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Anatomical insights into the adaptability and invasiveness of *Humulus scandens* in Romanian flora

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Abstract

The negative impact of invasive alien species on biodiversity, agriculture, ecosystem services, and human well-being is a significant concern for scientists. Despite extensive research in the past decade to understand their effects on native communities and ecosystems, predicting and managing future invasions remains challenging. *Humulus scandens*, a species native to Asia, was introduced to Europe in 1880 for ornamental purposes. This vigorous climbing vine thrives in wetlands, riparian habitats (especially river and stream sides), and ruderal habitats (roadsides, wastelands, abandoned, and disturbed areas). Our research aimed to investigate the vegetative body structure of *H. scandens* to understand its high resilience in Romanian habitats. The structural characterization of the vegetative organs involved detailed microscopic examination of the coloured cross-sections. The results revealed a well-developed secondary structure of the root and stem, granting robustness and resistance to the plant, favouring it in competition with other species for environmental resources.

Keywords: adaptive ability; environmental conditions; histo-anatomical analysis; invasive plant species

Introduction

Invasive alien species (IAS) have been considered by the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) as the fifth most important driver of global biodiversity and ecosystem service changes (Butchart *et al.*, 2010). Their impact on ecosystems is expressed through alterations in community structure, soil processes, hydrology, and ecosystem services (Levine *et al.*, 2004; Liao *et al.*, 2008; Pyšek and Richardson, 2010). These changes include hybridization with native species, competition, and altered species richness facilitated by mechanisms such as allelopathy (Vilà *et al.*, 2000; Hierro and Callaway, 2003; Vilà *et al.*, 2004; Gaertner *et al.*, 2009; Lázaro-Lobo *et al.*, 2023).

Given the global priority of managing invasive alien species for biodiversity conservation, research has focused on identifying the traits that enhance invasiveness and assessing which habitats are most susceptible to

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invasion (Pyšek *et al*., 1995; Alpert *et al*., 2000; Richardson and Pyšek, 2006; Pyšek *et al*., 2012). Plant productivity, influenced by competitive abilities and reproductive strategies, is particularly sensitive to environmental conditions (Daehler, 2003; Medina-Villar, 2020). Environmental stress, for instance, can increase the performance of native species and reduce opportunities for alien species invasions (Mueller-Dombois and Loope, 1990; Hobbs and Huenneke, 1992; Alpert *et al*. 2000).

Despite numerous studies, the latest reviews highlight that identifying specific traits linked to invasiveness remains challenging (Alpert *et al*., 2000; Richardson and Pyšek, 2006; Pyšek *et al*., 2012). However, key parameters such as species' evolutionary background, community structure, propagule pressure, habitat disturbance, and environmental stress are linked to invasiveness (Higgins and Richardson, 1999; Alpert *et al*., 2000; Richardson and Pyšek, 2006; Pyšek *et al*., 2012).

One of the species assessed by Pest Risk Analysis (PRA) as having a high potential for further spread and colonization in various biogeographical regions is *Humulus scandens* (EPPO, 2018). This species also poses a high phytosanitary risk due to allergenic pollens and is listed under the EU Regulation 1143/2014 (Park *et al*., 1999; EPPO, 2018).

Humulus scandens (Lour.) Merr. (syn. *Humulus japonicus* Siebold & Zucc.) belongs to the Cannabaceae family and is native to Asia (China, Taiwan, Japan, North and South Korea, Russian Far East and Vietnam) (EPPO, 2020). Introduced initially as an ornamental plant, it has been naturalized and even become invasive in various regions in Europe and North America (Small, 1997; Balogh and Dancza, 2008). The species was first introduced to Europe around 1880 in Paris, for growing over trellises, arbores or fences (Tournois, 1914; Chevalier, 1943; Balogh and Dancza, 2008; EPPO, 2019). By 1993, it was established in northern Italy, western Hungary, and possibly other regions, as indicated in Flora Europaea (Tutin and Akeroyd, 1993; Balogh and Dancza, 2008). Currently, the species is reported as invasive in France, Hungary, Italy, and Croatia (Balogh and Dancza, 2008; Fried *et al*., 2018; Galasso *et al*., 2018; Kiraly *et al*., 2021).

