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CURRENT STATE OF INVASIVE *ASCLEPIAS SYRIACA* IN ROMANIA: MORPHOLOGICAL AND ANATOMICAL INSIGHTS

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Abstract: Numerous ornamental plant species introduced to Europe have become invasive. *Asclepias syriaca* L. is a notable example and the focal point of our research. Native to North America, it was introduced to Europe in 1629 and is currently listed as an invasive species of concern under EU Regulation 1143/2014. In Romania, the species was first reported in 1836. *Asclepias syriaca* demonstrates a high capacity to adapt to various climatic and edaphic conditions. In Europe, it primarily inhabits abandoned agricultural lands, wet and dry meadows, road edges, and tree plantations, negatively impacting agriculture. The species exhibits competitive traits such as height, shade tolerance, vegetative propagation, drought resistance, and allelopathic potential. The aim of this study was to investigate the ecological and anatomical characteristics contributing to the invasive potential of *Asclepias syriaca* in Romania. The analyses of the current state of the species' populations highlighted the environmental conditions that support its establishment and spread. Additionally, anatomical investigations revealed structural traits that further emphasize its adaptability to diverse environmental conditions. This study enhances our understanding of the ecology and adaptive capacity of *Asclepias syriaca*, with significant implications for managing ecosystems affected by this invasive species in Romania.
Keywords: alien plants, anatomy, ornamental plants, structural adaptations.

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Introduction

Ornamental horticulture, through the cultivation and deliberate introduction of non-native species, has significantly contributed to the proliferation of alien plant species across diverse ecosystems (Nelufule et al. 2024). The introduction and spread of these non-native species pose serious threats to native biodiversity and disrupt ecosystem functioning (Vilá & Hulme 2017, Pyšek et al. 2020). Many ornamental species that were cultivated in the past now exhibit high invasive potential (Rouget et al. 2016). With climate change accelerating environmental shifts, the spread of invasive species is expected to continue far into the future (Beaury et al. 2023).

Asclepias syriaca belongs to the Apocynaceae family (https://gd.eppo.int/taxon/ASCSY) and is native to North America (Sîrbu & Oprea 2011).

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The species was first documented in Europe in 1629 when seeds of *Asclepias syriaca* have been sent to the pharmacyst Philip Cornut in Paris (France) to be studied and cultivated (Gaertner 1979).

Currently, the species is both naturalized and cultivated across several regions of Europe (Roşu et al. 2011, Gazoulis et al. 2022).

According to the Royal Botanic Gardens Kew, *Asclepias syriaca* is native to 45 countries globally, and has been introduced to 27 countries in Europe, including Romania (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:319076-2). The species is also listed on the EU's List of Invasive Alien Species of Union Concern (European Commision 2017, Ştef et al. 2022).

In Romania, it was first recorded in the Moldova region by Czihack in 1836, and later in Transylvania by Schur in 1866 (Sîrbu & Oprea 2011). Currently, it is widely distributed across Banat (Goia et al. 2014, Negrean & Ciortan 2014, Otves et al. 2014, Danciu et al. 2017), Crişana (Neacşu et al. 2017), Maramureş (Ţurca 1996, Marian et al. 2008, Szatmari 2012), Moldova (Sîrbu 2007), Oltenia (Răduţoiu & Stan 2013, Răduţoiu & Popescu 2020, Răduţoiu & Băloniu 2021, Răduţoiu et al. 2023), the Danube Delta (Anastasiu et al. 2014), and Transylvania (Drăgulescu 2007, Vrânceanu et al. 2010, Turcuş & Dărăban 2012, Sărăţeanu et al. 2019, Oroian et al. 2022, Sămărghiţan et al. 2022), being classified as an invasive species in Romania (Sîrbu & Oprea 2011, Urziceanu et al. 2021, Ştef et al. 2022). In addition to its broad distribution across various regions, *Asclepias syriaca* has been identified in several protected natural areas from Romania, such as the Carei Plain Natural Protected Area (Szatmari 2012), Lunca Mureşului Natural Park (Turcuş & Dărăban 2012, Sărăţeanu et al. 2019), Porţile de Fier Natural Park (Goia et al. 2014, Danciu et al. 2017), Mehedinţi Plateau Geopark (Negrean & Ciortan 2014), and the Danube Delta Biosphere Reserve (Anastasiu et al. 2014).

