Investigation of Ecophysiological and Phytosociological Parameters on the Populations of the Invasive Alien Species Asclepias Syriaca l. In the Danube Valley, Romania

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Research Article

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INVESTIGATION OF ECOPHYSIOLOGICAL AND PHYTOSOCIOLOGICAL PARAMETERS ON THE POPULATIONS OF THE INVASIVE ALIEN SPECIES *ASCLEPIAS SYRIACA* L. IN THE DANUBE VALLEY, ROMANIA

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Abstract: *Asclepias syriaca* L. commonly called common milkweed, butterfly flower, silkweed, silky swallow-wort, and Virginia silkweed ommon milkweed is a rapidly spreading invasive alien plant that threatens natural ecosystems, especially grasslands, but also some forest and agricultural ecosystems worldwide. This species is a flowering plant which can established viable populations across various regions. This study tries to evaluate the invasiveness of this specie in five localities from Oltenia region where, as a result of the observations made between March 2020 and September 2024, the presence of a large number of individuals was found. It was made determinations in 5 sites, located in the Danube basin in the south-western part of Romania. The size of the invasion with this species in the studied sites varies significantly, from a few individuals to a very large number. Phytosociological studies are essential to determine the presence, abundance-dominance and role of this species in invaded plant communities, in order to organize and manage management measures regarding the invasiveness of the species in the Danube Valley.

Results on physiological responses such photosynthetic rate, intensity of transpiration and chlorophyll content varies with density and area. In all of the sites studied, *A. syriaca* fructifies well and produces viable seeds with a high degree of dissemination and very good germination capacity. Considering the rather large number of the individuals in the analyzed localities in south-west Romania, it can be expected a future spread and invasion in the areas. Thus, *A. syriaca* should be treated as an invasive species with high potential for aggressiveness on diversity because it can form dense colonies that suppress the growth of other species through competition for light, water and nutrients. Continuous monitoring and management strategies are essential to mitigate its impact on native biodiversity and ecosystem health in the context of climate change.

Keywords: *Asclepias syriaca*, distribution, invasiveness, plant communities

1. Introduction

Invasive species have become widely distributed around the world through various pathways, having a significant impact on biodiversity and ecosystems [1]. Their number is constantly increasing due to climate change, as they are adaptable and much more resistant than native species, accustomed to a certain climate and therefore more sensitive to these changes.

Asclepias syriaca it is considered invasive because it can colonize a variety of habitats such as meadows, dunes, river valleys, forest edges, even wetlands, but more often in human-modified habitats (roadsides, railways areas, abandoned orchards, vineyards, abandoned arable land), with a wide range of soils of all textural groups – moist or dry, sandy, well-drained soil [2-7]. This species has the following characteristics: it is perennial and the height can reach up to 1.5-1.8 m, the leaves are long, elliptical, with a length of 10–28 cm and a width of 4–12 cm. The basal ones are light green, and the upper ones are dark green and acuminate, and the flowers areusually red or dark pink, arranged in dense inflorescences, with a diameter of about 1 cm. They have five horn-like hoods and are very fragrant, attracting numerous insects, including butterflies.They are grouped in rich umbelliferous inflorescences and the fruits are large follicles containing a large number of hairy seeds.The thick lateral roots, about 10-40 cm below the soil surface, can penetrate the soil to a depth of 1-1.20 meters [8]. The species is native to Canada and the

United States, but it can be identified in some Asian countries, namely Japan and Iraq [9]. Like most invasive species, A. syriaca was intentionallyintroduced to Europe for ornamentalpurposes and for otherslike source of fiber and rubber [10].It has established populations in at least 13 EU member states, including Austria, Bulgaria, Croatia, Czech Republic, Denmark, France, Hungary, Italy, Lithuania, Netherlands, Poland, Romania, and Slovakia. It is particularly prevalent in warmer areas of southern Europe and has been categorized as invasive due to its rapid spread and negative impacts on native flora and fauna [11]. Same source sustain that the European Union has recognized A. syriaca as an invasive alien species of concern under Regulation (EU) No. 1143/2014.

In Romania, the species occupies natural, semi-natural and artificial habitats, being recorded numerous presences in Dobrogea, Moldova and Banat[12], the species being also frequently observed in the south and south-west of the country.

Following the studies carried out in several areas in Romania, it was found that the species is very widespread, especially in the plains and hilly regions, very often inside Natura 2000 protected sites, having a negative impact on the conservation status of the habitats: 2130* Fixed coastal duneswith herbaceous vegetation (grey dunes); 2160 Dunes with *Hippophaë rhamnoides;* 2340* Pannonic inland dunes; 6240* Sub-pannonic steppic grasslands; 6260* Pannonic sand steppes; 6520 Mountain hay meadows; 40A0* Subcontinental peri-Pannonic scrub; 6430 *Hydrophilous* tall-herb fringe communities of plains and of the montane to alpine levels.

There is significant concern regarding the invasive nature of *A. syriaca* in certain regions. Studies have classified it as a "potentially invasive" species in parts of Romania, particularly in south-western, where it poses risks to native ecosystems. Its ability to spread aggressively makes it a candidate for their management in few areas from Oltenia where it could disrupt local flora. To assess the populations and invasiveness of this species, phytosociologica and physiological studies of this species were carried out in close correlation with habitat conditions.

The purpose of this study was to identify the distribution of the species in the communities from several areas where the degree of invasiveness is high and to find out the adaptation capacity by analyzing some physiological characteristics depending on density and temperatures.

2. Materials and Methods

2.1. Study area

In the Danube basin, located in the south-western geographical region of Romania, including the protected area known as the Danube Gorges, an integral part of the "Iron Gates" Natural Park, a series of populations of the invasive species *Asclepias syriaca* were studied. The analyzed populations were identified in six localities: Bigăr (44°39′55″N- 22°6′17″E), Baia Nouă (44°33′16″N-22°07′44″E), Radimna (44°47′42″N-21°33′49″E), Vânju Mare (44° 25' 31.01" N-22° 52' 10.99" E), Moldova Veche Island (44°40′56″N-21°38′05″E), belonging to Caraș-Severin and Mehedinți Counties. The populations were studied in situ during the summers of 2020-2024.

2.2. Field studies and data analysis

The identification of taxa was based on Romanian Flora [13] and Flora Europaea [14]. For the phytocoenotic research in the study area was used the method of phyto-sociologic research of the Central European School elaborated by Braun-Blanquet, (1932) [15]. The plant communities were identified and distinguished according to the characteristic, edifying, dominant and differential species. For the identification of the plant communities and the coenotaxonomic classification, synthesis works of Coldea (1991) [16],Sanda et al. (1997, 2001)[17, 18], as well as Oberdorfer (1992) [19], Mucina et al. (1993, 2016) [20, 21], Rodwell (2002) [22] were used.

A total of 40 sample plots of 200 m^2 each were considered in our studies, for the 6 plant communities in whose floristic composition *Asclepias syriaca* populations were analyzed.From the total of these plots, for each plant community 5 relevées (plots) were made, in which both the dominant abundance of the studied species, and the constancy of the species (K) were taken into account to express the presence of individuals of a species in sample plots and their degree of fidelity to the analyzed plant community.

There were performed calculations for the next Synthetic ecological indexes: Abundance, Dominance, Constancy and index of ecological significance [23].To determine the density of the *Sicyos angulatus* population, the number of plants (individuals per 1 m^2) was counted and then converted to the number of plants per 5000 m^2. The results were recorded according to the following five-point scale: 1 (1–20 plants), 2 (21–50 plants), 3 (51–100 plants), 4 (101–500 plants), 5 (more than 501 plants).

The following indicators were calculated: abundance (A), constancy (C), dominance (D) and ecological importance index (W). A dendrogram of the similarity of the study plots was delineated according to the values of these indices.