In Romania, *Humulus scandens* was first documented in 1942 from the southern region and subsequently, in 1952, was included in Flora R.P.R. as a subspontaneous ornamental species (Morariu, 1942; Săvulescu, 1952). Currently, the species' distribution spans from western and southwestern regions, along the Danube and its tributaries, to northwestern Romania (Anastasiu and Negrean, 2009; Sîrbu and Oprea, 2011; Urziceanu *et al*., 2022).

In its native habitat, *Humulus scandens* thrives in deciduous forests, forest margins, alluvial woods, streamsides, lowland meadows, and ruderal habitats, particularly along roads and wastelands (Monsi and Saeki 1953; Zhou and Bartholomew, 2003; Balogh, and Dancza, 2008). Its current distribution in introduced areas mirrors its native habitats, favouring riverbanks, floodplains, and disturbed areas, such as roadsides and railway sides (Zhou and Bartholomew, 2003; Balogh and Dancza, 2008; Fried *et al*., 2017). The species is a cosmopolite weed that prefers wet alluvial habitats with loamy sand-clay soils, especially invading open disturbed areas (Balogh and Dancza, 2008; EPPO, 2019; Urziceanu *et al*., 2022).

Humulus scandens is a climbing vine growing in large dense stands that can outcompete native communities by reducing species richness and modifying species composition (EPPO, 2019; Mahaut, 2014). Its anthesis period in Europe is from July to September, and it reproduces exclusively by seeds (Balogh and Dancza, 2008), which exhibit prolonged viability in the soil (more than one season). Additionally, the species can produce adventitious roots, as a perennial species (Balogh and Dancza, 2008; Urziceanu *et al*., 2022).

Although the biological effects and general morphology of *Humulus scandens* have been extensively studied, there is limited information on the anatomical structure of its vegetative body. This study aims to fill that gap by analysing and describing the anatomical features of *Humulus scandens*' vegetative body and correlating them with its ecological preferences in Romania. By investigating the species' anatomical traits, we aim to enhance our understanding of how these traits contribute to its adaptive capacity in various environmental conditions.

Materials and Methods

Study species

Humulus scandens (Lour.) Merr. is a dioecious herbaceous species, either annual or perennial (Balogh and Dancza, 2008). The root system consists of a taproot with a principal root that grows vertically and penetrates deep into the soil, accompanied by lateral roots, parallel to the surface (Ehara, 1955). The stem is branched, hexangular, pubescent with 6 sets of climbing hairs arranged spirally (Ehara, 1955; Balogh and Dancza, 2008). The bines twist clockwise around themselves and around the supports, using the rough hairs on the stems, petioles, and leaves (Balogh and Dancza, 2008). The leaves are opposite, 5-12 cm long, with light green cordate lamina, palmately lobed with 5-7(-9) lobes (Balogh and Dancza, 2008; EPPO, 2019). The leaves have a long petiole and are highly pubescent, particularly on the abaxial face, while the adaxial surface is covered with "cystolith hairs" (Carbone and Gervasi, 2022). Inflorescences are pale greenish-yellow, smaller than those of *Humulus lupulus*. Female inflorescences lack lupulin glands and are not used for fermentation (Balogh and Dancza, 2008; Carbone and Gervasi, 2022). However, recent studies have highlighted the species' medicinal properties, particularly in treating neurodegenerative diseases related to oxidative stress (Wang *et al*., 2022) and autism disorder (Park *et al*., 2021). The male inflorescence is represented by an erect, branched panicle measuring 15-25 cm in length, while the female inflorescence consists of ovoid cone-like spikes with cordate, significantly mucronate 10-16 bracts. The infructescence is pendulous, green, cone-like, ovoid to oblong, ranging from (1-)1.5-3.0(-4) cm in size, with yellow-brown, ovoid-orbicular, inflated to lenticular achenes (Balogh and Dancza, 2008).

Methodology

The biological material comprising the vegetative body of *Humulus scandens* was collected in 2023 from the banks of the Dâmbovița River, near Bălăceanca (44°23'53.36"N, 26°16'52.10"E), Ilfov County, and preserved in 70% alcohol.