Asclepias syriaca is an allogamous (Mulligan & Kevan 1973), self-sterile species (Moore 1947) that reproduces both via seeds and vegetatively through adventitious buds on its underground roots, contributing to its rapid spread (Evetts & Burnside 1973, Bhowmik & Bandeen 1976, Kelemen et al. 2016, Gazoulis et al. 2022).

The species grows in a range of disturbed habitats, including pastures, cultivated fields, wastelands (Gerhardt 1928), meadows, roadsides, railway edges (Woodson 1954, Baskin & Baskin 1977), river basin, fodder crops (Bhowmik & Bandeen 1976). It is also found along lakeshores, ponds, waterways, prairies, and forest edges (Gudźinskas et al. 2021). When it reaches maturity, it is particularly resilient to drought conditions (Berkman 1949, Bhowmik & Bandeen 1976). The species predominantly grows on well-drained loamy soils (Bhowmik & Bandeen 1976, Gudźinskas et al. 2021) but can also establish itself on sandy soils and on soils rich in organic matter, phosphorus and nitrate-nitrogen (Bagi 2008). *Asclepias syriaca* has also been observed in Romania in several protected and priority habitats, including the priority habitat 91EO* Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*) (Drăgulescu 2007, Răduţoiu et al. 2023), in habitat 92A0 *Salix alba* and *Populus alba* galleries (Răduţoiu et al. 2023), and habitat 8230 Siliceous rock with pioneer vegetation of the *Sedo-Scleranthion* or of the *Sedo albi-Veronicion dillenii* (Danciu et al. 2017).

In Romania, a series of studies have focused on various aspects of *Asclepias syriaca*, including the control of this species in relation to temperature and seed depth in soil (Ştef et al.2023), the potential for obtaining carotenoid pigments through the cultivation of *Rhodotorula* spp. in the presence of *Asclepias syriaca* extract (Hainal et al. 2012), its development on soils rich in copper (Vrânceanu et al. 2010), and the potential role of *Asclepias syriaca* extracts in enhancing plant growth and cadmium uptake in oat plants (*Avena sativa*) (Stingu et al. 2012). However, no comprehensive morpho-anatomical studies have yet been conducted on *Asclepias syriaca* in Romania. Additionally, there is a need for an updated evaluation of its current population status.

The aim of this study is to assess the current state of the invasive *Asclepias syriaca* in Romania by analyzing the habitats it occupies and the environmental conditions that support its survival and spread. Additionally, we provide new insights into the species' morpho-anatomical traits that may contribute to its invasive potential and inform future management strategies.

Material and methods

Study species. *Asclepias syriaca* L. (Apocynaceae) commonly known as beeswax, is a perennial herbaceous plant (Ţiţă 2003). Its root system initially grows horizontally in the first 20 cm, after which adventitious buds begin to form (Kiltz 1930, Evetts & Burnside 1973, Bagi 2008).

The stem is tall, robust, and typically unbranched, with fine hairs and can reach heights up to 2.5 meters (Ţiţă 2003, Pioarcă-Ciocanea et al. 2020, Gazoulis et al. 2022, Ştef et al. 2022). In some cases, the plant has been reported to reach up to 6 m in height (Gerhardt 1938).

The leaves are large, simple, elliptic, broad, and arranged oppositely. The upper surface of the leaf is nearly smooth, while the lower surface is covered with hairs. Leaves can grow up to 20 cm long and 10 cm wide (Ţiţă, 2003, Bagi 2008, Pioarcă-Ciocanea et al. 2020, Gazoulis et al. 2022).

The flowers are large with shades of pink and purple. Inflorescences consists of 20 to 130 small flowers (Ţiţă 2003, Pioarcă-Ciocanea et al. 2020), which grow in the axils of the upper leaves and are known for their pleasant fragrance (Howard 2018, Ţiţă 2003, Gazoulis et al. 2022). The fruit are represented by pods, which turn brown upon ripening. The seeds have tufts of 4-5 cm long silky hairs at the top, aiding in wind dispersal (Ţiţă 2003, Pioarcă-Ciocanea et al. 2020). Each plant produces, on average, 4-6 pods containing 150-425 seeds. At densities of 1-6 stems per square meter, *Asclepias syriaca* can yield up to 87 million seeds per hectare (Gazoulis et al. 2022).

