

Peculiarities of the metabolism of fruit vines *Actinidia arguta* (Siebold & Zucc.) Planch. ex Miq. and *Schizandra chinensis* (Turcz.) Baill. in mixed and mono-planting growth

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Purpose. A comparative study of the interaction between *A. arguta* and *S. chinensis* plants in mono- and mixed plantings was carried out. This study examined the pigment complex of the plants and the accumulation of flavonols and proline in their vegetative organs. The aim was to optimise the technology for growing these promising fruit plants and realise their productive potential. This research was conducted at the M. M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv). **Methods.** The quantitative content of pigments, flavonols and proline was determined using the spectrophotometric method with a Zalimp KF 77 spectrophotometer (Poland), in accordance with the relevant procedures. Plants that had grown for 40 years in grey forest soil (pH 6.5–7.0) were analysed. **Results.** Analysis of the experimental data revealed significant differences in flavonol content and accumulation dynamics between the studied species in mono- and mixed plantings during the growing season. Leaves of actinidia in mono-plantings had higher levels of these compounds than plants in mixed plantings. The accumulation of proline in actinidia leaves in combined plantings with magnolia vine was lower throughout the entire research period compared to mono-species plantings. Leaves of magnolia vines in monoculture accumulated 20% less proline than plants growing alongside actinidia. Mixed plantings were found to result in a 1.6-fold higher accumulation of chlorophyll in *A. arguta* compared to mono-species plantings. **Conclusions.** *A. arguta* plants grown alongside *S. chinensis* plants exhibit improved growth compared to those grown in mono-cultures. *S. chinensis* is an autotolerant plant for which mono-species plantings are preferable. The proline and flavonoid content of vine leaves, as well as their pigment complex, can serve as an indicator of the plants' physiological state and competitiveness in garden cenoses.

Keywords: allelopathy; biochemical indicators; secondary metabolites; photosynthetic pigments.

Introduction

Modern fruit plantations often rely on mono-culture orchards with closely spaced plants. This practice typically results in soil fatigue and a decline in the stability and productivity of orchard ecosystems. In order to counteract the detrimental effects of monoculture in fruit plantations, it is crucial to increase species diversity. According to the concept of ecological horticulture, the primary principle for developing orchard ecosystems is to optimise their structure by creating mixed, multicomponent plantations, thereby shifting from monoculture to polyculture. Ecosystems with greater species diversity tend to be more efficient, stable, productive and resilient than those with fewer species [2, 3].

At the current stage of horticulture development, the issue of introducing rare fruit plants into cultivation and developing effective cultivation technologies to improve the structure of garden phytocenoses and green horticulture in general remains important. Therefore, research into the interaction between perennial plants in mixed plantings and their subsequent effects is particularly relevant.

Experiments focusing on the joint cultivation of non-traditional and rare fruit crops are of particular interest. Specifically, these include the deciduous vines of *Actinidia arguta* (Siebold et Zucc.) Planch. ex Miq. and *Schisandra chinensis* (Turcz.) Baill. – magnolia vine. These plants produce fruits that are rich in biologically active substances and can be eaten fresh. They are also widely used in the production of various products. Despite their high nutritional and medicinal value, there is currently insufficient scientific information regarding their cultivation.

In order to introduce these new species into horticulture on a large scale, it is vital to assess their adaptive capabilities, study the conditions

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and factors that affect their growth and development, and investigate their mechanisms of resistance to adverse environmental conditions. Furthermore, it is essential to understand their allelopathic properties, which influence species compatibility in mixed plantings, in order to create production plantations.

Previous research on the allelopathic activity of fruit vines suggests that *Actinidia* and *Schisandra* (Chinese magnolia vine) are auto-intolerant crops. This means that the substances they secrete, such as leaf exudates, root diffusates, fallen leaves, shoots, flowers and fruits, contain compounds that inhibit the germination of their own seeds and the growth of various test plant seedlings [4].