Abundance (A) is the number of individuals of the study species occurring in a study plot. This value was expressed as an absolute number and was used to calculate other indices. According to the abundance value, the species occurring in each area were characterised as rare, quite rare, abundant and very abundant.

Constancy (C) (C) of a species in the study plot. This is a structural indicator that shows the proportion of a given species in defining the structure of the community. Higher values of this index reflect adaptation to the conditions offered by the habitat. The constancy is estimated using the relationship: $C = npA/Np$.

where:

C-constancy of the analysed species;

npA-number of sample plots where the species was found;

Np-total number of sample plots analysed.

Depending on the value of this index, the species were classified as follows: C1 accidental, present between 1–25% of the sample plots; C2-accessory, present between 25.1–50%; C3-constant present between 50.1–75% and C4-euconstant, present between 75.1–100% of the sample plots.

Dominance (D) shows the relationship between the population of the species studied and the sum of the individuals of the other species with which it is associated, expressing relative abundance. Dominance is considered an indicator of productivity because it shows the percentage of each species' contribution to the biomass production in the biocenosis. It is calculated according to the next formula: $D = (nAx100)/N$,

where: D-dominance of the analysed species;

nA-the total number of individuals of the analysed species found in the sample plot examined;

N-the total number of individuals of all species present in the sample plot.

Depending on the value of this indicator, the species were assigned to the following classes: D1 subrecessive (less than 1.1%); D2 recessive (between 1.1–2%); D3 subdominant (between 2.1–5%); D4 dominant (between 5.1–10%); and D5 eudominant (more than 10%).

The index of ecological significance (W) represents the ratio between the indices C and D and better illustrates the position of the study species in the biocenosis. It is calculated using the fallowing formula: $W = (CxD)/100$.

Depending on the value of this index, species were assigned to the following classes: W1 with values below 0.1% (accidental species); W2 with values between 0.1–1% (accessory species); W3 with values between 1.1–5%; W4 with values between 5.1–10% (species characteristic of the given phytocoenosis); and W5 with values above 10%.

Photosynthetic rate, transpiration rate and chlorophyll content were calculated after 10 determinations were carried out in the field on different plants from each location.The data were statistically analyzed using MS Office and IBM SPSS software. All data were recorded in five repetitions in randomized block design model and mean values were processed by analysis of variance (ANOVA). The significance of the differences was estimated with LSD multiple comparison test at the $p \leq 0.05$ level. The significance of differences between means was presented based on letters, being considered as significant the differences between means marked with different letters.

The correlation coefficients between the analyzed characters were also calculated, their significance being determined by Pearson values.

The Principal Component Analysis method explained by Harman (1976) [24] was followed in the extraction of the components. The percentage variability explained by each component was determined [25-26].

Principal component analysis was performed as well as biplot graphical display. Also it was performed a hierarchical dendrogram using Bray-Curtis similarity index.

The physiological parameters such as photosynthesis rate and intensity of transpiration were recorded with LCI 300 equipment and the chlorophyll content was recorded with Li-600 equipment.

3. Results

3.1. Phytosociological assessment of *Asclepias syriaca* in plant communities

The study of plant communities in the structure of which *Asclepias syriaca* is frequently encountered, most often with a high abundance-dominance, represents a vital aspect that helps to understand the ecology, syndynamics, distribution, and structure of each analyzed phytocenosis, with particular importance regarding the relationships between native and allogeneic species. in current global climatic conditions.

In the studied area, populations of common milkweed are found in the structures of the plant communities that build the habitat of sunny and dry places in the south-west of Romania, Slovakia, namely habitats within degraded meadows, on abandoned, uncultivated orchard or vineyard lands, along of time roads, ditches, at the edge of thickets and forests, on dilapidated and littered trains. Many times, the species is also accompanied by other invasive species: *Ailanthus altissima, Erigeron annuus, Oenothera glazioviana, Abutilon theophrasti, Ambrosia artemisiifolia, Xanthium orientale subsp. italicum, Amorpha fruticosa,* especially in the phytocoenoses analyzed on Moldova Veche Island, where the degree of invasiveness is much increased.

1. *Festucetum vaginatae* Rapaics ex Soó 1929

This plant community found on Moldova Veche Island on a land with bare sands forming open meadows, sometimes windy, due to the more or less strong currents from the Danube, meadows characterized by a large number of therophytes. In this plant community, the species: *Corynephorus canescens, Thymus serpyllum, Viola tricolor subsp. tricolor, Cerastium semidecandrum, Spergula morisonii, Alyssum montanum* subsp. *gmelinii, Bassia laniflora, Cynodon dactylon*.The structure and floristic physiognomy of the phytocoenoses within this plant community undergo profound changes due to the installation of very large populations of *Asclepias syriaca* (Figure 1).

The phytocoenoses of this plant community are installed on the southern slopes of the island, with semi-stabilized dunes, weakly fixed with vegetation, based on characteristic, dominant species such *as Festuca vaginata, Carex liparicarpos* and *Fumana procumbens* whose dominant abundance decreases over large areas due to the growth of the abundant-dominant this invasive species. Vegetation coverage reaches up to 90-100%, the abundance-dominance of the *Asclepias syriaca* species reaching up to 60% in some places, practically leading to an extreme degradation of the characteristic vegetation built by Festuca vaginata, a characteristic type of vegetation of Moldova Veche Island, which builds a priority habitat 6260 * – Pannonian steppes on sands, within the Iron Gates National Park.

Figure 1. *Asclepias syriaca* in *Festucetum vaginatae* Rapaics ex Soó 1929 plant community, Moldova Veche Island, June 2021 (photo, M. Niculescu)

In the floristic structure of these phytocoenoses, there are also other species with low abundance-dominance, but with a high constant: *Bromus tectorum, Thymus pulegioides, Acinos arvensis, Cynodon dactylon, Plantago arenaria, Petrorhagia saxifraga, Astragalus onobrychis, Tribulus terrestris, Teucrium chamaedrys, Euphorbia segueriana, Alyssum desertorum, Polygonum arenarium*. Within these phytocoenoses, there are also some rare, protected species - *Colchicum arenarium, Silene borysthenica, S. conica*, their populations being greatly affected by the existing anthropogenic pressures on the island, but especially by the excessive development of the populations of the common milkweed species. And other invasive species were observed within the phytocoenoses of the plant community *Festucetum vaginatae* Rapaics ex Soó 1929, especially *Erigeron annuus, Oenothera glazioviana* and *Xanthium orientale* ssp. *italica*.

The increased share of invasive species, especially the *Asclepias syriaca* species, is closely related to the constantly changing eco-pedo-climatic conditions, which allow a very good exposure of individuals. Also, *Asclepias syriaca* fruits very well, the number of capsules on each individual is 2-4, the capsules are large, well developed and contain a very large number of viable seeds, with a morphology adapted to dispersion with the help of the wind, on the island there are currents of air, favoring the development of populations and their spread over ever larger areas. The proliferation of individuals of Asclepias syriaca have an impact on the plant community and the entire protected habitat, as a priority, so that conservative management measures must be applied quickly and correctly, taking into account that the conservation value of semi-fixed dunes with characteristic vegetation is very high.

2. *Calamagrostio epigeji-Hippophaëtum rhamnoidis* Popescu et al. 1986 is the second plant community that we studied due to the presence of the invasive species *Asclepias syriaca* within the structure of phytocoenoses. Such phytocoenoses were analyzed on Moldova Veche Island, both in the north-west and south-west, in interdunal depression areas. This plant community builds the natural habitat 2160 Dunes with *Hippophaë rhamnoides* (Palearctic Code - CLAS. PAL.: 16,251), thus having a special importance in terms of conserving and protecting the natural capital.