Asclepias syriaca also produces latex, a characteristic feature of the genus (Gazoulis et al. 2022).

Methodology. Occurrences of *Asclepias syriaca* in Romania were extracted from the following published materials: Sărăţeanu et al. (2020), Ştef et al. (2022), Anastasiu et al. (2022), Anastasiu et al. (2023a, b, c), Anastasiu et al. (2024), and analyzed using ArcMap v.10.4. The analysis incorporated pedological, hydrological, and human impact-related raster data. When available, population size data were also extracted. Population size was categorized according to the scale proposed in the *Protocol de inventariere a speciilor de plante invazive și potențial invazive*, developed within the Project *Managementul adecvat al speciilor invazive din România, în conformitate cu Regulamentul UE 1143/2014 referitor la prevenirea și gestionarea introducerii și răspândirii speciilor alogene invazive* (Project code: POIM2014+ 120008). The population size categories used were: 1 (1-10 individuals), 2 (11-50 individuals), 3 (51-100 individuals), 4 (101-500 individuals), 5 (>500 individuals).

For spatial analysis, various vector data sets were incorporated, including roads, rivers, lakes, land use classes, soil classes, and soil texture. These datasets were sourced from Open Street Map Contributors (OSM) at a scale of 1:1000, Corine Land Cover 2018 at a scale of 1:100,000, and the European Soil Data Centre (ESDAC) (Atlas of Romania Soils Map 1:1,000,000). The Corine Land Cover dataset was reclassified to create categories more suitable for the environmental preferences of *Asclepias syriaca*. Distance variables from linear elements (roads and water) were generated and adjusted for elevation variations using a digital elevation model (DEM) in ArcGIS v.10.4. The DEM was sourced from EU-DEM v1.1 Copernicus (European Environment Agency – EEA).

The Human Impact Index (HII) was obtained from the Center for International Earth Science Information Network (CIESIN) - Wildlife Conservation Society (Last of the Wild Data v2-2005 LTW2 Global Human Footprint Data set), at a resolution of 1 km^2.

For the anatomical studies, plant material was collected from the Botanical Garden "D. Brandza" in Bucharest in June 2024 (Fig. 1). Specimens, approximately 1.5 meters in height, were preserved in 70% ethyl alcohol.

Structural analysis was performed by preparing manual cross-sections of the stem (in the upper, median, and lower third) and leaf. The sections were stained using a double coloration technique with Iodine Green and Carmin Alaun. Starch was highlighted by soaking the sections in IIK solution (Şerbănescu-Jitariu et al. 1983). The resulting cross-sections were examined and photographed using an optical microscope.

Fig. 1. *Asclepias syriaca* in Botanical Garden "D. Brandza" in Bucharest (habitus)

Results and discussions

We analyzed 306 verified occurrences of *Asclepias syriaca* from Romania. The species is currently distributed across all major geographical regions, including Banat, Oltenia, Transylvania, Moldova, Maramureș, Crișana, and Dobrogea (Danube Delta) (Fig. 2). The highest concentration of occurrences was recorded in the western (Crișana, Banat), northwestern (Maramureș), central (Transylvania), and southwestern Romania (Oltenia), accounting for over 90% of the total occurrences (Fig. 2). Population size data were available for 243 occurrences, with the largest populations (categories 4: 101-400 individuals and 5: >500 individuals) primarily located in these same regions (Fig. 2).

A significant concern is the invasion of *Asclepias syriaca* into Protected Areas across Romania, particularly in Sites of Community Importance (SCIs) within the Natura 2000 Network and several Natural Parks (Fig. 2). Our analysis identified 110 populations within SCIs, with 15 populations exceeding 100 individuals. Additionally, 15 populations were found within Natural Parks. Notably, no populations were found within National Parks.

