

ANATOMICAL RESEARCH OF THE VEGETATIVE BODY OF *IMPATIENS GLANDULIFERA*, AN ORNAMENTAL PLANT THAT HAS BECOME INVASIVE

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Abstract

Ornamental horticulture is a significant contributor to the introduction of invasive plant species, such as *Impatiens glandulifera*. Native to the temperate and humid regions of Asia, particularly the Himalaya Mountains, the species was first reported in Europe in 1839, introduced for ornamental and nectar-producing purposes. Its presence in Romania dates since 1882. Over the past few decades, the species' invasive nature has led to its inclusion in the List of Invasive Alien Species of Union concern. The existing studies have systematically examined the plant's morphology, habitat requirements, ecology, life cycle, and genetic aspects, emphasizing the traits associated with its high invasive potential. The species exhibits a preference for moist to wet and nutrient-rich soils, commonly found in river valleys and associated riparian habitats. Our research, based on samples collected from Romania, aims to identify and detail the structural features of the vegetative body of *Impatiens glandulifera* that contribute to its adaptive ability to specific environmental conditions. The anatomical investigations revealed a secondary root and stem structure, enhancing this annual species' robustness and resistance in plant communities.

Key words: alien plants, structural adaptations, wet environment, environmental requirements.

INTRODUCTION

The increase of invasive alien species is a growing concern, with their spread posing significant threats to ecosystems worldwide. These invaders can affect native species by altering their abundance, diversity, and even driving them towards extinction. Furthermore, the disruptive effects of invasive species extend to ecosystem functioning, with consequences that are expected to increase in the future (Pyšek et al., 2020).

The introduction of invasive species into new regions can result from various human activities, either deliberate or unintentional (Pyšek et al., 2020; Pyšek et al., 2004; Richardson et al., 2000).

The horticultural industry, widely recognized as one of the main pathways for introducing invasive species worldwide, plays a significant role in this (Bayón & Vilà, 2019).

Invasive alien species are defined as non-native species that establish themselves in ecosystems or natural habitats, posing threats to native biological diversity. Such alien species, also referred to as allochthonous, non-indigenous, or exotic species, have the ability to disperse or reproduce beyond their native range (IUCN, 2000).

Impatiens glandulifera Royle, a species of the Balsaminaceae family (Clements et al., 2008; Kraehmer, 2013; Caris et al., 2006; Helsen et al., 2021), is native to northeastern Pakistan, northwestern India, and western Nepal, thriving in temperate climates, between 2000 and 4000 m above sea level (Beerling & Perrins, 1993; Helsen et al., 2021; CABI, 2023).

In Europe, the species was first reported from England in 1839 (Drescher & Prots, 2003). Within approximately four decades, it became naturalized, spreading across nearly all European countries (Pyšek et al., 1995; Helsen

et al., 2021). Currently, the species is distributed in 34 European countries, including Romania (CABI, 2023).

In Romania, *Impatiens glandulifera* was documented for the first time in 1882, from Sibiu (Balog, 2008; Sîrbu & Oprea, 2011). According to the literature, the species is widely distributed throughout the country, excluding Banat, Oltenia and Dobrogea regions (Oprea, 2005; Sîrbu & Oprea, 2011; Dumitrașcu et al., 2014; Anastasiu et al., 2020). Notably, it has also been reported from natural or national parks (Dumitrașcu et al., 2014; Anastasiu et al., 2022). The species' rapid growth rate is highlighted by its ability to reach up to 1.3 meters in height within 72 days of germination (Perrins et al., 1993; Helsen et al., 2021). *Impatiens glandulifera* thrives in riparian habitats, including moist forests, swamps, stream sides, ditches, in full light, or partial shade (Balogh, 2008; Sîrbu & Oprea, 2011; Anastasiu et al., 2019). It commonly inhabits hilly and mountainous regions (Sîrbu & Oprea, 2011; Anastasiu et al., 2005; Anastasiu et al., 2019). This species can impede the growth and development of native plants and can spread along watercourses, thus constituting a danger for nature conservation (Pyšek & Prach, 1995; Anastasiu et al., 2019).