3. *Hippophaë rhamnoides* develops very well on Moldova Veche Island, occupies quite large areas,

especially in the form of very long strips, but approximately 3 m wide. The edifying species of the plant community is very well developed and has abundant fruiting. Within the floristic structure of this plant community, several invasive species were identified, including *Asclepias syriac*a, this time with a much lower dominant abundance. However, the invasive species disrupts the floristic composition of the phytocoenoses, so it is important that the size of the populations and the degree of development, including vitality and viability of the invasive species is permanently controlled.

4. *Plantaginetum arenariae* (Buia et al., 1960) Popescu et Sanda 1987 is a plant community characteristic of flat or uneven sandy lands, at the base of the dunes, occupying very large areas on Moldova Veche Island, both in the northern part of the island, but also in the southwestern part. The phytocoenoses within the plant community generally have a coverage of 80-95% and a monodominant aspect, the edifying species having high abundance-dominance values (AD=4-5) compared to the other characteristic species. The floristic composition includes a number of psammophilous species, belonging to the order *Festucetalia vaginatae* Soó 1957, mainly to the alliances *Bassio laniflorae-Bromion tectorum* (Soó 1957) Borhidi 1996 and *Festucion valesiacae* Klika 1931. This plant community, in addition to the role of fixing sand, also has a very high conservation value building the habitat 2130* Fixed coastal dunes with herbaceous vegetation (grey dunes) (Paleartic code: CLAS. PAL.: 16.221 to 16.227, 16.22B).

Asclepias syriaca is present in the floristic structure of the phytocoenoses in the southwestern part of the island, especially on the dike areas and the higher areas. The degree of invasiveness of this species is higher in the phytocoenoses in the southwest of the island, in the northern part of the island individuals of *Asclepias syriaca* are found scattered, due to the fact that the phytocoenoses in this part of the island are more protected from currents, have an north exposure, some are on the border of willow, black alder groves or are found on beaten lands, much more affected by the ever-growing population of wild horses existing on Moldova Veche Island.

Individuals of *Asclepias syriaca* have a negative impact on all the species in the floristic structure of this plant community and the entire protected, priority habitat built by it, the structural changes appearing with the increase in the number of individuals within the population of this invasive species, which has a waist much higher, which can reach up to 200 cm, practically suffocating the layer of edifying and characteristic species.

5.*Sambucetum ebuli* (Kraiser 1926) Felföldy 1942 is a plant community found in places with ruderals next to households, roadsides, ponds, on the banks of waters, on soils with a high nitrogen content. Phytocoenoses of this plant community, including *Asclepias syriaca*, were identified in the Radimna locality on the side of the road, at the border of habitat 91E0, built by *Salix alba* in places with relatively high humidity. Thus, in conclusion, following the studies carried out, it was found that common milkweed can also be found in rural vegetation communities, especially on the sides of roads, paths or next to abandoned buildings. From the point of view of physiognomy and floristic composition, the phytocoenoses of this plant community are dominated by the edifying species, *Sambucus ebulus*, popularly called danewort, alongside which *Asclepias syriaca* (Figure 2) can be found with great abundance-dominance, thus being the co-dominant species. Along with these two species, a number of ruderal species are also frequently encountered: *Urtica dioica, Glechoma hederacea, Geum urbanum, Senecio vulgaris, Cardaria draba* the predominant ones being mesophilic, mesothermic and weakly acid-neutrophilic hemicrypotphytes. As we mentioned, *Asclepias syriaca* is very well adapted within the ruderal phytocoenoses of this type, the individuals have a massive development, they are very vigorous, fruiting is very good, there are favorable conditions for the dissemination and germination of the seeds, which determines the development of new populations in the Danube Valley.

Figure 2. *Asclepias syriaca* in the danewort plant community

5. *Medicagini minimae-Festucetum valesiacae* Wagner 1941, is a meadow community frequently found in the studied area. Phytocoenoses of this plant community were analyzed in Baia Nouă and Bigăr localities. This type of meadow is very widespread in the southern part of Banat.

The analyzed phytocoenoses have a rich, heterogeneous floristic composition, with poor conservation status. In some phytocoenoses that build these meadows, they are invaded by bushes of *Prunus spinosa, Rubus fruticosus, Rosa canina*. The invasive species *Asclepias syriaca* is found in the floristic composition of these phytocoenoses with a much lower abundance-dominance compared to the other plant communities analyzed in this study, and which are in direct contact with the Danube River. Thus, it is found that the area in the immediate vicinity of the Danube offers much better conditions for the development of this species, but also for the dissemination and expansion of the invaded surfaces. In some of the phytocoenoses of this type of meadow, he also found the presence and development of the colonizing species *Pteridium aquilinum*. The phytocoenoses identified and included in the plant association *Festucetum valesiaco-rupicolae* Csuros et Kovacs 1962, build the priority habitat Natura 2000 - 6240*, and for its conservation, it is necessary to apply some conservation measures that primarily stipulate that the exploitation of the meadows is done only by mowing, to avoid the establishment of bushes and then forest formations, which lead to the degradation of this type of meadow.

6. *Calamagrostietum epigeji* Juraszek 1928 is a plant community whose phytocoenoses they can often be found on the side of the roads, in areas with degraded, ruderalized land, waste deposits, on different types of soil: skeletal, sandy (loose sand and slightly loamy sand), clayey. The field studies regarding this plant community, in which the invasive species taken in the studio was identified, were carried out in the town of Vânju-Mare, on a degraded land, which was originally an abandoned vine plantation, on a land with sandy soil, close to the Calafat-Șimian national road and a short distance from the Danube Valley. In the *Calamagrostietum epigeji* plant community, the phytocoenoses have a floristic structure with high heterogeneity, comprising a series of species belonging especially to the class *Molinio-Arrhenateretea* and *Artemisietea vulgaris* and *Festuco-Brometea*. However, the high abundance-dominance of the *Asclepias syriaca* species is noted (Figure 3). The studied phytocoenoses occupy considerable areas in this area, the populations of *Asclepias syriaca* being extremely well developed, the sandy land, with an increased degree of ruderalization, creating favorable conditions for the development of this species.

The specimens have a high degree of vitality, are extremely vigorous, fruit very well, over 60% of individuals produce 4-5 capsules/individual (Figure 4), with fertile seeds. The favorable eco-pedo-climatic conditions, in which the species took root, in the ruderal areas of the Vânju Mare locality, favor the morpho-anatomical development of the individuals of *Asclepias syriaca* populations, but also the development regarding the size and density of the populations, as well as the size of the areas occupied by easily conquering new territories with a direct impact on the state of conservation of several types of ecosystems.

Figure 3. *Asclepias syriaca* in *Calamagrostietum epigeji* Juraszek 1928plant community, June 2022 (photo, M. Niculescu)

Figure 4. Fruiting of the *Asclepias syriaca* species in *Calamagrostietum epigeji* Juraszek 1928plant community, Vânju Mare, September 2023 (photo, M. Niculescu)

3.2. Variability of some physiological characteristics depending on density

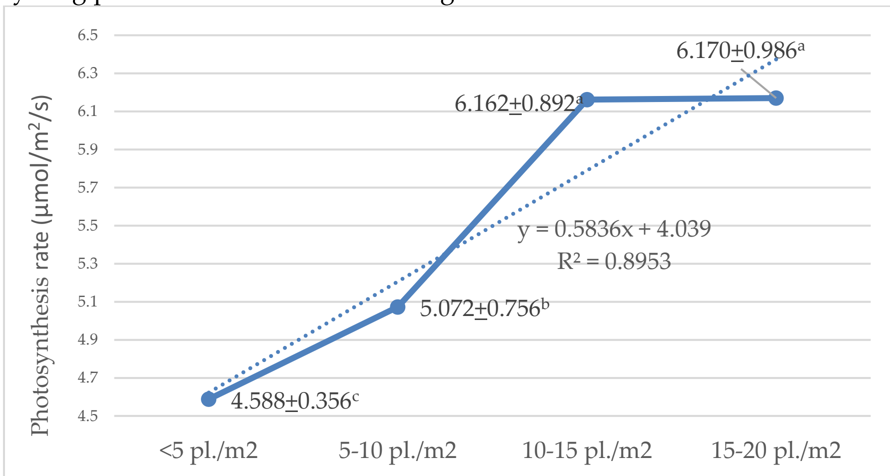
As concern the abundance of the *Asclepias syriaca* species, the most individuals were identified in the Moldova Veche Island and Vânju Mare areas while the fewest in the Bigăr area. Regarding the dominance analysis, in the Radimna, Vânju Mare and Moldova Veche Island areas, the species is eudominant, having very high values of this index while in the Bigăr and Baia Nouă areas the calculated values place it is a recedent species (Baia Nouă area) and respective, subrecedent species (Bigăr).
Highest values for constancy (C%) and index of ecological significance (W) were registered in Vânju Mare and Moldova Veche Island areas (Table 1).