An examination of the phytotoxicity of soil beneath *A. arguta* and *S. chinensis* after long-term monoculture growth revealed interesting results. Soil from under *A. arguta* plants did not exhibit any phytotoxic effects on the test plants, whereas soil from under *S. chinensis* plants showed high levels of phytotoxicity [5].

There is substantial evidence that each organism within a phytocoenosis has the ability to produce various metabolic products that affect the surrounding environment. These products can be toxic, beneficial or neutral for nearby plants. As they grow and develop, plants create an allelopathic sphere around themselves, leading to allelopathic interactions within plant communities [6, 7]. Allelochemicals, which can be primary or secondary metabolites, are produced by plants during growth and are influenced by environmental conditions.

It is essential to study the mutual influence of plants as an environmental factor, particularly the way in which allelochemicals enter the environment and affect nearby plants [8]. When plants are exposed to adverse conditions, they experience stress, which can manifest at genetic, metabolic, morphological and physiological levels [9]. This stress can affect the health and development of plants, leading to changes in photosynthetic activity, increased levels of the amino acid proline and the accumulation of phenolic compounds [10]. These changes indicate the degree to which plants can resist various negative factors.

This study focuses on the biochemical characteristics of plants grown in monocultures and mixed plantings. The aim of this research is to reveal the patterns of interaction between different plant species, with the potential to optimise cultivation techniques and enhance fruiting in the future.

Specifically, the research compares the interactions between *A. arguta* and *S. chinensis* in

garden ecosystems under mono- and mixed planting conditions. It also assesses the aftereffects of these interactions by examining the plants' pigment complexes and measuring flavonol and proline accumulation in the leaves of perennial species.

Materials and Methods

The study was conducted in the collection areas of the M. M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (NBG) using standard scientific fruit-growing methods [11]. The subjects of the study were the twenty-year-old perennial vines *A. arguta* 'Sentiabrskaia' and *S. chinensis* 'Sado-vyi-1'. The vines were planted in rows 2 metres apart with 3 metres between each row.

Samples were collected in June, August and early October 2022. The experimental variants included:

- I) a mixed planting of 50% actinidia and 50% Chinese magnolia vine;
- II) a mono-planting of 100% actinidia;
- III) a mono-planting of 100% Chinese magnolia vine;
- IV) a magnolia vine planted after previously grown actinidia;
- V) a Chinese magnolia vine planted after a Chinese magnolia vine that previously grew in this plantation.

To analyse the flavonols, 0.5 g of dried, crushed raw materials was placed in a flask. Next, 3 ml of 80% ethanol was added and the mixture was heated in a water bath for 45 minutes. Once heating was complete, the flask was left to cool to room temperature. The resulting suspension was filtered through a paper filter into a 100 ml volumetric flask. The solution was adjusted to a final volume of 100 ml with more 80% alcohol, resulting in solution A.

Two millilitres of solution A were transferred to a 25-millilitre volumetric flask. Then, 1 ml of a 2% aluminium chloride solution in 95% ethanol was added and the volume was made up to 25 ml with 95% alcohol. After waiting for 20 minutes, the optical density of the solution was measured using a Zalimp KF 77 spectrophotometer (Poland) at a wavelength of 390 nm, using a cuvette with a 10 mm light path length. For the control, a mixture of aluminium chloride and acetic acid solutions was used [12].

The concentration of flavonols, denoted as C_{flav} , in terms of rutin in dried raw materials is calculated using the following formula:

$$C_{flav} = (R \times D \times K \times A) / m,$$

In this formula:

R represents the tangent of the slope of the calibration graph showing the relationship be-

tween the optical density of the test solution and the flavonol concentration (with a value of 0.1062); D is the measured optical density; K is the conversion factor for dried weight (1 in this case); A is the volume of the sample; and m is the sample weight in grams.