Several factors contribute to the success of the invasion of *Impatiens glandulifera*. One such factor is the species' ability to grow up to 4 meters in height, being the tallest annual plant in Europe, making it the strongest competitor against native grass species. Additionally, the species' prolific seed production, with a single plant capable of producing up to 2500 seeds dispersed over distances of up to 7 meters, further amplifies its invasive potential (Balogh, 2008; Ab Razak et al., 2023). Moreover, the seeds can persist as a seed bank for at least 18 months (Mumford, 1990).

Despite extensive research on the morphology, habitat requirements, and ecology of *Impatiens glandulifera*, information regarding the structural characteristics of its vegetative organs remains scarce. Thus, in this paper we aim to analyse the vegetative body's structure of *Impatiens glandulifera* to identify the histo-anatomical features that highlight its adaptability to diverse environmental conditions and habitats.

MATERIAL AND METHODS

Study species. *Impatiens glandulifera* Royle [*I. royle* Walp.] (Fam. Balsaminaceae), commonly known as Himalayan balsam, is an adventitious and invasive plant (Ciocârlan, 2009; Sîrbu & Oprea, 2011). Initially introduced as an ornamental plant, the species has escaped cultivation and became naturalized (Sîrbu & Oprea, 2011; Georgescu & Luchian, 2023).

Impatiens glandulifera is an annual, glabrous plant that can reach heights up to 2-2.5 meters (Beerling & Perring, 1993; Anastasiu & Negrean, 2007; Pioarca-Ciocanea et al., 2020). The stems are hollow, and the nodes are thickened (Balogh, 2008; Helsen et al., 2021).

The primary roots are cylindrical with a diameter of 2-3 mm, while the adventitious roots are conical and can grow up to 15-20 cm long. These roots can reach into the soil to depths of 10-50 cm (Clements et al., 2008; Balogh, 2008; Helsen et al., 2021).

The leaves are petiolate, arranged in whorls of three, ovate-lanceolate in shape, with serrate margins. Dark red glands can be observed at the nodes of the petiole (Săvulescu, 1958; Pioarca-Ciocanea et al., 2020).

The flowers are formed in the axils of the upper leaves, arranged in erect racemes, with 2-14 large flowers that can reach up to 3-3.5 cm and are lilac pink in color. The flowers are zygomorphic (Săvulescu, 1958; Pioarca-Ciocanea et al., 2020).

The fruit is a capsule that twists into a spiral when ripe, facilitating the dispersal of seeds over long distances (Săvulescu, 1958; Balogh, 2008; Anastasiu & Negrean, 2007; Anastasiu et al., 2019; Pioarca-Ciocanea et al., 2020).

The seeds are pale grey-brown, turning black when mature. They are oval-shaped, measuring 3-4(7) mm in length, 2-4.8 mm in width, and 1.5-2 mm in diameter (Balogh, 2008; Helmisaari, 2010).

Methodology. Plant material, consisting of specimens measuring 1.5-2 m in height, was collected on 22nd of July 2023 from the DJ101P 173, Răgman (45.143111°N, 25.696358°E). The collected biological material was fixed in 70% ethyl alcohol.

Cross-sections for structural analyses were performed through adventitious roots, principal stem in the basal, median, and tip areas, as well

as through the petiole, and leaf. The cross-sections were further processed using the double coloration technique (Iodine Green and Carmine Alum). Starch was highlighted by soaking the sections in IIK solution (Șerbănescu-Jitariu et al., 1983).

After analyzing the resulting microscope slides, they were photographed with the optical microscope.

RESULTS AND DISCUSSIONS

Root Structure: The root system of *Impatiens glandulifera* comprises a cylindrical primary root, along with numerous lateral and adventitious roots (Helsen et al., 2021). The analyzed adventitious roots exhibit a secondary structure resulting from the activity of the secondary meristems, cambium and phellogen. The phellogen produces several layers of cork outward and phelloderm inward. The vascular cambium generates secondary phloem externally and secondary xylem internally. In cross-section, the adventitious root displays a circular shape and the following distinct anatomical regions: the epidermis (rhizodermis), as the outermost layer featuring

root hairs, a cortex, and a central vascular cylinder (Figure 1 - A).