Table 1. The variation of ecological indices on the *Asclepias syriaca* species in the researched areas

Crt. no.	Area	Abundance (pl./5000 m^2)	Dominance (%)		Constancy (%)		W (index of ecological significance)		Density (pl./100 m^2)
1.	Bigăr	334^{e}	3.21^{d}	D_3	10.25^{e}	C_1	0.33^{d}	W_2	7.45±0.56^{d}
2.	Baia Nouă	511^{c}	5.62^{d}	D_4	23.27^{d}	C_1	1.31^{d}	W_3	11.41±1.52^{d}
3.	Radimna	2258^{c}	17.42^{c}	D_5	38.14^{c}	C_2	6.64^{c}	W_4	88.48±12.48^{c}
4.	Vânju Mare	8796^{b}	31.25^{b}	D_5	82.14^{b}	C_4	25.67^{b}	W_5	385.89±75.4^{b}
5.	Moldova Veche Island	10256^{a}	45.45^{a}	D_5	98.11^{a}	C_4	44.59^{a}	W_5	478.01±112.58^{a}
	LSD 5%	107.23	6.57		12.47		3.21		10.29

Different letters denote significant differences (LSD test, $p < 0.05$)

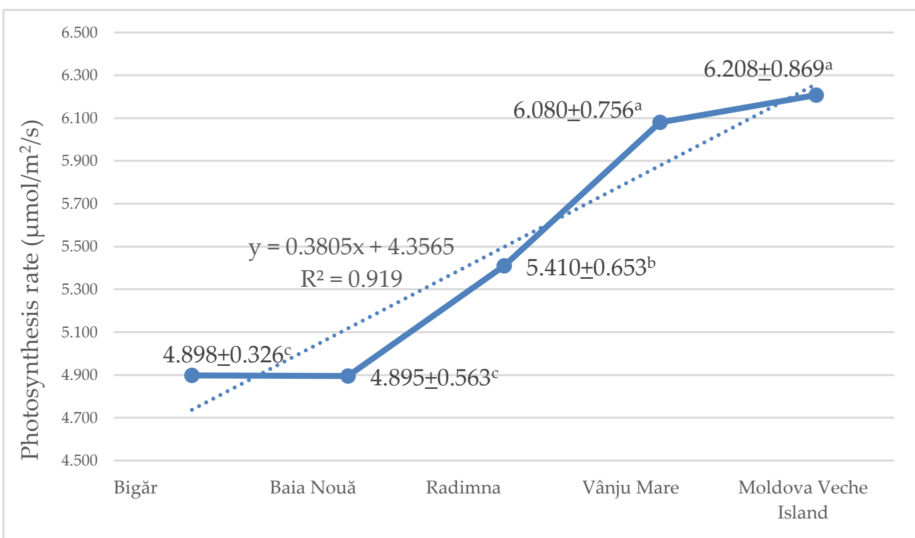
The variation of photosynthesis rate depending on plant density/m^2 is, in general, larger in higher densities, comparative with low densities (Figure 5). The explanation is that, in high densities, the plants shaded each other, thus protecting themselves from the effect of excessive light and sun radiation, especially during severe drought, a fact that did not happen in the case of specimens at low density, exposed for very long periods of time in excessive light and in conditions of lack of humidity.



LSD 5%= 0.326 µmol/m²/s. Different letters denote significant differences (LSD test, $p < 0.05$)

Figure 5. The variation of average values of photosynthesis rate (µmol/m²/s) depending of plant density on the *Asclepias syriaca* species, at temperatures above 38.5 ⁰C

The analysis of the variation of the photosynthesis rate depending on locationpresents highest average values in Moldova Veche Island and Vânju Mare areas, these being significantly different from the other average values (Figure 6).



Different letters denote significant differences (LSD test, $p < 0.05$)

Figure 6. The variation of average values of photosynthesis rate (µmol/m²/s) depending on location, at the temperature above 38.5 ⁰C.

The variability of physiological characteristics in *Asclepias syriaca* is significantly influenced by density and environmental conditions. Understanding these relationships is essential for managing ecosystems where this species has a very large number. Further research could provide deeper insights into how these dynamics affect the ecological interactions of *A. syriaca* within its habitat.

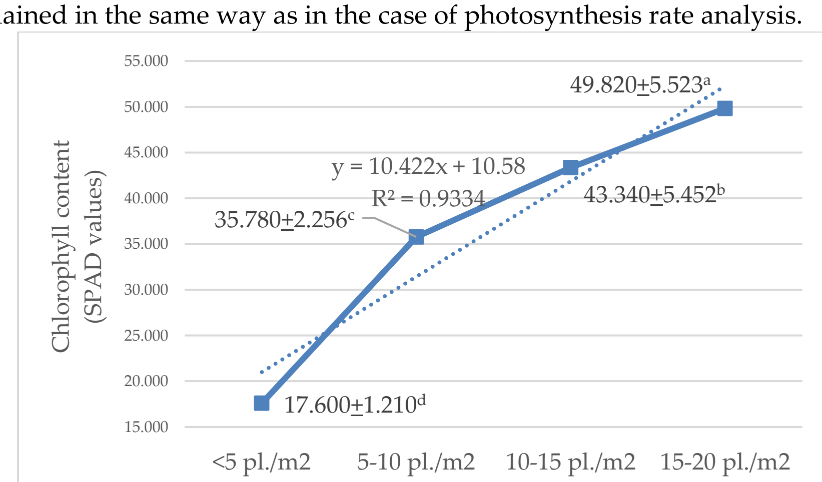
Concerning the analysis of the photosynthesis rate depending on location and plant density, the highest values are recorded in the locations with the highest number of plants, at highest densities, respectively in the Moldova Veche Island and Vânju Mare locations, respectively at densities of 15-20 pl./m² (Table 2).