The quantitative content of proline was determined using the method described in reference [13]. First, 0.5 g of plant material was homogenised in 10 ml of a 3% sulfosalicylic acid solution and the resulting mixture was filtered through double filter paper. In a test tube, 2 ml of the filtrate was reacted with 2 ml of acidic ninhydrin and 2 ml of ice-cold acetic acid for one hour at 100 °C. The reaction was stopped by placing the test tube in an ice bath and then combining the reaction mixture with 4 ml of toluene for 20 seconds. The resulting coloured toluene solution was separated from the aqueous phase and analysed using a spectrophotometer at a wavelength of 520 nm, with toluene serving as the control.

The proline concentration (Cpr, $\mu\text{mol/g}$ wet weight) was determined using a standard curve and calculated according to the following formula:

$$Cpr = (D \times K \times V) / m$$

where: D is the optical density of the solution, K is the calibration curve coefficient (217.49), V is the extract volume in millilitres and m is the sample weight in grams.

Experiments were performed with three biological and three technical replicates for each variant. The resulting data were processed using variational statistical methods. The results are presented as the mean $\pm$ standard error ($M \pm SE$). The significance of the differences in means was assessed using a Student's t -test. A level of statistical significance of $p < 0.05$ was considered significant. Statistical data processing was performed using the IBM SPSS Statistics package (version 27.0.1).

The content of photosynthetic pigments was determined by spectrophotometry. The pigment complex was studied in the first decade of June, which is the period of most intensive shoot growth. Carotenoids were measured at $\lambda = 440.5$ nm, a at $\lambda = 665$ nm, and chlorophyll b at $\lambda = 649$ nm. Pigment extracts from leaves were prepared in 96% alcohol for the measurement. Measurements for each pigment extraction were carried out in tenfold replicates. The concentrations of chlorophylls a and b (mg/L) in the extract were calculated using the Vernon formula. The Vettstein formula was used to determine the carotenoid concentration (mg/L) in the total pigment extract [14]. The pigment content (A , mg/g of wet weight) in the extract was determined using the following formula:

$$A = C \times V / (P \times 1000),$$

where: C – pigment concentration (mg/L); V – extract volume (mL); P – plant material weight (g).

Results and Discussion

Phenolic compounds are among the most important classes of allelochemical due to their widespread presence in the plant kingdom and their diverse effects on key physiological and biochemical processes, such as respiration, photosynthesis, growth and development [15]. They play a crucial role in chemical interactions between plants and are among the allelopathic substances found in fruit crops. The accumulation of flavonoids is considered an indicator of non-specific plant resistance to stress. Analysis of experimental data revealed significant differences in flavonol content and accumulation dynamics between the studied plant species under various cultivation conditions. At the beginning of the growing season (in the first or second decade of June), flavonol content in plant leaves is at its maximum, corresponding to growth and metabolic processes. Interestingly, leaves of *Actinidia* grown in mono-plantings exhibited higher levels of flavonoids than those grown in mixed plantings. Conversely, the opposite trend was observed with magnolia vines, where the flavonol content was higher in leaves growing under joint conditions.

A decrease in flavonols was observed in the leaves of both *A. arguta* and *S. chinensis* across all experimental variants. This decline lasted until mid- to late August, after which these compounds accumulated actively in the leaf mass of the plants, a trend that continued until November. This accumulation is likely related to the plants' excretory functions, the cessation of metabolic activity and their preparation for winter. Furthermore, comparing the average flavonol content with weather conditions reveals that the highest levels of these compounds were present in the leaves during pronounced moisture deficits in June, when daytime temperatures reached 30 °C. This suggests that an increase in flavonol content is a plant's response to drought as a stress factor. Conversely, an increase in flavonol content in the leaves of *S. chinensis* (under mono-planting conditions) was only observed at the end of the dry period. This may suggest that *A. arguta* is more adaptable than *S. chinensis* in response to stress caused by high temperatures and drought.