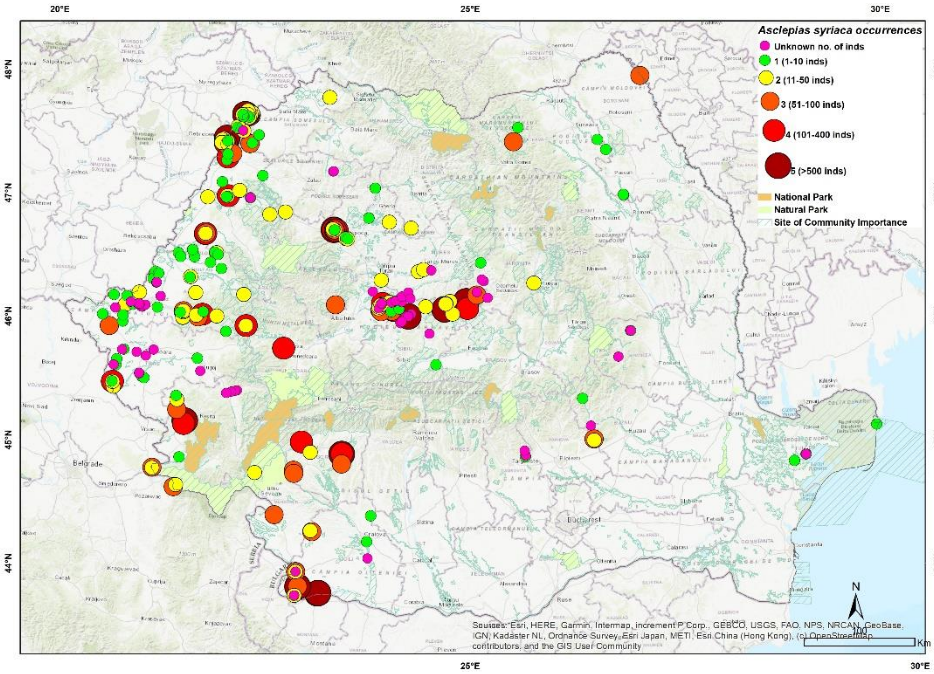

Fig. 2. Updated distribution of *Asclepias syriaca* in Romania, based on occurrences and population size data sourced from https://zenodo.org/records/6577809, https://zenodo.org/records/10396235, https://zenodo.org/records/10396155, https://zenodo.org/records/10396292

The environmental conditions that seem to favor *Asclepias syriaca* in Romania align with the species' documented habitat preferences, while also highlighting regional variations that reflect the local landscape characteristics and land-use practices.

Our findings showed that nearly 50% of *Asclepias syriaca* occurrences were found on truncated soils (Fig. 3), a soil class typically characterized by significant erosion or anthropogenic modifications (Blaga et al. 2005). Notably, more than 50% of the largest populations were established on these truncated soils, indicating they provide an advantageous environment for the species, likely due to their disturbance which creates favorable conditions for colonization and growth.

Approximately 30% of *Asclepias syriaca* occurrences were located on mollisols and hydromorphic soils (Fig. 3). These results align with its known ability to also establish on soils rich in organic matter, phosphorus and nitrate-nitrogen (Bagi 2008).

In terms of soil texture, the species exhibited a preference for heterogeneous soils in Romania, with almost 30% of occurrences recorded on such soils (Fig. 3). This reflects its adaptability to a variety of substrate conditions. Additionally, the species is found on sandy silty and silty clay loam textures, which indicates its ability to establish on both well-drained and moisture-retentive soils. Notably, large populations (exceeding 100 individuals) were most commonly associated with heterogeneous soil textures (almost 40%), suggesting that these conditions are particularly favorable for supporting large populations.