Table 2. The variation of average values of photosynthesis rate (μmol/m²/s) depending on location and plant density on *Asclepias syriaca* species at temperatures above 38.5⁰ C

Area \ Plant density	<5 pl./m²	5-10 pl./m²	10-15 pl./m²	15-20 pl./m²
Bigăr	4.61 ± 0.316^{ghi}	4.92 ± 0.251^{defgh}	5.05 ± 0.456^{cde}	5.01 ± 0.400^{cde}
Baia Nouă	4.35 ± 0.632^{i}	4.95 ± 0.601^{defg}	5.11 ± 0.658^{cd}	5.17 ± 0.521^{cd}
Radimna	4.65 ± 0.553^{fghi}	4.96 ± 0.589^{def}	5.98 ± 0.547^{b}	6.05 ± 0.687^{b}
Vânju Mare	4.58 ± 0.655^{hi}	5.25 ± 0.782^{c}	7.28 ± 0.720^{a}	7.21 ± 0.658^{a}
Moldova Veche Island	4.75 ± 0.725^{efgh}	5.28 ± 0.552^{c}	7.39 ± 0.653^{a}	7.41 ± 0.723^{a}
		LSD 5% = 2.612 μmol/m²/s		

Different letters denote significant differences (LSD test, $p < 0.05$)

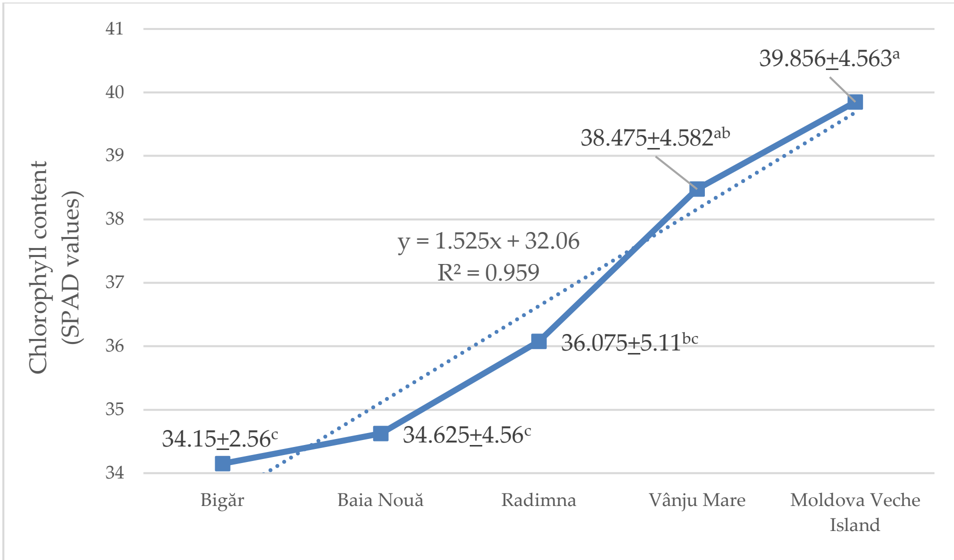
Variations in chlorophyll content of *Asclepias syriaca*in relation to plant density have been studied, revealing significant insights into how environmental factors influence plant physiology. Chlorophyll content is a critical indicator of plant health and photosynthetic capacity.Variation of chlorophyll content depending on plant density is high in higher densities, comparative with low densities (Figure 7), a fact that can be explained in the same way as in the case of photosynthesis rate analysis.



LSD 5%=5.638 SPAD values. Different letters denote significant differences (LSD test, $p < 0.05$)

Figure 7. The variation of average values of chlorophyll content (SPAD values) depending on location

Related to chlorophyll content analysis depending on location, the highest average value was recorded in Moldova Veche Island area, which differs significantly from all other recorded values, except the average from Vânju Mare area (Figure 8).



LSD 5%=3.263 SPAD values. Different letters denote significant differences (LSD test, $p < 0.05$)

Figure 8. The variation of chlorophyll content (SPAD values) depending on location

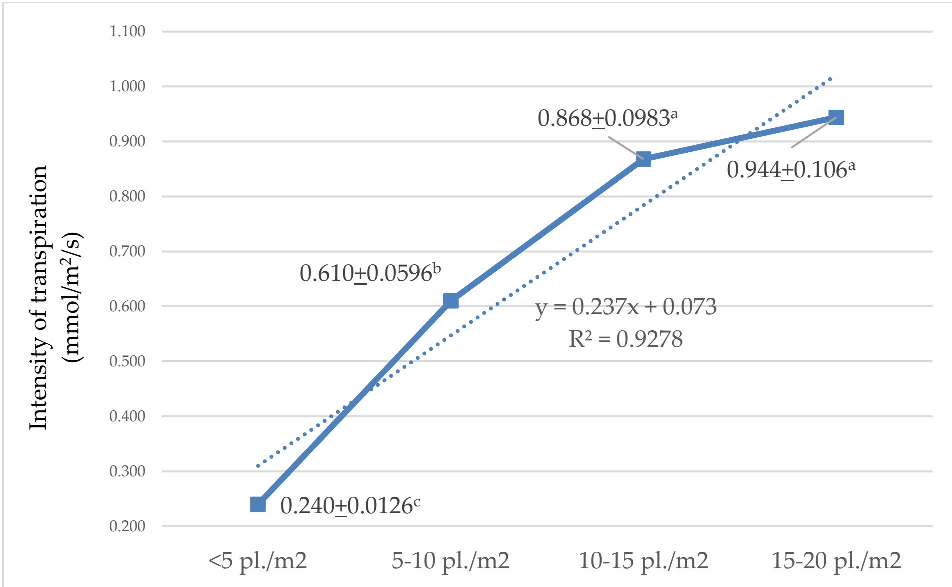
Chlorophyll content analysis depending on location and plant density is higher in the locations with the highest number of plants, in highest densities, respectively in Moldova Veche Island and Vânju Mare (Table 3).

Table 3. The variation of chlorophyll content (SPAD values) depending on location and plant density

Area \ Plant density	<5 pl./m^2	5-10 pl./m^2	10-15 pl./m^2	15-20 pl./m^2
Bigăr	17.5±1.95^{hi}	30.4±3.52^{f}	40.2±3.96^{d}	48.5±4.52^{b}
BaiaNouă	15.5±1.69^{i}	32.1±3.59^{f}	41.5±7.21^{d}	49.4±4.33^{ab}
Radimna	16.4±2.56^{hi}	35.7±3.69^{e}	42.4±4.22^{cd}	49.8±6.25^{ab}
Vânju Mare	18.4±2.23^{gh}	39.5±3.44^{d}	44.8±4.02^{c}	51.2±7.21^{a}
Moldova Veche Island	20.2±2.89^{g}	41.2±4.23^{d}	47.8±4.01^{b}	50.2±6.21^{ab}
LSD 5%=2.612 SPAD values				

Different letters denote significant differences (LSD test, $p < 0.05$)

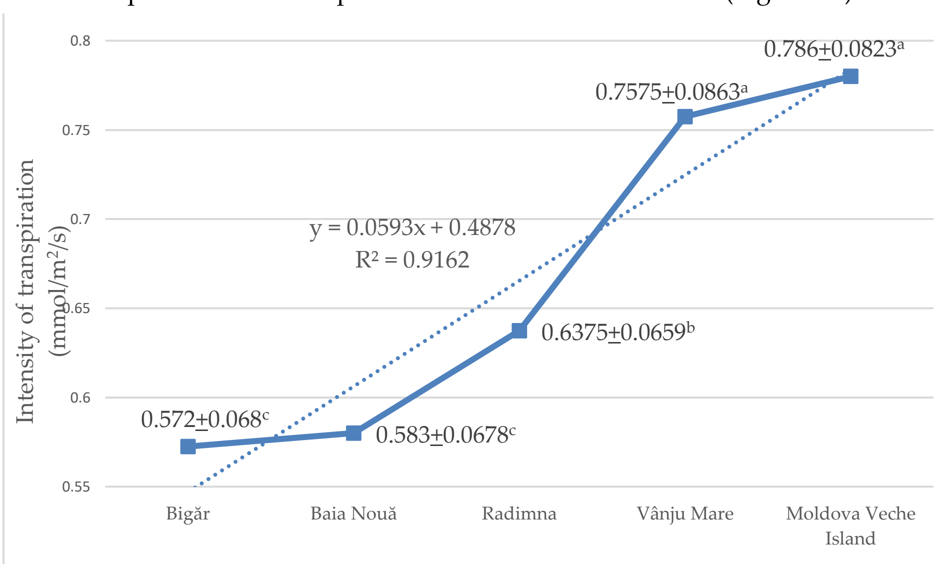
Related to the intensity of transpiration analysis depending on plants density/m^2, at temperatures above 38.5^0 C, the highest average values were recorded at the highest densities, due to the fact that, only at high densities the plants keep their leaves longer green because of the shade, so they are still able to carry out photosynthesis. At low densities, due to excessive exposure to light and sun radiation, the leaves dry out, so their capacity for photosynthesis and evapotranspiration also decreases drastically (Figure 9).