Vegetative organs were manually cross-sectioned in the upper third of both the root and stem. The sections were bleached and then stained with two differential colorants applied successively: Carmine Alum (colour the cellulose - pectic cell walls in tones of pink and Iodine Green (colour in green the cell walls that contain lignin), followed by IIK to highlight the starch (Șerbănescu-Jitariu *et al*., 1983).

The microscopic slides were analyzed with DOCUVAL CARL ZEISS JENA optical microscope and micro-photographed with an incorporated NIKON D90 digital camera.

Results

The root of *Humulus scandens* exhibits a well-developed secondary structure resulting from the activity of the two secondary meristems: the vascular cambium, located within the vascular cylinder, and the phellogen, found in the cortex. This structure is characterized by concentric rings of secondary vascular tissues crossed by medullary rays. These rays are narrowing at the level of the xylem and widen at the level of the phloem.

In cross-sections, the root displays a circular shape and differentiates the following distinct histo-anatomical areas: the periderm, cortex, and the vascular cylinder. Notably, the vascular cylinder occupies almost 95% of the root's area (Figure 1 A, B).

The periderm, produced by phellogen, functioning as the secondary cortex, is located outside the primary cortex (Figure 1 A, B). Within the vascular cylinder, both primary and secondary vascular tissues, and medullary rays, are evident. The center of the vascular cylinder is filled in by the primary xylem; the pith is lacking (Figure 1 A).

Both the secondary xylem and phloem parenchyma contain bundles of sclerenchymatous fibers and calcium oxalate crystals (Figure 1 C). Additionally, the older vessels located in the center of the root are characterized by the presence of tyloses (Figure 1 D).

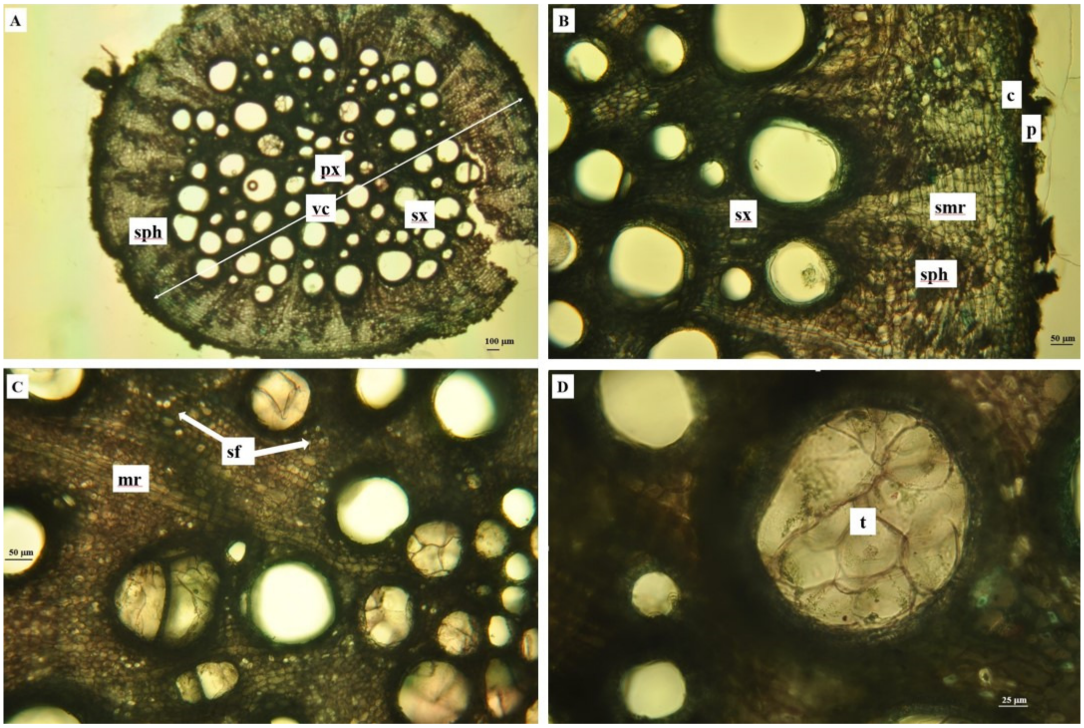

Figure 1. Cross section of *Humulus scandens* root, highlighting the different anatomical areas. (A) - overview; (B, C, D - detail); (colorants: Iodine Green and Carmine Alum): c - cortex, mr - medullary rays, p - periderm, px - primary xylem, sf - sclerenchymatous fibers, smr - secondary medullary rays, sph - secondary phloem, sx - secondary xylem, vc - vascular cylinder, t - tyloses