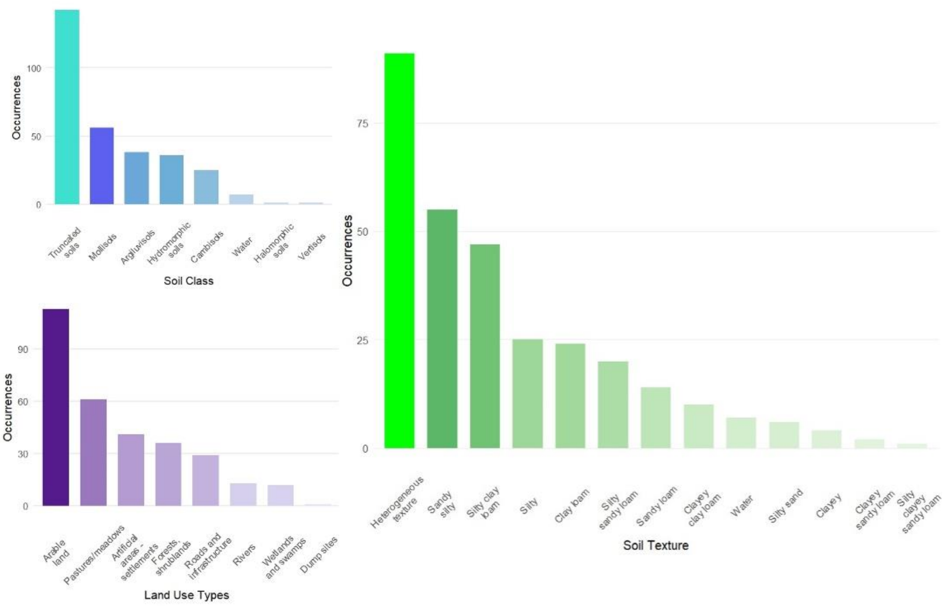

Fig. 3. Distribution of *Asclepias syriaca* occurrences across soil classes, land use types, and soil textures in Romania

As previously noted, *Asclepias syriaca* thrives in disturbed habitats such as cultivated fields, pastures, roadsides, and waterways (Gerhardt 1928, Woodson 1954, Bhowmik & Bandeen 1976, Baskin & Baskin 1977, Gudźinskas et al. 2021). In Romania, this pattern is consistent, with agricultural land supporting approximately 37% of the recorded occurrences (Fig. 3). The species also establishes in pastures/ meadows and in artificial environments such as settlements (Fig. 3).

When considering the largest populations (categories 4 and 5), arable land was found to host over 40% of these occurrences, followed by pastures/meadows, as well as forests, shrublands, each contributing approximately 15% of the largest populations. In contrast, heavily urbanized or disturbed areas, such as settlements, supported only 3 occurrences in categories 4 and 5 (9.37%), suggesting that urban areas may not provide optimal conditions for large populations of the species.

Another notable aspect of *Asclepias syriaca* distribution in Romania is its frequent proximity to water bodies and roads (Fig. 4). The species' higher density along roadsides and waterways aligns with findings from other regions where it thrives in similarly disturbed environments (Woodson 1954, Bhowmik & Bandeen 1976, Gudźinskas et al. 2021).