LSD 5%=0.082 mmol/m²/s. Different letters denote significant differences (LSD test, $p < 0.05$)

Figure 19. The variation of the intensity of transpiration (mmol/m²/s) depending on location at temperatures above 38.5 ⁰C

Referring to the analysis of the intensity of transpiration depending on plants density/m², at temperatures above 38.5⁰ C, the highest values were recorded in the areas of Moldova Veche Island and Vânju Mare, areas where the most specimens of this species were otherwise identified (Figure 10).



LSD 5%=0.049 mmol/m²/s. Different letters denote significant differences (LSD test, $p < 0.05$)

Figure 10. The variation of the intensity of transpiration (mmol/m²/s) depending on plant density/m² at temperatures above 38.5 ⁰C

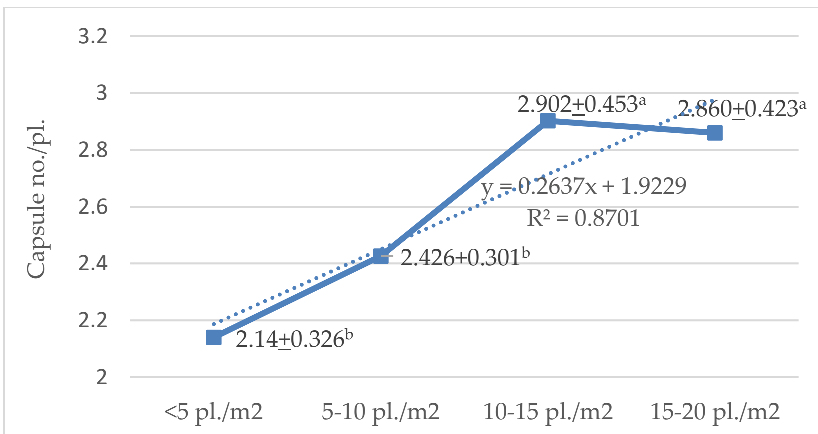
As concern the analysis of intensity of transpiration depending on location and density of plants, the highest values are recorded in the locations with the highest number of plants, in highest densities, respectively in Moldova Veche Island and Vânju Mare locations (Table 4).

Table 4. The variation of intensity of transpiration (mmol/m²/s) depending on location and plant density, at the temperature above 38.5⁰ C

Area \ Plant density	<5 pl./m^2	5-10 pl./m^2	10-15 pl./m^2	15-20 pl./m^2
Bigăr	0.220±0.0125^{j}	0.520±0.0425^{h}	0.750±0.0896^{e}	0.800±0.763^{d}
Baia Nouă	0.210±0.0128^{j}	0.500±0.0452^{h}	0.800±0.0863^{d}	0.810±0.0986^{cd}
Radimna	0.230±0.0186^{j}	0.650±0.0563^{g}	0.820±0.0638^{cd}	0.850±0.0763^{c}
Vânju Mare	0.250±0.182^{ij}	0.680±0.0561^{fg}	0.990±0.0826^{b}	1.110±0.983^{a}
Moldova Veche Island	0.290±0.0365^{i}	0.700±0.0561^{ef}	0.980±0.0845^{b}	1.150±0.108^{a}
LSD 5% = 0.0456 mmol/m^2/s				

Different letters denote significant differences (LSD test, $p < 0.05$)

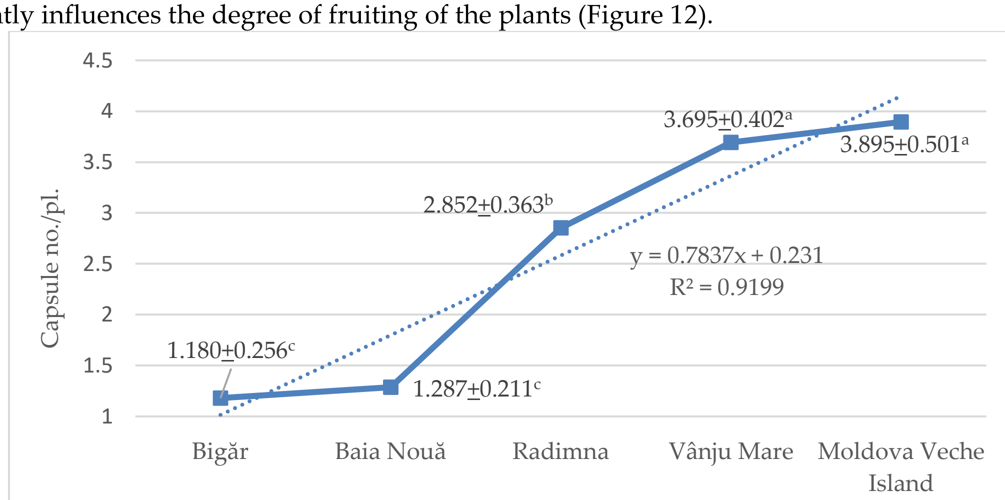
The number of fruits can vary significantly depending on the habitat type and environmental conditions. Referring to the analysis of number of fruit/plants depending on density/m², the highest values were recorded at highest densities (Figure 11).



LSD 5%= 0.367 fruits/pl.Different letters denote significant differences (LSD test, $p < 0.05$)

Figure 11. The variation of average number of fruits/plant depending on plant density/m²

The number of fruits/plant variation depending on location, is higher in Moldova Veche Island and Vânju Mare, areas where the most specimens of this species were identified, which means clearly thatsite significantly influences the degree of fruiting of the plants (Figure 12).



LSD 5%= 0.311 fruits/plant. Different letters denote significant differences (LSD test, $p < 0.05$)

Figure 12. The variation of number of fruits/plant depending on location

The number of fruits produced by *Asclepias syriaca* can vary significantly based on environmental conditions, habitat type and plant density, with averages ranging from 1.13 to 4.56. It is also obvious that the site played a critical role in terms of fruiting, Vânju Mare and Moldova Veche Island being areas that favor plant fruiting. Then, the density favors the degree of fruiting especially within the same location, the density of 10-15 pl./m² being very favorable from this point of view (Table 5).

Table 5. The average number of fruits/plant depending on location and plant density

Area \ Plant density	<5 pl./m²	5-10 pl./m²	10-15 pl./m²	15-20 pl./m²
Bigăr	1.14 ± 0.192^{l}	1.16 ± 0.182^{kl}	1.29 ± 0.236^{jkl}	1.13 ± 0.171^{l}
Baia Nouă	1.18 ± 0.201^{kl}	1.2 ± 0.211^{jkl}	1.32 ± 0.298^{ijkl}	1.45 ± 0.511^{ij}
Radimna	2.22 ± 0.235^{h}	2.87 ± 0.263^{g}	3.23 ± 0.320^{def}	3.09 ± 0.311^{fg}
Vânju Mare	3.01 ± 0.269^{fg}	3.45 ± 0.362^{d}	4.11 ± 0.399^{c}	4.21 ± 0.401^{bc}
Moldova Veche Island	3.15 ± 0.278^{ef}	3.45 ± 0.301^{d}	4.56 ± 0.478^{a}	4.42 ± 0.420^{ab}
LSD 5%=0.260 fruits/pl.				

Related to the analysis of the correlation coefficients calculated for the analyzed physiological parameters depending on density, from table 6, it can be seen that the values increase with plant density, regardless of the analyzed links. Thus, at the density of5 plants/m², all calculated values are insignificant, while at the density of 15-20 plants/m², the coefficients are distinctly significant.

