

## WHAT STRUCTURAL TRAITS ENSURING *SOLIDAGO CANADENSIS* L. TO INVADE HETEROGENEOUS HABITATS SUCCESSFULLY?

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### Abstract

*Solidago canadensis* is a non-native weed that has invaded China, which has had a large ecological impact on the areas. In this paper, we attempt to understand the morphological and anatomical characteristics of *Solidago canadensis* that ensure it to invade to heterogeneous habitats. We found that the characteristics that facilitate *S. canadensis* invasion and adaptation to heterogeneous ecological environments are as follows: low leaf porosity, thick cuticle, and long trichomes, which play a role in water retention and protection. The developed vascular bundles and bundle sheath extensions within the leaf blades increase the transport capacity of photosynthetic products through the leaf blade, thereby fulfilling the developmental needs of underground vegetative organs. In addition, the secondary structures of the stem differentiate early. *S. canadensis* has septate fibers and large amounts of alternating tissues and vestured pits. Simple and transverse perforation plates account for 94.9 and 51%, respectively, and alternate pitting accounts for 53.8%. The primary root stops developing relatively early to adapt to the development needs of underground structures. Flowering, inflorescence structure, and failure to establish continuous access to local insects, lead to failure in the development of capitulum. The exterior of mature pollen grains is enclosed by a callose wall, which facilitates pollen transmission. Rhizomes have become the substance and structural basis of independent propagules.

**Key words:** *Solidago canadensis*; Morphology; Anatomical structure; Characteristic.

### Introduction

*Solidago canadensis*, an herbaceous perennial plant belonging to the family Asteraceae, is native to North America and was introduced to China in 1935 as an ornamental plant. Since the 1980s, it has spread to provinces in East China, Xinjiang, and Northeast China, and is considered an exotic, invasive plant species (Qiang *et al.*, 2021).

The relationship between the invasive ability and traits of an exotic plant is the fundamental question in invasion ecology, and common characteristics of invasive species can be determined primarily through analyzing their morphological, cytological, and genetic traits (Huang *et al.*, 2003). Traits that are more emphasized include epidermal structure, spores, pollen grain structure, and vascular tissue structure, distribution, and proportions. The adaptability, fecundity, and spreading ability of plants in diverse habitats are based on the plants' internal structure (Heywood, 1979). Therefore, studies on invasive plant structure, ecological adaptability, and invasion mechanisms, especially reproduction, are needed for their control and prevention of spread. To our knowledge, most of the *S. canadensis* studies focus on hazard assessment (Mei *et al.*, 2020; Yang *et al.*, 2020), control and prevention (Ma *et al.*, 2007; Li *et al.*, 2017), biological characteristics and morphological description (Ruan *et al.*, 2004; Xu *et al.*, 2018), and ecology (Wang & Chu, 2007; Zhang *et al.*, 2019), whereas reports on how its morphological characteristics and internal structure relate to its ecology, invasion, reproduction, and spread are relatively few.

This study used light microscopic and electron microscopic analyses to perform more detailed observation of the morphological characteristics and organ structure of *S. canadensis*. The relationship between its biological traits and ecological characteristics was also discussed, by which the adaptability, the structural mechanism of invasion, and structural features were deduced. This study provides anatomical and ecological bases for the control and prevention of *S. canadensis*.

### Materials and Methods

Field observation was conducted in the suburbs of Bengbu city, where the experimental materials were collected.

**Paraffin sections:** Fresh main roots, lateral root tips, upper and middle parts of the stem, underground rhizomes including nodes, and suitable parts of the leaf midrib of *S. canadensis* were collected and cut into small pieces of approximately 0.5 cm in length. Samples were washed with tap water, air dried, and fixed in FAA for 24 h, followed by dehydration through an ethanol gradient, clearing with xylene, and conventional paraffin sectioning. Sections (thickness 8-12  $\mu$ m) were stained with sarranine-fast green (Li & Zhang, 1983; Li, 1996), observed under a darkfield microscope (Tektronix, XS-200) and a light microscope (Olympus, IX51), and photographed.