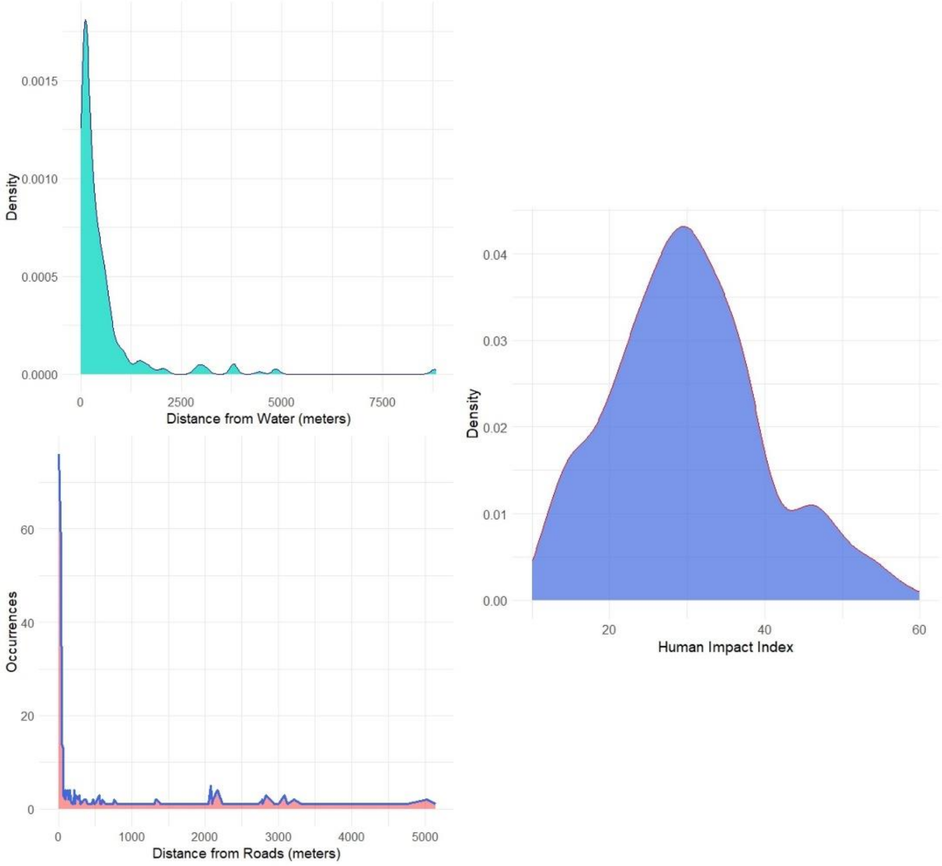

Fig. 4. Relationship between *Asclepias syriaca* distribution and anthropogenic factors in Romania: occurrence density relative to distance from water bodies, and Human Impact Index (HII), and occurrences relative to distance from roads

Regarding anthropogenic influence, *Asclepias syriaca* appears to reach its highest density in areas with a Human Impact Index (HII) of 25-35 (Fig. 4), which corresponds to moderate levels of human disturbance. This finding aligns with the species' ecological strategy of thriving in environments that experience regular disturbances, such as agricultural activities or infrastructure development, but are not entirely degraded.

The anatomical investigations, revealed that the **stem** cross-sectioned at the three levels: lower, median and upper third, showed a circular outline in the lower and median and a slightly ridged shape with a prominent lateral ridge in the upper third (Fig. 5 A, C, E). The stem of *Asclepias syriaca* presents a secondary structure across the entire stem, resulting from the vascular cambium activity (Fig. 5 A - F). The structure displays the following anatomical areas: epidermis, cortex and vascular cylinder with a medullary lacuna in the center (Fig. 5 C, E).