The cortex of the adventitious root contains an aerenchyma formed by isodiametric cells with large gaps (Figure 1 - A, B). The endodermis, composed of tangentially elongated cells with Casparian strips, forms the innermost layer of the cortex (Figure 1 - B). Notably, some cortex cells contain raphides.

The pericycle, consisting of parenchyma cells of varying sizes, forms the outermost layer of the vascular cylinder, positioned beneath the endodermis (Figure 1 - B).

Within the vascular cylinder, the secondary phloem and xylem have a circular disposition, with a reduced phloem, located outward. In the cross-section of the adventitious root, 16-20 vascular bundles are arranged in a circle (Figure 1 - A). The xylem comprises wood vessels of various diameters, along with lignified wood parenchyma, and wood fibers.

At the center of the vascular cylinder there is a parenchymatous pith, formed of large, polygonal cells with small intercellular spaces (Figure 1 - A, B), some of which contain raphides.

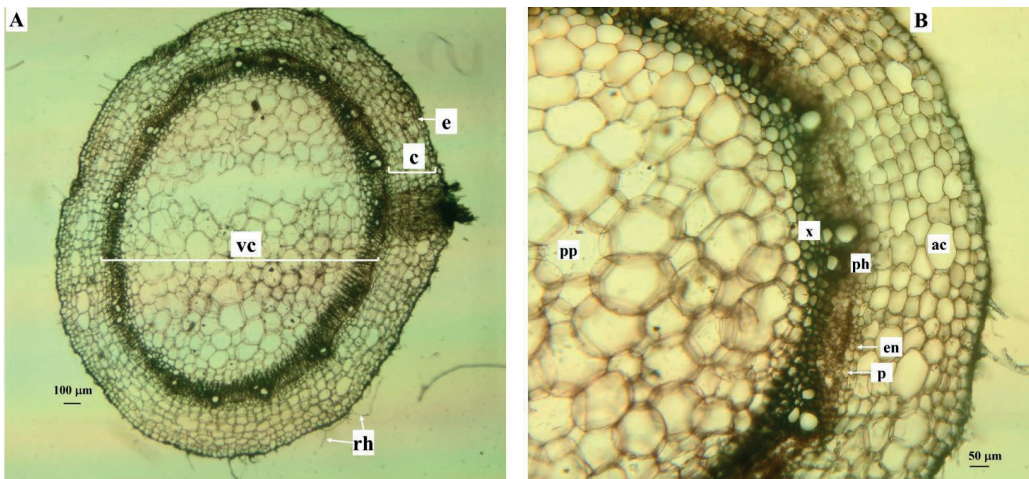


Figure 1. Cross-sections of *Impatiens glandulifera* adventitious roots, highlighting the the different anatomical areas (A - overview, B - detail) (colorants: Iodine Green and Carmine Alum): ac - aerenchyma, c - cortex, e - epidermis, en - endodermis, p - pericycle, ph - phloem, pp - parenchymatous pith, rh - root hairs, vc - vascular cylinder, x - xylem

Stem structure: In cross-sections performed at three levels of the stem (lower, median, and upper third), the contour shifts from nearly circular in the lower third to slightly ridged in the median third and prominently ridged in the

upper third, where 6 ridges differentiate (Figure 2 - A, B, C).

The stem has a secondary structure resulting mainly from the activity of the vascular cambium. At all three levels analyzed, the stem

displays the following anatomical areas: epidermis, cortex, and vascular cylinder with a large medullary lacuna in the center (Figure 2 - A, B, C).

The epidermis is unstratified, formed of small isodiametric cells covered by a thin cuticle. The cortex comprises parenchymatous polygonal cells that increase in size towards the vascular cylinder. The number of cortex layers increases from 5-6 in the lower third to 6-8 in the upper third and 10-12 in the ridge area (Figure 2 - D, E, F).

Beneath the epidermis, the outer cortex comprises an angular collenchyma. The number of layers of this mechanical tissue increases from 2-3 in the lower third of the stem, to 3-5 in the upper third. The innermost layer of the cortex accumulates starch and differentiates an amyliiferous sheath (Figure 2 - F, G).