Table 6. The variation of the correlation coefficients between the analyzed physiological parameters depending on plant density

Plant physiology indexes	**<5 pl./m²**	**5-10 pl./m²**	**10-15 pl./m²**	**15-20 pl./m²**
Photosynthesis rate/Chlorophyll content	0.549^{ns}	0.612^{*}	0.725^{*}	0.783^{**}
Photosynthesis rate/Intensity of transpiration	0.423^{ns}	0653^{*}	0.783^{**}	0.827^{**}
Chlorophyll content/Intensity of transpiration	0.489^{ns}	0.672^{*}	0.764^{**}	0.847^{***}
Photosynthesis rate/Capsule no./pl.	0.511^{ns}	0.612^{*}	0.627^{*}	0.756^{**}
Chlorophyll content/Capsule no./pl.	0.427^{ns}	0.556^{ns}	0.628^{*}	0.781^{**}
Intensity of transpiration/Capsule no./pl.	0.563^{ns}	0.611^{*}	0.756^{**}	0.856^{***}
P 5%=0.602; P 1%=0.735; P 0.1%=0.847				

ns, *, **, *** = not significant, significant, distinct significant and very significant at P level of 0.05, 1 and 0.01.

Regarding the PCA, the first two components explain 96.573% of total variance, the first component registering 78.497% and the second component 18.076% (Table 7).

Table 7. Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.140	78.497	78.497	3.140	78.497	78.497
2	0.723	18.076	96.573	0.723	18.076	96.573
3	0.124	3.088	99.661			
4	0.014	0.339	100.000			
Extraction Method: Principal Component Analysis.						

Related to the first component, the index that obtain both high positive values are Photosynthesis rate and Intensity of transpiration. The first component is the ability of the plant to produce high photosynthesis rate with a high level of transpiration. For the second component, high positive values are on fruits number/plant index. So, the second component is the ability of plants to produce a high number of fruits (Table 8).

Table 8. Component Matrix-Extraction Method: Principal Component Analysis, 2 components extracted

Comp. value / Index	Component 1	Component 2
Photosynthesis rate	0.944	0.188
Chlorophyll content	0.883	-0.443
Intensity of transpiration	0.956	-0.279
Capsule no./plant	0.745	0.643

The four groups based on the value of the two components were (Figure 13):

- *The variants with both positive components*:
  - Vânju Mare with 5-10 pl./m^2 density
  - Vânju Mare with 10-15 pl./m^2 density
  - Vânju Mare with 15-20 pl./m^2 density
  - Radimna with 15-20 pl./m^2 density
  - Moldova Veche Island with 5-10 pl./m^2 density
  - Moldova Veche Island with 10-15 pl./m^2 density
  - Moldova Veche Island with 15-20 pl./m^2 density
- *The variants with the first component positive and the second one negative*:
  - Radimna with 10-15 pl./m^2 density
  - Baia Nouă with 15-20 pl./m^2 density
- *The variants with both negative components*:
  - Bigăr with 5-10 pl./m^2 density
  - Bigăr with 10-15 pl./m^2 density
  - Bigăr with 15-20 pl./m^2 density
  - Baia Nouă with 5-10 pl./m^2 density
  - Baia Nouă with 10-15 pl./m^2 density
- *The variants with the first component negative and the second one positive*:
  - Bigăr with <5 pl./m^2 density
  - Baia Nouă with <5 pl./m^2 density
  - Radimna with <5 pl./m^2 density
  - Radimna with 5-10 pl./m^2 density
  - Vânju Mare with <5 pl./m^2 density
  - Moldova Veche Island with <5 pl./m^2 density

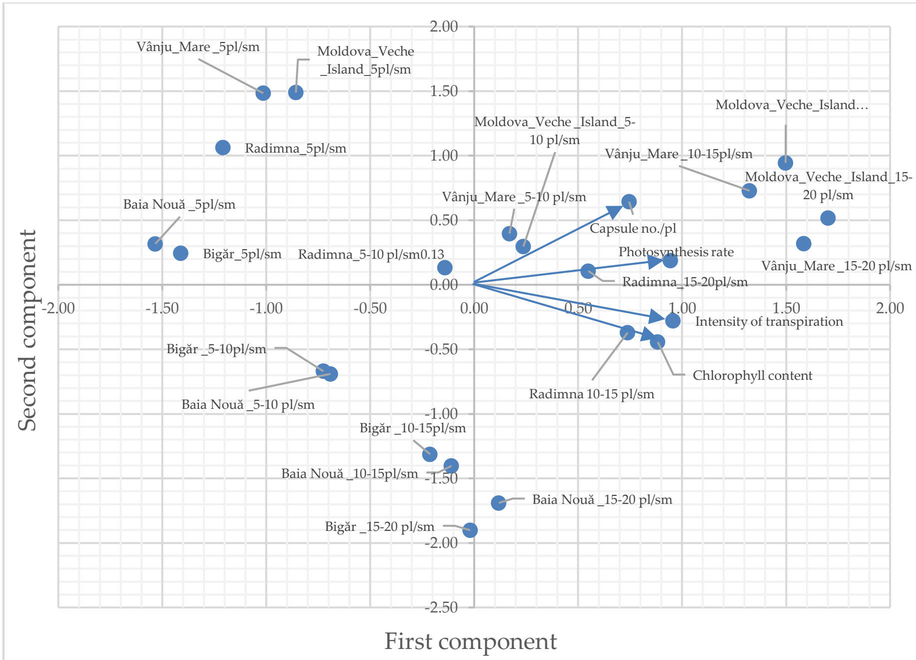


Figure 13. Classification of variants according to the score of the two components

Related to the analysis of the similarity index between the areas, index made based on dendrogram, (Figure 14), it can be seen that 4 large groups of variants were identified. Thus, the first group includes the variants from the areas that offer superior ecological conditions for this species, Moldova Veche Island and Vânju Mare, but in conditions of high plant density.

The second group, which is closest to the first group, includes the variants in which the plants are found in the highest densities in areas with lower favorability for this species, being clear that, in conditions of strong drought and exposure to prolonged sun light, plant density is a crucial factor for the species.

The third group includes the variants in which the plants are found in the highest densities in areas with the lowest favorability.

The fourth group includes the variants in which the plants are found in the lowest densities, in areas with the lowest favorability.

It is therefore clear that, although this species is very sun-loving, in conditions of extreme drought and very high temperatures, plant density plays a crucial role in terms of the evolution of this species in an area, in the sense in which, high densities ensure a more favorable microclimate for plants, while prolonged exposure to the sun and drought conditions leads to a reduction in the degree of development, going as far as canceling the favorability of the ecological zone.