In cross-section, the **stem** of *Humulus scandens* has a hexagonal shape with six prominent ridges, and displays the distinct anatomical areas: epidermis, cortex, and vascular cylinder, which occupies approximately 90% of the stem's surface (Figure 2 A, C). The stem features a secondary structure with growth rings resulting from the vascular cambium activity.

The epidermis consists of a single layer of compactly arranged cells, covered by a thick, striated cuticle (Figure 2 C). At the level of ridges, short, stiff cystolithic hairs with noticeably thickened cell walls are present (Figure 2 B).

Below the epidermis, the cortex is composed of an angular collenchyma (forming 8-10 layers in the ridge areas) and a parenchyma of thin-walled isodiametric cells with small intercellular spaces, which forms the internal cortex. The innermost layer of the cortex function as primary endodermis (Figure 2 C).

A thick ring of pericyclic sclerenchymatous fibers defines the boundary of the vascular cylinder (Figure 2 C). The vascular tissues constitute approximately 20% of the stem's area. Within the vascular cylinder, bundles of primary xylem extend into the medullary parenchyma, while the secondary vascular tissue forms a thinner outer ring of phloem and a thicker inner ring of xylem. The well-developed vascular cambium is prominent (Figure 2D). The wood parenchyma is lignified and lacks wood fibers. Additionally, the medullary rays are thin and mostly uniseriate (Figure 2 C, D). In the phloem parenchyma, secretory idioblasts and druses can be observed (Figure 2 B).

The medullary parenchyma consists of cells with thin cellulosic walls. These cells become disorganized in the central part of the stem, leading to the formation of an air cavity with a relatively circular outline (Figure 2 A).