The pericycle, consisting of small parenchyma cells, forms the outermost layer of the vascular

cylinder and it is positioned beneath the amyliiferous sheath (Figure 2 - D, E, F). The secondary vascular tissues are arranged concentrically, with a thin area of phloem (outward) and a thick area of xylem in the lower third of the stem. In the secondary xylem, the predominant wood fibres (libriform) have a radial arrangement (Figure 2 - D).

Secondary xylem development is more pronounced in the lower third of the stem, with an almost concentric arrangement, contrasting with the fascicular organization observed in the median and upper thirds of the stem (Figure 2 - D, E, F). In the median and upper third, at the ridges level and between these areas, vascular bundles are distinguished, concentrically arranged, with 2-3 in the median third and 3-4 in the upper third of the stem (Figure 2 - E, F).

Needle-like crystals composed of calcium oxalate (raphides) were observed in the cells of the cortex and vascular cylinder (Figure 2 - G).

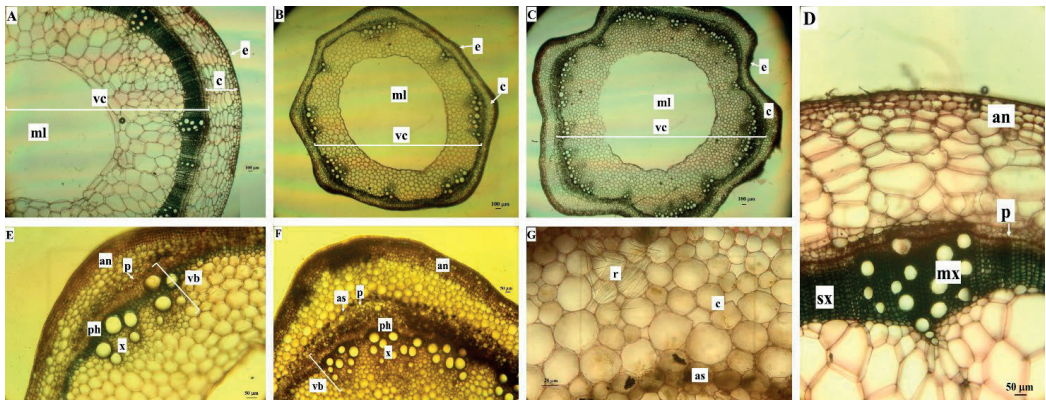


Figure 2. Cross-sections of *Impatiens glandulifera* stem (A, D - lower third; B, E - median third; C, F, G - upper third) (colorants: Iodine Green and Carmine Alum, IIK): an - angular collenchyma, as - amyliiferous sheath, c - cortex, e - epidermis, ml - medullary lacuna, mx - metaxylem, p - pericycle, ph - phloem, r - raphides, sx - secondary xylem, vc - vascular cylinder, x - xylem

The lamina of *Impatiens glandulifera* exhibits a bifacial dorsiventral structure. In cross-sections, the lamina displays prominent features on both faces around the midbrid, with an elongated contour on the adaxial face (Figure 3 - A).

The upper and lower epidermis, corresponding to the adaxial and abaxial faces, consist of isodiametric cells covered by a thin cuticle.

At the midbrid level, vascular tissues are organized into a closed collateral vascular bundle arranged in an open arch (Figure 3 - A). The phloem is positioned towards the abaxial

face and is bordered by an amyliiferous sheath. The vascular bundle is supported by an angular collenchyma located beneath both epidermis around the midbrid (Figure 3 - A).

Between the angular collenchyma and the vascular bundle, several layers of parenchymatic cells with cellulosic walls and small to larger intercellular spaces can be distinguished (Figure 3 - A).

The mesophyll comprises one layer of palisade cells beneath the upper epidermis, a layer of collector cells beneath the palisade tissue, and a

lacunose tissue consisting of 5 to 6 layers of isodiametric cells with large gaps, situated above the lower epidermis (Figure 3 - B).

The leaf is amphystomatic, with stomata located at approximately the same level as the epidermal cells (Figure 3 - B). Additionally, certain parenchymatic cells within the lamina contain raphides of calcium oxalate.