One of the primary non-specific responses of plant cells to stress factors is an increase in the amino acid proline. During the study, it was ob-

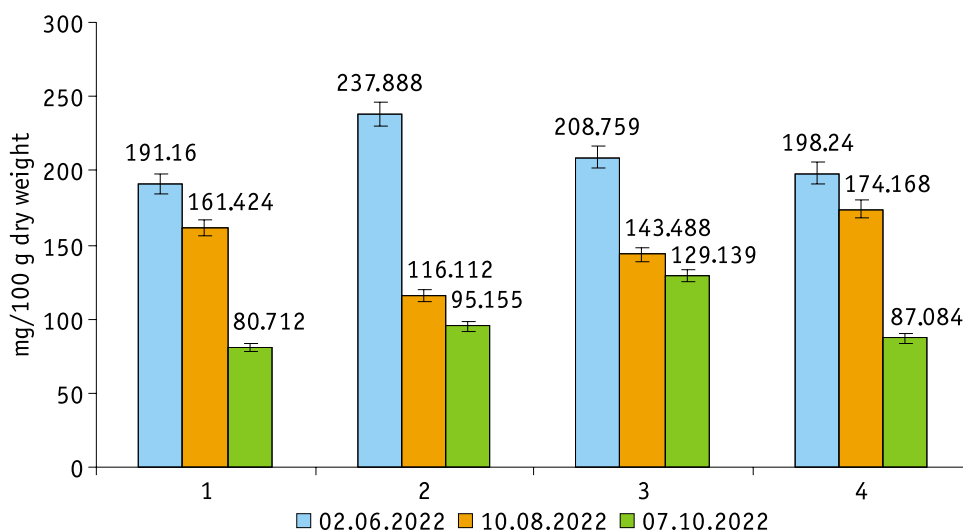


Figure 1. The flavonol content in the leaves of *A. arguta* and *S. chinensis* during the growing season, comparing mono- and mixed plantings.

The groups are as follows: 1 – Actinidia in mixed planting (I); 2 – Actinidia in mono-planting (II); 3 – Magnolia vine in mixed planting (I); 4 – Magnolia vine in mono-planting (III)

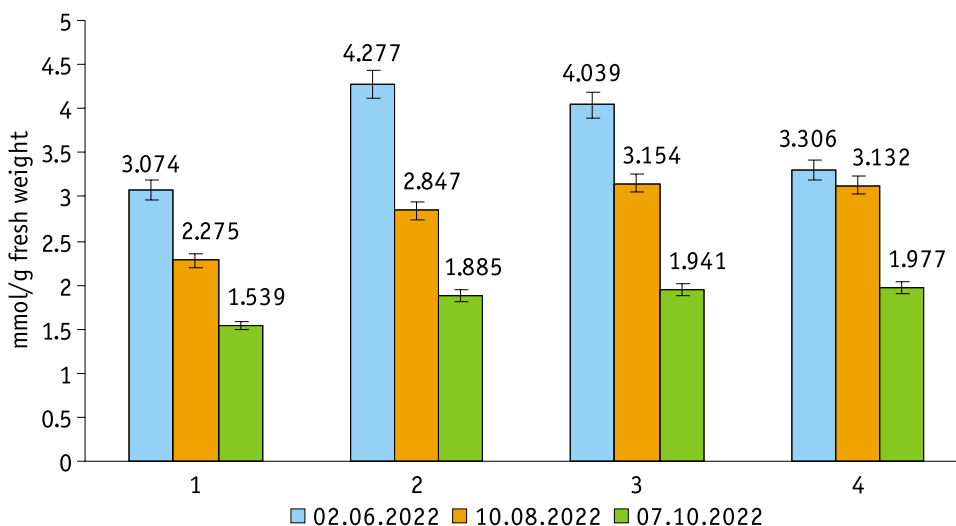


Figure 2. Proline content in the leaves of *A. arguta* and *S. chinensis* during the growing season was measured in both mono- and mixed plantings.