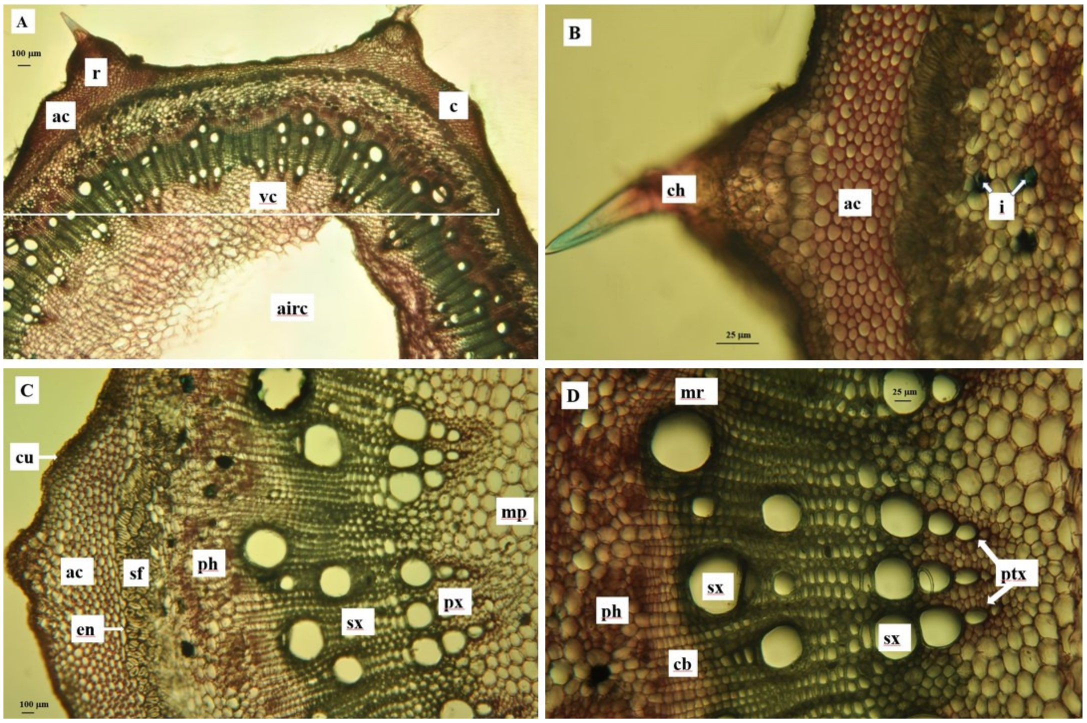

Figure 2. Cross section of *Humulus scandens* stem, highlighting the different anatomical areas. (A) - overview; (B, C, D) - detail; (colorants: Iodine Green, Carmine Alum, IIK): airc - air cavity, ac - angular collenchyma, c - cortex, cu - cuticle, cb - vascular cambium, ch - cystolithic hair, en - endodermis, i - idioblasts, mp - medullary parenchyma, mr - medullary ray, ph - phloem, px - primary xylem, ptx - protoxylem, r - ridge, sf - sclerenchymatous fibres, sx - secondary xylem, vc - vascular cylinder

The **lamina** is bifacial, dorsiventrally differentiated. In cross section, the midrib is more prominent towards the abaxial face, while it presents a groove on the adaxial face (Figure 3 A). Both upper and lower epidermis are uniseriate and feature unicellular trichomes. The upper epidermis consists of large isodiametric cells interspersed with short cystolithic conic trichomes (Figure 3 B). Conversely, the lower epidermis contains smaller cells and trichomes that are either short or long and acute with a dilated base (Figure 3 B, C). Stomata were observed exclusively on the lower epidermis (hypostomatic lamina).

The mesophyll comprises a single layer of elongated palisade cells located beneath the upper epidermis, and three to four layers of dense spongy parenchyma. The palisade cells contain calcium oxalate druses (Figure 3 B, C).

The vascular tissues form collateral – type vascular bundles, with a larger bundle in the midrib, where xylem and phloem are arranged in an open arch formation. This vascular bundle is enclosed by a few layers of meatic parenchyma. On the periphery of the phloem, various secretory idioblasts were observed. Angular collenchyma is present under both the upper and lower epidermis layers of the midrib (Figure 3 A).