The petiole of *Impatiens glandulifera* exhibits a fistulous structure with a polygonal external outline, featuring two lateral elongations (Figure 3 - C). Both, the upper and lower epidermis consist of a single layer of isodiametric cells, covered by a thin cuticle (Figure 3 - C).

The petiole is supported by a mechanical tissue comprised of 4-5 layers of continuous angular collenchyma situated beneath the entire surface of epidermis (Figure 3 - D).

The vascular tissues are organized in several vascular collateral bundles, forming a semicircular pattern towards the lower epidermis (Figure 3 - C). Adjacent to the phloem, a distinct amyliiferous sheath was observed (Figure 3 - D). The vascular bundles are surrounded by a lacunose tissue composed of isodiametric cells with small to large gaps, accompanied by angular collenchyma (Figure 3 - D). Towards the adaxial face, this tissue defines a lacuna (Figure 3 - C).

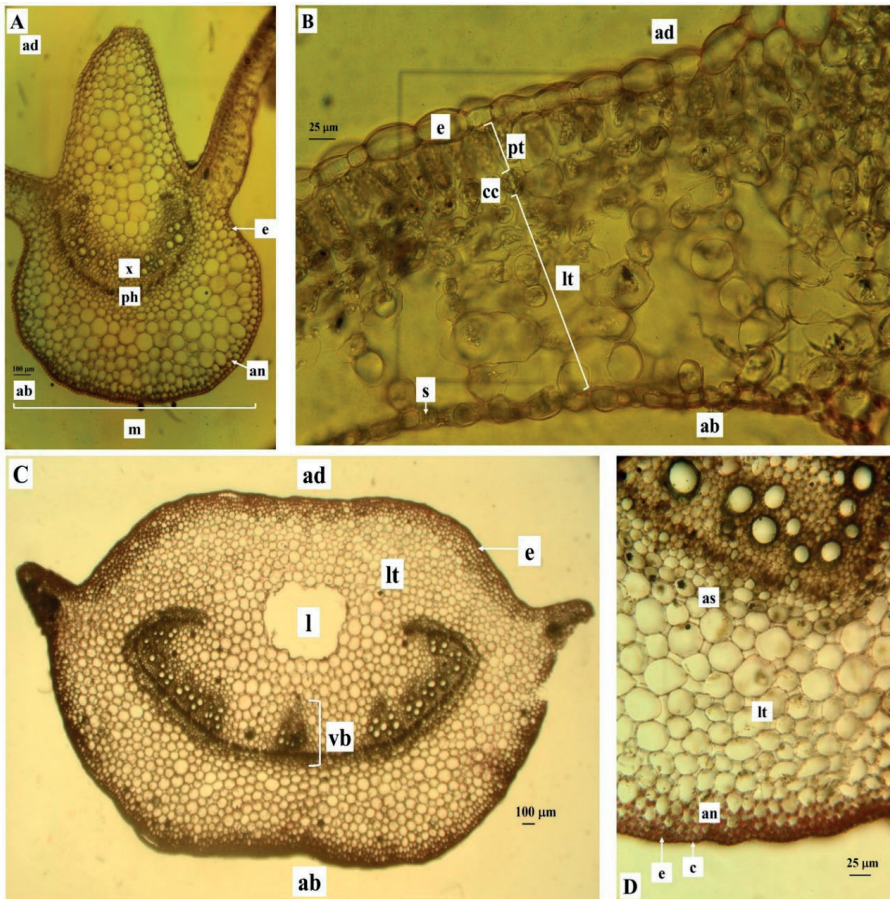


Figure 3. Cross-sections of *Impatiens glandulifera* leaf (A - lamina, highlighting the structure of the midrib; B - lamina, highlighting the mesophyll structure; C, D - petiole), (colorants: Iodine Green and Carmine Alum, IIK): ab - abaxial face, ad - adaxial face, an - angular collenchyma, as - amyliiferous sheath, c - cuticle, cc - collector cells, e - epidermis, l - lacuna, lt - lacunose tissue, m - midrib, ph - phloem, pt - palisade cell, s - stomata, vb - vascular bundle, x - xylem

The anatomical analysis of *Impatiens glandulifera*'s vegetative body has revealed a secondary structure of both root and stem systems. These characteristics, associated with a structural robustness and rapid growth of the corm, provide notable advantages to this annual species, ensuring its competitiveness over native flora within its habitat (Wang et al., 2022; Dumitrașcu et al., 2023).