The study included the following setups: 1 – Actinidia mixed planting (I); 2 – Actinidia mono-planting (II); 3 – planting (II) Magnolia vine mixed planting (I); 4 – Magnolia vine mono-planting (III)

served that the accumulation of proline in the leaves of actinidia was lower in mixed plantings with magnolia vine than in monoculture. Conversely, the amount of proline accumulated in the leaves of magnolia vines grown in monoculture was 20% lower than in vines growing alongside actinidia. This suggests that magnolia vine enjoys more favourable growing conditions in monoculture.

The photosynthetic activity of plants is a key factor in determining the productivity of garden agroecosystems. It is well established that the photosynthetic apparatus of plants responds to various agronomic practices by undergoing specific changes. These changes can include alterations in total chlorophyll content, the ratio of

chlorophyll *a* to chlorophyll *b* and carotenoid levels. Carotenoids are pigments that protect the photosynthetic apparatus from photooxidation caused by adverse environmental conditions [17]. Research on pigment accumulation dynamics has shown that mixed plantings have a higher chlorophyll content than mono-species plantings (see Fig. 3a, b). Notably, the chlorophyll *b* content of *A. arguta* leaves was found to be 59% higher in mixed plantings than in mono-species plantings.

Research has shown that the total amount of chlorophyll *a* and *b* in the leaves of *A. arguta* in single-species plantings is 5.75 mg/g of raw plant weight. In mixed plantings, this figure increases by 20% to 6.90 mg/g. In magnolia vines,