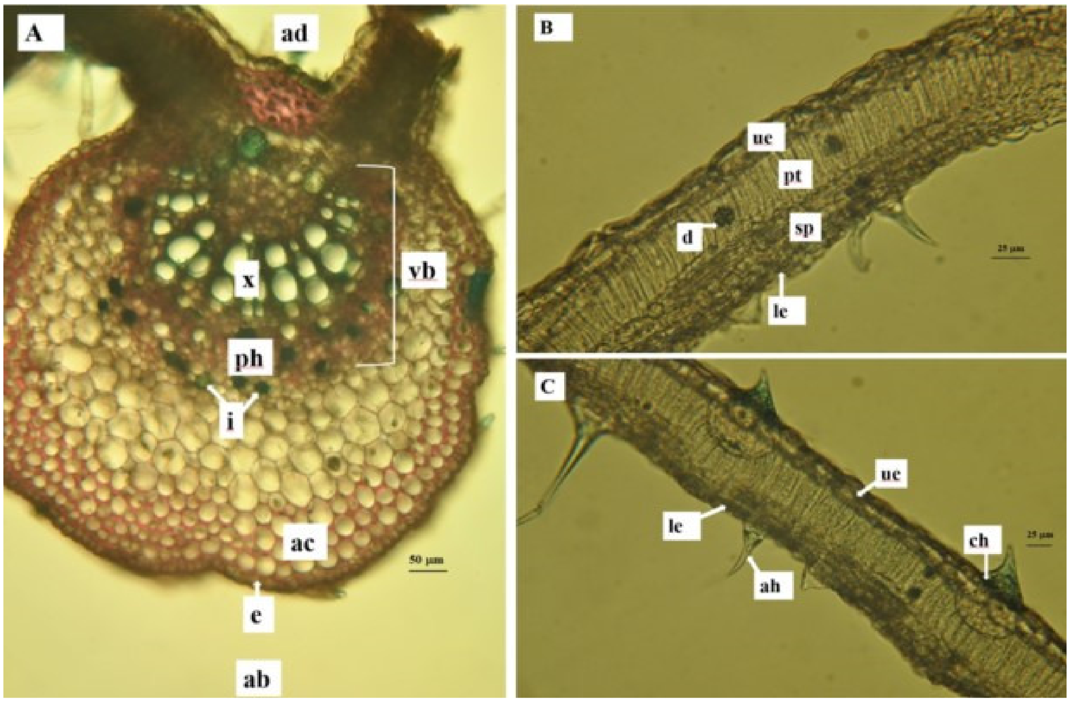

Figure 3. Cross section of *Humulus scandens* leaf, highlighting the different anatomical areas (A - midrib, B, C - mesophyll) (colorants: Iodine Green and Carmine Alum): ab - abaxial face, ac - angular collenchyma, ad - adaxial face, ah - acute hairs, ch - cystolithic hair, d - druse, e - epidermis, i - idioblasts, le - lower epidermis, ph - phloem, pt - pallisade parenchyma, sp - spongy parenchyma, ue - upper epidermis, vb - vascular bundle, x - xylem

In cross-section, the **petiole** of *Humulus scandens* exhibits a semicircular outline with a slightly concave adaxial face and a distinctly ridged abaxial face marked by seven ridges, each with cystolithic hairs (Figure 4 A).

The epidermis consists of a single layer of cells covered by a thin cuticle. It features unicellular tector trichomes and rare stomata.

The cortex is differentiated into two distinct areas: a continuous angular collenchyma beneath the epidermis, followed by a parenchyma. The angular collenchyma is more extensive in the ridge areas, consisting of 9-10 layers of cells (Figure 4 A, B).

The vascular tissues of the petiole are represented by 11 collateral vascular bundles of varying sizes, displaying a secondary structure. These bundles are arranged in a ring formation with two central bundles surrounded by meat fundamental parenchyma (Figure 4 A). Narrow parenchymatic rays cross all vascular bundles (Figure 4 B), and in proximity to the central bundles, larger parenchyma cells exhibit signs of disorganization.

An amyliferous sheath encloses the vascular bundles (Figure 4 A, B). Additionally, numerous secretory idioblasts are present in the outer region of the phloem (Figure 4 B).