Additionally, similar features, such as a well-developed secondary structure of roots and stems, have been observed in other annual alien species like *Symphyotrichum ciliatum* and *S. squamatum* (Sârbu & Smarandache, 2015; Dumitrașcu et al., 2023).

Successful plant invasion relies upon efficient nutrient absorption and utilization, as well as adaptation to diverse environmental conditions. This often includes effective competition for resources such as water and nutrients. Therefore, plants with strong competitive abilities often possess advanced root systems (Wang et al., 2022).

The root system of *Impatiens glandulifera*, characterized by a wide stem base acting as a rigid anchor point, numerous adventitious roots with fleshy composition and strengthening elements increase the mechanical efficiency of the roots for anchorage (Ennos et al., 1993). Additionally, the presence of aeriferous tissue from the adventitious roots, enables it to thrive in wet environments, indicating its adaptation to such conditions. These structural adaptations, along with the well-developed adventitious roots with secondary structure, allow *Impatiens glandulifera* to outcompete native species by enhancing its anchorage, increasing resistance to pullout and bending forces, and ensuring stable growth within its habitat (Ennos et al., 1993).

The stem of *Impatiens glandulifera* exhibits structural adaptations that support its invasive characteristics, in line with findings regarding plant growth goals, adaptation to heterogeneous environments, and fruit production observed in invasive species (Wang et al., 2022). Similar to other invasive plants, *Impatiens glandulifera* demonstrates robust stem architecture, allowing it to support the inflorescences produced mainly on the upper parts of the stem. This structural strength is essential for bearing the load of the inflorescences and ensuring efficient nutrient transport, as highlighted by studies on other

invasive plants such as *Solidago canadensis* (Wang et al., 2022). The presence of highly differentiated secondary xylem in *Impatiens glandulifera*'s stem further enhances its support function, similar to the advanced supporting fiber structures found in other invasive plants (Sârbu & Smarandache, 2015; Wang et al., 2022; Dumitrașcu et al., 2023). These structural adaptations contribute to *Impatiens glandulifera*'s invasive success by facilitating its growth, reproduction, and competitive ability in diverse ecological environments.

Furthermore, the leaf structure of *Impatiens glandulifera* exhibits adaptations for effective sunlight absorption and nutrient transport. Its bifacial leaves with a well-developed layer of palisade tissue ensure efficient sunlight absorption, while the presence of thick leaf veins, as well as the presence of the collector tissue, enhances nutrient transport efficiency (Wang et al., 2022).

The presence of angular collenchyma in above-ground organs provides considerable strength and elasticity, allowing the plant to bend without breakage (Carrillo-López & Yahia, 2019). Moreover, the presence of calcium oxalate raphides in all examined vegetative organs most likely enhances their resistance to environmental stress factors.

These structural adaptations observed in *Impatiens glandulifera* and other invasive plant species, contribute to their invasive success by enabling effective colonization and dominance in new habitats. These findings emphasize the importance of understanding the anatomical basis of plant invasiveness for effective management and control strategies.

CONCLUSIONS

Overall, *Impatiens glandulifera* exhibits a robust anatomical structure characterized by secondary growth, which confers competitive advantages in resource acquisition and growth rates compared to native plant species. The presence of the aeriferous tissue in adventitious roots, enables adaptation to diverse soil conditions, especially wet environments. Additionally, the presence of angular collenchyma in above-ground organs provides mechanical support and flexibility, facilitating the plant's ability to withstand environmental stresses.

While this invasive plant has been extensively studied, the literature contains insufficient data regarding the structure of *Impatiens glandulifera*'s vegetative body. Our detailed analysis enhances the understanding of the adaptive capacity *Impatiens glandulifera* to environmental conditions and underscores the importance of considering anatomical traits in invasive plant species management strategies.

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