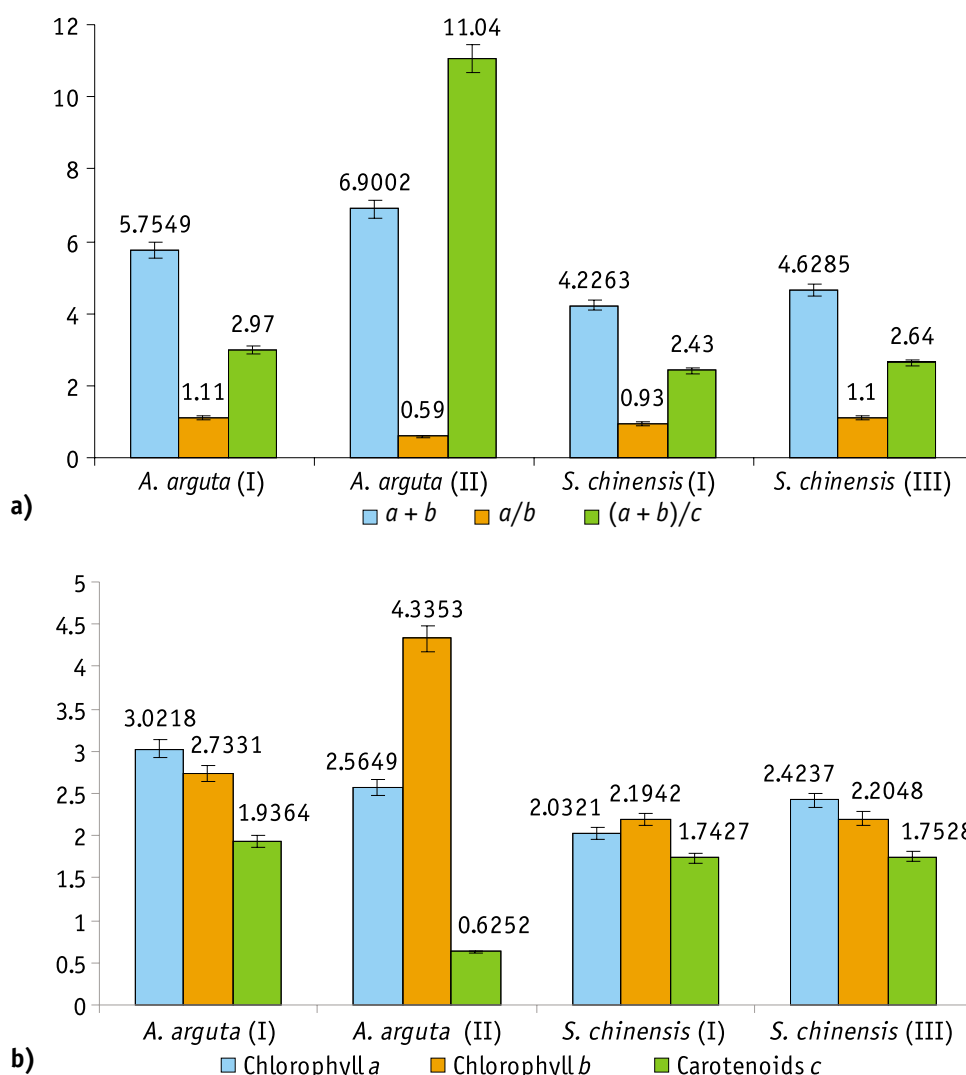


Figure 3. The content of photosynthetic pigments in the leaves of *A. arguta* and *S. chinensis* (measured in mg/g of raw weight) is presented along with their ratios in mono- and mixed plantings: *A. arguta*: (I) Mixed planting, (II) Mono planting; *S. chinensis*: (I) Mixed planting, (III) Mono planting

the sum of chlorophyll *a* and *b* in single-species plantings is 4.22 mg/g, whereas in mixed plantings it is 4.63 mg/g. The ratio of chlorophyll *a* to chlorophyll *b* in *A. arguta* is 1.11 in a single-species planting and drops to 0.59 in a mixed planting. In contrast, the ratio for *S. chinensis* is 0.93 in a single-species planting and increases to 1.10 in a mixed planting. *A. arguta* has slightly higher levels of chlorophyll *a* than *S. chinensis*. As chlorophyll *a* is more efficient in the photosynthesis process of converting carbon dioxide and water into organic substances, *A. arguta* is considered a more productive crop than *S. chinensis* in terms of biomass. The amount of chlorophyll *b* in *A. arguta* plants increases significantly (by 1.6 times) in mixed plantings compared to mono-species plantings. Conversely, the amount of chlorophyll *a* in *S. chinensis* leaves in mixed plantings increases by 20%, while the chlorophyll *b* content does not differ

significantly between mixed and mono-species plantings. In summary, although the ratio of chlorophyll *a* to chlorophyll *b* decreases, the leaves of *A. arguta* in mixed plantings have a higher overall chlorophyll content.

Maintaining carotenoid levels supports photosynthesis during stressful conditions. Carotenoids protect plant cells from increased reactive oxygen species and stabilise chloroplast membranes [18]. Additionally, changes in the chlorophyll-to-carotenoid ratio indicate the restructuring of light-harvesting complexes in photosystems and the enhanced role of carotenoids as supplementary light-gatherers in the blue-violet region of the solar spectrum. In *A. arguta*, the ratio of total chlorophyll to total carotenoids in the leaves ranges from 2.97 to 11.04, whereas in *S. chinensis* it ranges from 2.43 to 2.64. The highest ratio (11.04) is observed in *A. arguta* leaves in mixed planting conditions,

whereas *S. chinensis* reaches a maximum ratio of only 2.64.

The data obtained are consistent with the results of an experiment conducted by Japanese scientists which demonstrated that tomato plants (*Solanum lycopersicum*) thrive when grown alongside members of the *Lamiaceae* family. This mixed planting enhances growth through changes in secondary metabolite and amino acid content. Specifically, root exudates from *Lamiaceae* improve soil properties and positively influence its microbiota [19]. Studies suggest that *A. arguta* is a dominant species compared to *S. chinensis*. Dominant species typically experience strong intraspecific competition and tend to thrive better when grown alongside other plant species. In such scenarios, intraspecific competition is often replaced by weaker interspecific competition [20].