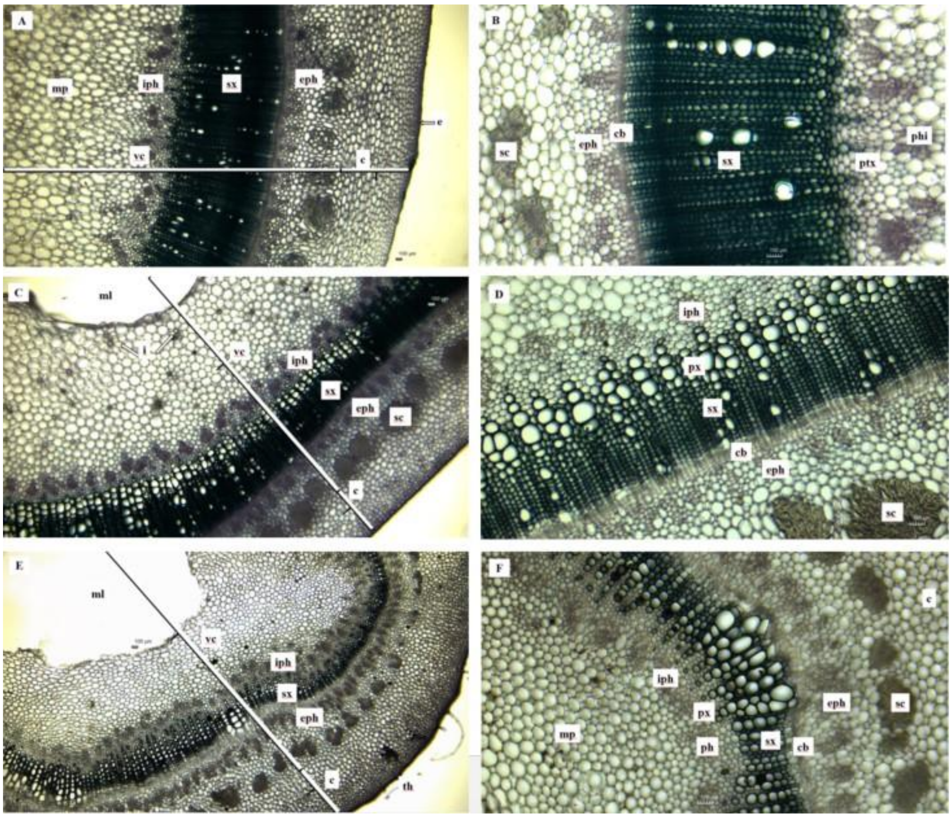

Fig. 5. Cross-sections of *Asclepias syriaca* stem, highlighting the different anatomical areas (A, B - lower third, C, D - median third, E, F - upper third), (colorants: Iodine Green, Carmine Alum, IIK): c - cortex, cb - vascular cambium, e - epidermis, eph - external phloem, id - idioblast, iph - internal phloem, ml - medullary lacuna, mp - medullary parenchyma, px - primary xylem, ptx - protoxylem, sc - sclerenchymatous caps, sx - secondary xylem, th - trichomes, vc - vascular cylinder

The epidermis consists of a single layer of isodiametric cells, with thicker cell walls inwards. In the upper third, among the epidermis cells, pluricellular mineralized trichomes were noticed (Fig. 5 E).

The cortex is composed of parenchyma cells with intercellular spaces. The first 1-2 layers presents thick cellulosic walls.

The vascular cylinder is outlined by a ring of sclerenchymatous caps formed by sclerenchymatous fibers which sustain the vascular bundles (Fig. 5 A - F). The vascular tissue is organized in open bicollateral vascular bundles, concentrically arranged, with a prominent vascular cambium between the outer phloem and xylem (Fig. 5 A - F). In the secondary xylem, the woody vessels have a radial arrangement. The area of the secondary xylem decreases from the base to the top of the stem (Fig. 5 A, C, E). The bundles of primary xylem are extended towards the inner phloem (Fig. 5 B, D, F).

The vascular cylinder is filled with a fundamental parenchyma of different sizes of parenchymatic cells with intercellular spaces, which became disorganized in the central part, resulting in a medullary lacuna. Additionally, both areas, cortex and vascular cylinder contain frequent secretory idioblasts (Fig. 5 C).

The **lamina** of *Asclepias syriaca* presents a bifacial dorsiventral structure. In cross section, the leaf exhibits a flattened shape on both faces: adaxial and abaxial, excepting the midrib, which has a semicircular outline with a rounded abaxial face (Fig. 6 A).

Both epidermis are single-layered, consisting of isodiametric cells with thick cell walls, interspaced with long pluricellular mineralized trichomes (Fig. 6 A - C). The epidermis is covered by a thin cuticle which displays cuticular ridges (Fig. 6 C). The leaf is amphystomatic, with stomata located at the same level with the epidermal cells. Paradermal sections displayed the anomocytic type of stomata, identified on the adaxial surface (Fig. 6 D).

The mesophyll is differentiated into 1-2 layers of palisade cells located beneath the upper epidermis and a large spongy parenchyma with large gaps, above the lower epidermis (Fig. 6 A - B).

The vascular tissues are organized into bicollateral vascular bundles with a primary structure. In the midrid, the xylem and phloem are larger with a semi-circular arangement, exposing the phloem towards the both faces. Various secretory idioblasts were observed between xylem and phloem and at the periphery of the phloem (Fig. 6 A). Few layers of angular collenchyma, located under both the upper and lower epidermis, supports the midrib (Fig. 6 A, C). The minor vascular bundles are enclosed by parenchymatous perifascicular sheaths (Fig. 5 B). The internal phloem is bounded by an amyliferous sheath (Fig. 6 A).

The potential risk of invasion of *A. syriaca*, considered one of the most dangerous invasive species (Bakacsy & Bagi 2020), is attributed to a fast-growing rate associated with a tall habitus, the vegetative spread by rhizome propagation, drought tolerance, and a high resistance to herbivory (Bhowmik & Bandeen 1976, Agrawal 2004, Bagi 2008, Tao et al. 2016).

