologic variant (V2) stood out for the highest concentration of polyphenols. These results suggest that both genetic factors and the cultivation system significantly influence the synthesis and accumulation of these beneficial compounds.

Study 4 - Determinations of total anthocyanin content (TAC) and antioxidant activity (AAOX) of identified anthocyanin compounds

1. The aim of the study

The study aim is to determine the total anthocyanin content (TAC) in aronia berries using various analytical approaches (differential pH method), evaluating the antioxidant activity (AAOX) specific to anthocyanin compounds in order to identify how crop systems can influence the content of bioactive compounds in aronia.

2. Material and methods

The total anthocyanin content (TAC) was determined by the differential pH method, a rapid and accurate technique that quantifies monomeric anthocyanins. The method is based on the reversible structural changes of the anthocyanin chromophore as a function of pH. At pH 1.0, anthocyanins are colored pink (cationic flavylium form), and at pH 4.5 they become colorless (carbinol-hemiketal or chalcone form). A UV-Vis spectrophotometer calibrated and set to the wavelength corresponding to the maximum absorption of anthocyanins (515 nm) was used.

The antioxidant capacity (AAOX) of aronia berries was measured by the ABTS method. This method evaluates the total antioxidant capacity of various samples (pure substances, aqueous mixtures, beverages) by directly generating the blue-green chromophore ABTS•+, which absorbs light maximally at 734 nm. The value obtained for each solvent was reported to the average antioxidant capacity of the entire set of compounds analyzed by the ABTS test, by summing the results.

3. Results regarding the total anthocyanin content (TAC)

The analyses regarding the total anthocyanin concentration (CTA) in 2023 indicated that the 'Viking' and 'Galicjanka' cultivars had a higher content of total anthocyanins (TAC) in the ecologic system (V2) in 2023, while 'Nero' and 'Melrom' presented similar values in the two cultivation systems (conventional and organic) but also in the untreated variant (V3). The analysis of the total anthocyanin content (CTA) in aronia fruits in 2024 revealed a complex influence of the cultivar and climatic factors, with a different pattern than was observed in 2023.

The results indicate a high variability depending on the cultivar, of the total anthocyanin content in 2023 and 2024. The 'Viking' and 'Galicjanka' cultivars had a higher anthocyanin content in the ecologic system (V2) in 2023 while 'Nero' and

'Melrom', presented close values in both cultivatios systems but also in the untreated variant (V3).

The severe drought in 2024 had a generalized negative impact on the accumulation of anthocyanins, causing a decrease in content in most variants and cultivars. Notable exceptions, where higher values were recorded than in 2023, were in the conventional system of the 'Galicjanka' cultivars. These results suggest a specific interaction between genotype and environmental conditions regarding anthocyanin synthesis, with a significant impact on this process.

4. Results on the antioxidant activity (AAOX) of anthocyanin compounds

The antioxidant activity of anthocyanins in aronia berries showed significant variability between cultivars in response to the cultivation system applied during the two-year study. The 'Galicjanka' and 'Nero' cultivars showed superior antioxidant activity in the organic/V2 system, followed by the conventional/V2 in 2023. In contrast, the cultivars 'Viking' and 'Melrom' showed the highest antioxidant activity of anthocyanin compounds in the untreated/V3 variant, surpassing the ecologic/V2 and conventional/V3 systems. These results highlight the specific influence of the cultivar and the climatic conditions specific to the experimental year on the antioxidant potential of anthocyanins in chokeberry berries.

Under the conditions of 2024, 'Nero' and 'Melrom' showed stability of antioxidant activity, with relatively similar values in the two cultuvation systems and very close to the values of the untreated variannt/V3, suggesting a significant influence of the specific conditions of the year on the quality of anthocyanin compounds.

The notable decrease in antioxidant activity in 2024 in 'Galicjanka' in both cultivation system and for 'Viking' in the ecologic system (V2) highlights the need to consider the complex interaction between genotype and environmental conditions in optimizing the antioxidant activity of chokeberry fruits. An exception to this trend is the untreated variant/V3 of the 'Nero' and 'Galicjanka' varieties, which under the same year conditions recorded an increase in antioxidant activity of approximately 1.21%.

The conclusion is that annual variations in antioxidant activity were observed to be dependent on the cultivar and specific climatic conditions, emphasizing the influence of environmental factors.

CONCLUSIONS AND RECOMMENDATIONS

1. The studied aronia cultivars 'Nero', 'Melrom', 'Viking' and 'Galicjanka' have adapted remarkably to the pedoclimatic conditions in the Bistrița area, and can be successfully cultivated in both conventional and organic cultivation systems.
2. The biochemical profile of aronia cultivars is complex and rich in ascorbic acids and flavonols in the 'Nero' cultivar, carbohydrates and polyphenols in the 'Melrom' cultivar, anthocyanins and high antioxidant activity in the 'Viking' and 'Galicjanka' cultivars.
3. In order to ensure constant and quality production, it is recommended to cultivate several aronia cultivars in combination. Thus, early cultivar with high yields such as

'Nero' and 'Melrom' can be combined with cultivars intended for fresh consumption and processing such as 'Viking' and 'Galicjanka'.
4. Due to its versatility and high adaptability to various cultivation systems and geographical areas, aronia is an excellent option for growers and is highly appreciated.

ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

Aronia is a species of fruiting shrub recently introduced for cultivation, which makes information about its behavior in different regions of Romania limited. Through the studies presented in the thesis, additional information and the most recent research on the adaptability of this species to the pedoclimatic conditions specific to the Bistrița area are brought, as well as to identify the most efficient cultivation and processing methods.

Through the research, new information was brought about about the behavior of four aronia varieties in different cultivation systems with the aim of maximizing the production and quality of the fruits. Through these efforts, the doctoral thesis aims to provide an in-depth and useful perspective for growers and processors in Romania, facilitating the development of aronia culture and promoting its health benefits.

Aronia species represents a novelty for the Fruit Research and Development Station Bistrița (SCDP Bistrița) marking the resumption of research on fruiting shrubs after a long period of absence. Through this initiative, the aim is to explore the adaptability of chokeberry cultivars to local conditions, develop optimal cultivation systems and provide specialized support to growers in the area.

The comparative evaluation of conventional and ecologic cultivation systems applied to romanian and foreign aronia cultivars represents a rigorous scientific approach, essential for the sustainable development of this crop in the Bistrița area.

The study of the chemical composition of four aronia cultivars provides valuable information about their biochemical profile, cultivated in an area where the scpecie is still a novelty. The results obtained complement existing knowledge and may contribute to the development of aronia cultivation in the region.

Research conducted under the pedoclimatic conditions at SCDP Bistrița has generated valuable data on the behavior and adaptation of aronia cultivars, highlights both the strengths and vulnerabilities of this species.