Like all plants, fruit crops absorb essential mineral nutrients and organic compounds during their life cycles. They also actively release various metabolites into their environment. These metabolic products can accumulate in the soil, creating a unique biochemical environment that influences the growth of both related and unrelated plant species [16]. A study evaluating the effects on *S. chinensis* plants of Actinidia (*A. arguta*) and magnolia vine plants that had been growing for over 40 years and were subsequently removed showed interesting results. The leaves of *S. chinensis* accumulated higher amounts of the stress-related compound proline than those of plants that were planted afterwards. This suggests that *S. chinensis* may have a degree of self-tolerance and indicates the strong allelopathic activity of Actinidia root secretions.

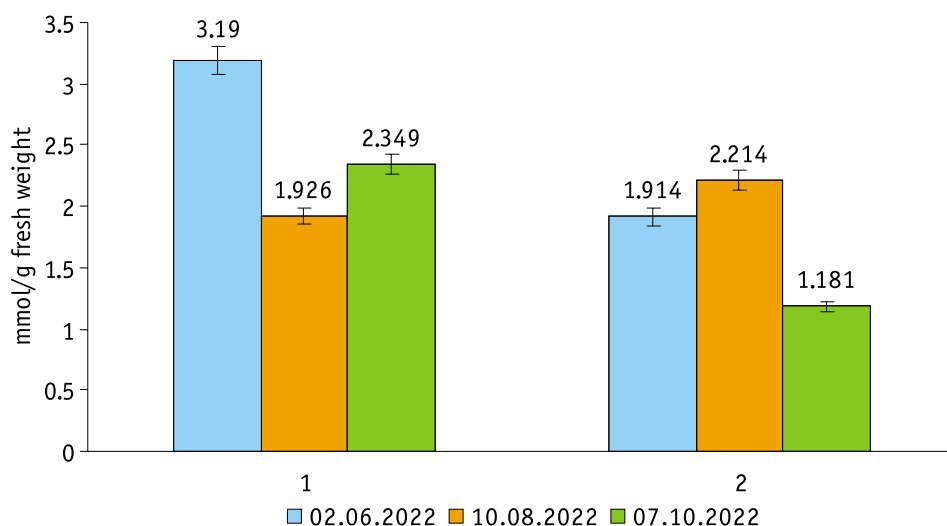


Figure 4. Proline content in the leaves of *S. chinensis* during the growing season:
1 – magnolia vine after actinidia (IV); 2 – magnolia vine after another magnolia vine (V)