Our observations on the vegetative organs anatomy revealed a vigurous secondary structure of the stem. The presence of the rhizome supports the vegetative spread and fragmentation facilitating the adaptation to various environmental conditions, contributing to its high competitivity (Bhowmik 1994, Dvirna 2018, Follak et al. 2021). *Asclepias syriaca* clonal's activity is an important strategy for colonization of new ecological niches and succesful invasion (Anderson 1999, Nowiński & Latowski 2003, Podbielkowski & Sudnik-Wójcikowska 2003, Rowe & Speck 2005, Borders & Lee-Mäder 2015).

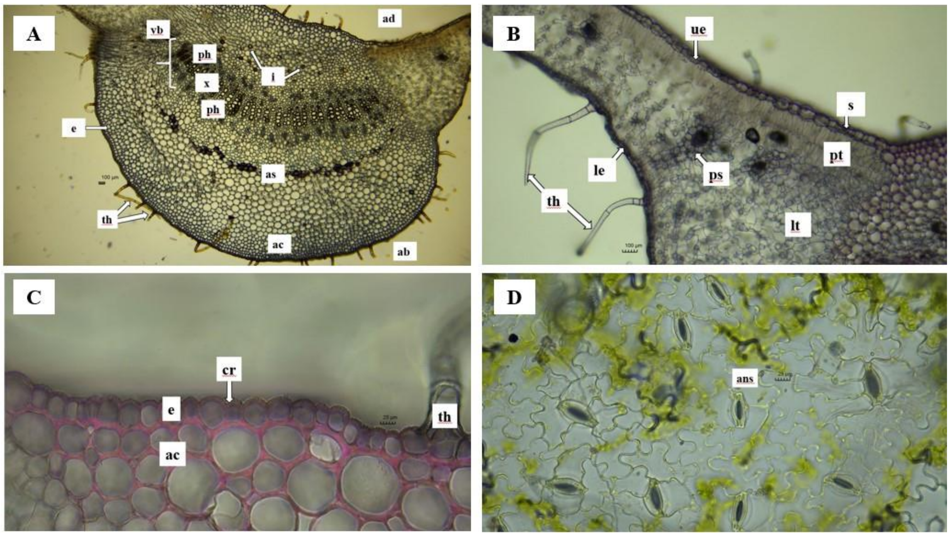

Fig. 6. Cross-sections of *Asclepias syriaca* leaf, highlighting the different anatomical areas (A, C - midrid, B - mesophyll, D - paradermal section of adaxial epidermis), (colorants: Iodine Green, Carmine Alum, IIK): ab - abaxial face, ad - adaxial face, ac - angular collenchyma, ans - anomocytic stomata, as - amyliferous sheath, cr - cuticular ridges, e - epidermis, id - idioblast, le - lower epidermis, lt - lacunose tissue, ph - phloem, ps - perifascicular sheaths, pt - palisade tissue, s - stomata, th - trichomes, ue - upper epidermis, vb - vascular bundle, x - xylem

Agrawal et al. (2012) have shown that when competing for light, *Asclepias syriaca* exhibits significant shoot elongation. The long trichomes and ridged and thick cuticles enables the resistance of this species in full sun (Xiao et al. 2017).

The orthotropic stem exhibits a strong stem structure with a large area of secondary xylem which sustains the leaves and reproductive organs promoting thus the settlement and resilience of this invasive species (Sârbu & Smarandache 2015, Wang et al. 2022, Dumitrașcu et al. 2023, Mihai et al. 2024).

The calcium oxallat druses, substances noticed in the stem and leaves, and also by leaf trichomes serve as a defense mechanism against the herbivores (Agrawal 2004). The leaf structure pointed once again the high rate of specialization of this species.

Conclusions

In Romania, *Asclepias syriaca* is primarily found in disturbed habitats, highlighting its adaptability to human-modified environments. Large populations are often associated with regions featuring truncated soils, heterogeneous soil textures, and other anthropogenic habitats, which seem to facilitate its successful establishment and spread.

A concerning finding is that significant populations of *Asclepias syriaca* are present within Protected Areas. Given the potential ecological risks posed by this invasive species, it is essential to implement mitigation measures to control its spread in these sensitive areas.

Anatomical investigation of *Asclepias syriaca* vegetative structures provides further insight into its invasive potential. The species possesses versatile traits that contribute to its ability to withstand environmental stress and outcompete native species for resources in diverse ecological environments.

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