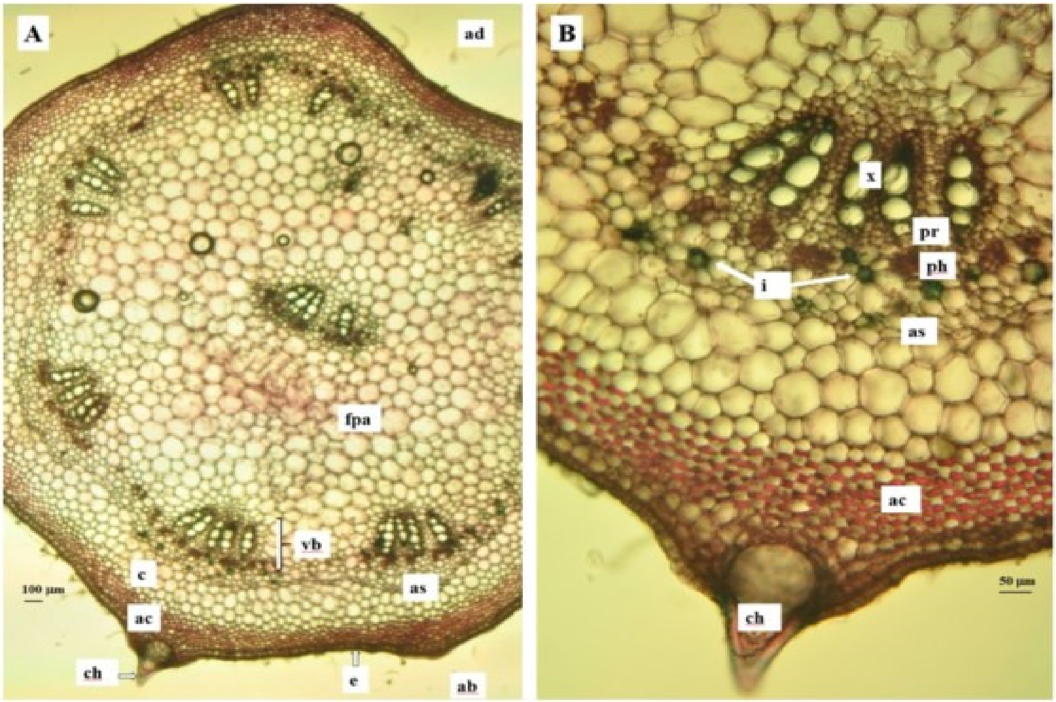

Figure 4. Cross section of *Humulus scandens* petiole, highlighting the different anatomical areas (A - overview, B - detail) (colorants: Iodine Green, Carmine Alum, IIK): ab - abaxial face, ac - angular collenchyma, ad - adaxial face, as - amyliferous sheath, c - cortex, ch - cystolithic hair, e -epidermis, fpa - fundamental parenchyma, i - idioblasts, ph - phloem, pr - parenchymatic ray, vb -vascular bundle, x - xilem

Discussion

The anatomical investigations of *Humulus scandens*' vegetative organs demonstrate its robustness as an adaptation to diverse environmental conditions, contributing to its competitive vigour and successful establishment.

Both the root and stem develop an early secondary structure, providing this annual plant species with a solid vegetative body. Alien plant species often exhibit increased vigour and height in introduced areas (Blossey and Notzold, 1995; Dumitrașcu *et al*., 2023), enhancing their competitive capacity and facilitating successful invasion and settlement in heterogeneous habitats (Blossey and Notzold, 1995; Wang *et al*., 2022; Dumitrașcu *et al*., 2023).

The root system, deeply anchored in soil, is well adapted for efficient nutrient absorption, enabling the species to effectively compete with native species for environmental resources and space. Xylem vessels containing tyloses are indicative of the plant's defense responses, activated under various abiotic and biotic stresses such as flooding (Davison and Tay, 1987), mechanical injury (Sun *et al*., 2022), or pathogen infection (Dimond, 1955). This defensive mechanism of *Humulus scandens* in combating threats such as hop's powdery mildew (*Podosphaera macularis* (Wallr.) U. Braun & S.Takaram), has been noted in previous studies (Georgescu *et al*., 2021). Furthermore, different experiments showed that tylose formations were considered a response to loss of vessel water (Böhm, 1867; Tyree and Zimmermann, 2002).

The premature development of the secondary structure of the stem provides advantages for leaf support and reproductive organs, crucial for completing the plant's life cycle and ensuring species dispersal across different ecological conditions (Wang *et al*., 2022).

The hexagonal stem shape, reinforced by the angular collenchyma, enhances flexibility and prevents damage to vascular tissues during bending (Leroux, 2012). The stem's structural outline, defined by the strong cystolithic hairs containing calcium carbonate deposits, serves important purposes such as calcium excess elimination (Freisleben, 1933) and defense against herbivore attack (Villard *et al*., 2019).

The bifacial leaves with a large palisade layer between the upper epidermis and spongy parenchyma, optimize sunlight absorption and utilization (Wang *et al*., 2022). Furthermore, trichomes from both the upper and lower epidermis, along with ridged and thick cuticles, play essential roles in protecting against water loss (Xiao *et al*., 2017).

Conclusions

The anatomical investigations of *Humulus scandens*' revealed a robust vegetative body that enhances its adaptability and resilience across diverse habitats. This species exhibits distinct morpho-anatomical traits that not only enhance its ecological success but also contribute to its competitive advantage. This adaptability is evident in its early development of secondary structures in both roots and stems, which provide strength and support for survival and growth, in the competition for living space. These anatomical features, coupled with ecological data, provide valuable insights for monitoring the distribution of *Humulus scandens* and assessing its potential invasiveness in new environments. Understanding these traits and their ecological implications is essential for formulating effective management strategies and conservation efforts aimed at mitigating potential impacts on native ecosystems. However, continuous research and monitoring of the species' reproductive biology, seed dispersal mechanisms, and population dynamics are crucial to fully understand its invasiveness potential and long-term ecological impact.

Authors' Contributions

Conceptualization: MD, AS; Formal analysis: MD, AS; Investigation: MD, AC, DCM; Methodology: MD, AS; Resources: MD, AC, DCM; Supervision: AC, AS; Writing: MD, AC, AS, DCM.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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