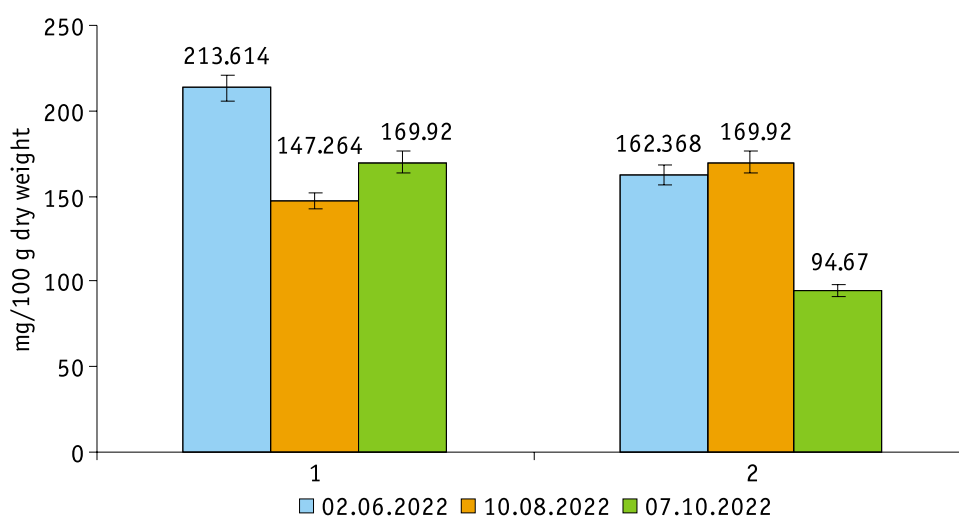


Figure 5. Flavonol content in the leaves of *S. chinensis* during the growing season:
1 – magnolia vine following actinidia (IV); 2 – magnolia vine following another magnolia vine (V)

The discrepancy in research outcomes concerning the tolerance of experimental plants, as compared to prior findings [4], could be attributed to the extended growth period of the predecessor plants and the uncontrolled conditions under which the studied plants were cultivated within the garden ecosystem. Throughout the growing season, the accumulation of flavonols in the leaves of *S. chinensis* following the magnolia vine was lower than in plants grown after actinidia (Fig. 5).

By the end of the growing season in October, the flavonol content of magnolia vine plants that had previously grown in the same area had decreased significantly. This is in contrast to magnolia vine plants, which were planted after actinidia and do not exhibit this decrease. Waste products from the previously grown actinidia plants suppress the growth of *S. chinensis*.

Conclusions

Interplanting *A. arguta* with *S. chinensis* has a positive impact on the productivity and stress resistance of Actinidia vines. Compared to plants grown in isolation, the growth of *A. arguta* improves when it is grown alongside *S. chinensis* due to alterations in the content of secondary metabolites and the pigment system. The levels of proline and flavonoids in the leaves of these vines, along with their pigment composition, can serve as biochemical indicators of the plants' physiological state and competitiveness in mono- and mixed plantings. Furthermore, *S. chinensis* is a self-tolerant species and mono-species plantings are more beneficial for it.

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Мета. Вивчити взаємодію між плодовими лозами *A. arguta* 'Сентябрьская' та *S. chinensis* 'Садовий' у моно- та змішаних насадженнях, дослідивши пігментний комплекс рослин та особливості накопичення флавонолів і проліну у вегетативних органах. **Методи.** Дослідження проводили в Національному ботанічному саду імені М. М. Гришка Національної академії наук України (м. Київ). Кількісний уміст пігментів, флавонолів і проліну визначали методом спектрометрії, послуговуючись відповідними методиками. **Результати.** Аналіз одержаних експериментальних даних показав значні відмінності між досліджуваними видами в моно- та змішаних насадженнях як за вмістом флавонолів у період вегетації, так і за динамікою їх накопичення. Рослини актинідії більшою кількістю цих сполук відрізнялися в одновидових насадженнях, порівнюючи із сумісними. Вміст проліну в листках *A. arguta* за умови росту разом із *S. chinensis* був нижчим, ніж у монокультурі, впро-

довж усього періоду досліджень. На 20% меншим накопиченням вказаної амінокислоти характеризувалися й листки лимонника в одновидових насадженнях, як порівняти зі змішаними. Якщо плодові лози *A. arguta* росли разом із *S. chinensis*, то накопичували в 1,6 раза більше хлорофілу, ніж у монокультурі. **Висновки.** Вегетативні органи рослин *A. arguta* у змішаних із *S. chinensis* насадженнях, як порівняти з однокультурними, вирізняються вищими показниками фотосинтезу та нижчим умістом проліну й флавоноїдів. Лимонник характеризується аутоolerантністю, тому для нього прийнятнішим є монокультурне вирощування. Особливості пігментного комплексу, показники вмісту проліну та флавоноїдів у тканинах можуть слугувати біохімічними індикаторами стану рослин та їхньої сумісності у змішаних насадженнях.

Ключові слова: алелопатія; біохімічні індикатори; вторинні метаболіти; фотосинтетичні пігменти.

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