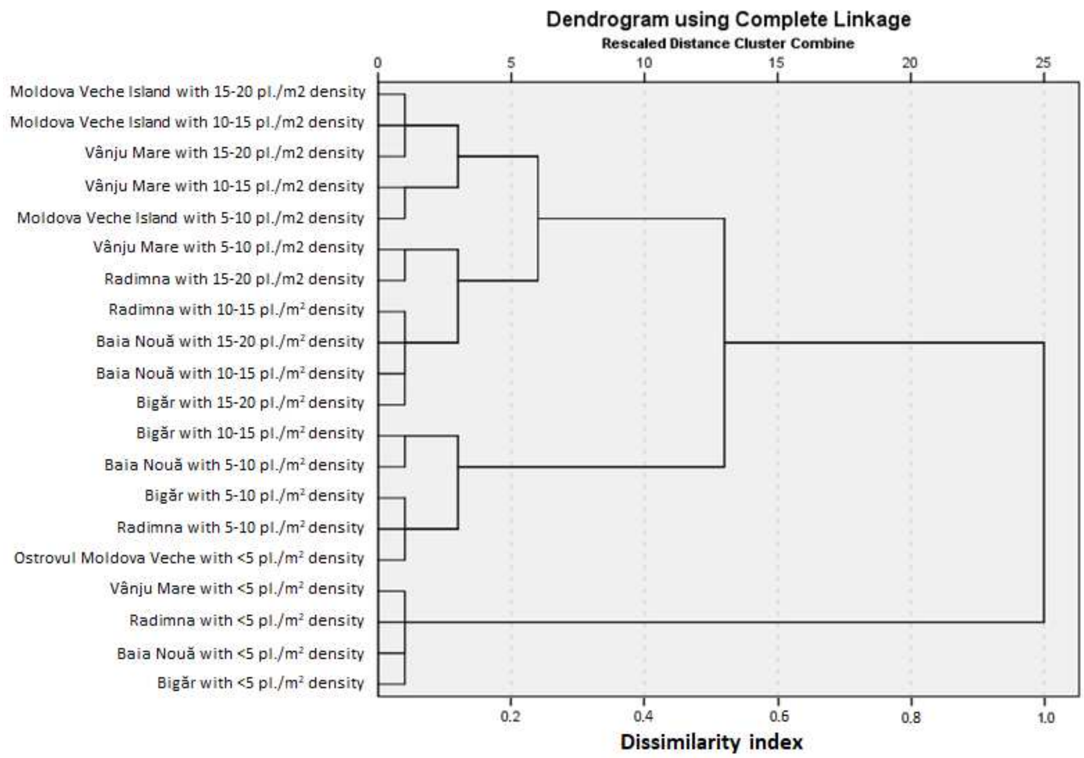


Figure 14. Dendrogram made after similarity index analisys between the areas.

Hierarchical clustering of the study areas according to the values of the analysed ecological indices using the Bray-Curtis dissimilarity index and UPGMA linkage.

3. Discussion

In recent years, *Asclepias syriaca* has invaded large areas of natural vegetation in Oltenia region, south west of Romania, which has led to the disruption of several ecosystems, especially meadows, but also bushes and ruderals. Climate change in recent years has played a significant role in the spread and development of populations of this invasive species, as well as in the increase in the degree of aggressiveness, especially in the Danube basin, an important access route for invasive species.

The high temperatures and long warm periods had a great influence on the increase in the number of individuals within the populations of *Asclepias syriaca*, as well as its spread over larger areas, having in mind seeing that this is a thermophilic species.

As a result of the studies, it was found that *Asclepias syriaca* develops very well in the Danube Valley, invading six types of plant communities. As concerned meadows, the species competes very well with the native species, often becoming dominant, having a negative effect on their pastoral value. At the same time, the toxicity of this species is increased, the source of food for ruminants being affected.

To assess the adaptation capacity of *Asclepias syriaca*, analyzing various physiological characteristics and density is essential. The species has a robust underground system of thick, white horizontal storage roots, which allows it to spread effectively and adapt to different soil conditions [27]. The plant's ability to produce vegetative sprouts from these roots contributes significantly to its adaptability.Also, the opposite leaves, broadly ovate-lanceolate, and can grow up to 28 cm long. Their waxy surface helps reduce water loss, which is advantageous in varying moisture conditions [28].The prominent midrib and overall leaf structure support efficient photosynthesis, crucial for growth in diverse environments.

Assessing the density of *A. syriaca* in different habitats can reveal how density affects competition for resources like light, water, and nutrients. Higher densities may lead to increased competition but can also enhance clonal spread through root systems [29]. Higher density also might exhibit reduced individual growth due to competition but could show increased reproductive output due to enhanced pollinator attraction [30]. This study indicates that the density can significantly influence traits such as number of fruits. Higher densities often lead to increased competition for resources, which can affect overall plant vigor and reproductive success [31]. Studies have shown that at lower densities, plants tend to exhibit greater heights and potentially higher fruit set rates due to reduced competition [32].

Study have shown that chlorophyll content in *A. syriaca* does not only depend on density but also varies with area. Some authors sustain that chlorophyll content also varies due to competition for resources such as light, water, and nutrients. Higher densities often lead to reduced light availability per plant, which can result in lower chlorophyll levels as plants adapt to shaded conditions [33]. Lower chlorophyll levels in high density areas can lead to reduced photosynthetic rate, affecting the overall growth and reproductive success of the plants [33]. Also, as density increases, individual plants may exhibit a decrease in leaf area and chlorophyll concentration. This is a common response among many species, including *A. syriaca*, where denser populations may lead to a phenomenon known as "self-shading," reducing the amount of light each plant receives [34].Other environmental factors such as soil quality, moisture availability, and temperature also interact with plant density to influence chlorophyll levels. For instance, optimal moisture and nutrient conditions can enhance chlorophyll production even at higher densities [35].

The intensity of transpiration is influenced by plant density per square meter, as well as environmental conditions. Factors such as soil moisture and temperature significantly impact transpiration. In conditions of drought or high temperatures, stomatal closure may occur to conserve water, leading to decreased transpiration rates [36]. Other authors highlighted that as plant density increases, overall biomass per area rises while individual plant growth may be stunted due to shading effects. This interplay suggests that at high densities, individual plants may experience reduced transpiration efficiency due to limited access to light and nutrients [37].

Recommendations for reducing *A. syriaca* densities include appropriate road management (eg. mowing regimes) and species control in the habitat. It is supposed that *A. syriaca* is more susceptible to mowing before flowering comparative with an early growth stage [38].

An even wider spread of the species is possible. In Vanju Mare and Radimna, the spread of *A. syriaca* is along roads and adjacent unused land, which facilitates the spread in the study area where there was a high presence of the species. The obtained results indicate that the road network contributes to the spread of *A. syriaca* in the study area, most likely by providing suitable habitats. Its reproductive behavior, characterized by a high production of wind-dispersed seeds and also the propagation of creeping lateral rhizomes, allows the species to proliferate rapidly.

In a study from Lithuania referring to *A. syriaca*, it is mentioned that the specie invades anthropogenic, semi-natural, and natural habitats and most frequently, various types of grasslands and only a small fraction of stands occupy woodland regrowth and agricultural habitats of winter or summer crop fields [39].

5. Conclusions

Considering the studies carried out in the area of the Danube Valley, the results highlighted the existence of a very high risk of invasion by *Asclepias syriaca* in Romania, especially due to the influence of recent climate changes. The identification of the species in the floristic composition of six different plant communities, from six different localities, suggests that the species has adapted and is in a continuous expansion, having a severe impact on protected habitats but also on agricultural and forestry ecosystems. The massive development and uncontrolled proliferation of individuals from the populations of this species has a negative impact on the entire biodiversity, with socio-economic effects.

From a phytosociological point of view, the results of the studies have highlighted the high adaptability of the species to integrate and develop in several types of plant communities, having a particularly negative influence on the structure, physiognomy and syndynamics of phytocenoses, with a direct impact on the entire biodiversity.

From the point of view of studies on the analyzed ecophysiological parameters, important results were obtained, the chlorophyll content of *Asclepias syriaca* is significantly influenced by plant density, with higher densities potentially leading to lower chlorophyll levels due to competition for light and resources. Further research could explore specific thresholds of density that optimize chlorophyll production and overall plants development.

Increased density can reduce photosynthetic capacity, influencing transpiration negatively. The relationship between plant density and transpiration is complex, involving interactions between physiological responses to environmental stressors and competitive dynamics among plants.

Asclepias syriaca demonstrates significant physiological resilience at high temperatures, making it an important ecologically species. Further studies could provide deeper insights into its adaptive mechanisms and potential impacts on ecosystems where it becomes invasive.

The combination of efficient reproduction, adaptability to diverse conditions, aggressive competition and lack of natural enemies makes *Asclepias syriaca* a species with a high potential for invasiveness when introduced outside its native range. The management of this invasive species requires an integrated approach, including mechanical, chemical and biological methods adapted to each situation.

Asclepias syriaca poses a significant risk as an invasive species in southwestern Romania, necessitating monitoring and management efforts to protect local biodiversity and ecosystems. We recommend prompt action (rapid removal of any new emergence of the species) to keep *Asclepias syriaca* under control in the long term.

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