**Sample maceration:** The samples were macerated with nitric acid-chromic acid solution (Li, 1996). Stem xylem tissues were collected, cut into small rods of approximately 1 mm in length, and placed in nitric acid-chromic acid solution (1:1, v/v) for 48 h. The solution was removed and distilled water was added to the sample, which was ground into a flocculent mass with a glass rod and centrifuged (2000 r/min, 15 min). This step was repeated several times until the yellow color disappeared. Samples were dehydrated through an ethanol gradient and stored in 100% ethanol.

A small amount of sample was taken up by a dropper and loaded onto the center of the slide, covered with a cover slip and observed under a microscope. We recorded indicators of tubular molecule type (length, diameter, and quantity) and obtained an average value, based on 20 fields.

**SEM sample preparation:** To examine xylem vessels, a small amount of macerated stem xylem material was transferred to a piece of double-sided tape using a dropper. After the ethanol evaporated, samples were coated with gold to a thickness of approximately 10 nm, and then

observed under a scanning electron microscope (SEM) (Hitachi, S-4100) and photographed.

For analysis of the leaf epidermis, leaves were gently brushed to remove dust and cut into small pieces (0.5 cm × 0.5 cm). The pieces were attached to double-sided tape on the sample holder and sprayed with gold. Then, they were observed under an SEM and photographed.

To examine acetolysis of pollen grains (Erdtmann, 1978), flowers were soaked in glacial acetic acid for 48 h, ground, and centrifuged (2000 r/min, 15 min). The supernatant was poured off, and the acetic anhydride-sulfuric acid mixture (9: 1, V/V) was added. Samples were incubated in a water bath (100°C, 8 min), followed by centrifugation (2500 r/min, 5 min). The acetic anhydride-sulfuric acid mixture was decanted, and distilled water was added, followed by centrifugation (2000 r/min, 15 min). This step was performed three times. Then, 100% ethanol was added to dehydrate the sample for 15 min, and this step was performed three times. Final samples were stored in 100% ethanol.

A small amount of pollen sample was deposited onto the double-sided tape on the sample holder. After the ethanol evaporated, samples were attached to double-sided tape, sprayed with gold to a thickness of approximately 10 nm. Samples were observed under an SEM and photographed.

**Leaf epidermis:** Leaf epidermis preparation followed an improved, modified method (Hong & Chen, 2002). The stomatal distribution pattern and epidermal cell characteristics were observed from the prepared permanent slides. Twenty epidermal cells in a field were selected and their sizes (maximum length × maximum width) were measured to calculate the mean value. Stomatal index ( $I$ ), number of stomata per field ( $S$ ), and number of normal epidermal cells per field ( $P$ ) were determined:

$$I = \frac{S}{S + P} \times 100\%$$

**Field observation:** Field observation was conducted in the suburbs of Bengbu city. A total of six plants were randomly selected. Plant height (cm), main root length (cm), the number of lateral roots and rhizomes (>1 cm), and the total length of the rhizomes (cm) were measured. The mean number of lateral branches ( $b$ ) was calculated by averaging the number of lateral branches of the six plants. One lateral branch was collected from the upper, middle, and lower parts of each plant, and the number of cymose inflorescences were counted and the mean ( $c$ ) was calculated. One cymose inflorescence was collected from the upper, middle, and lower parts of the lateral branch, and the number of capitula were counted and the mean ( $h$ ) was obtained. From each plant, 10 capitula were randomly collected and the number of tubular florets were counted to calculate the mean ( $t$ ). The mean number of florets produced by each plant was calculated:  $n = b \times c \times h \times t$ .

## Results and Discussion

### Vegetative organs

**Leaf:** The leaf surface of *S. canadensis* had relatively tall ridges, and trichomes were found on both the upper and lower epidermis. Trichomes consisted of 3-4 cells, and

there were inflection points at the junction between the last two cells and at the terminal cell (Figs. 1a-1c). The maximum length of trichomes on the upper epidermis reached 328 μm. The trichomes on the lower epidermis, shorter than that on the upper epidermis, reached 253 μm in length. Trichomes were more distributed near the veins on both sides (Fig. 1b). At the base of the trichome on the upper epidermis, 7-9 epidermal cells surrounded the basal trichome cell (Fig. 1d). These cells were arranged differently, but their size and shape were not different from those of the regular epidermal cells. However, these structural features were not observed in the lower epidermis.

Here were schizogenous secretory ducts in the leaves and petioles. Epithelial cells with secretory function surrounded the secretory duct. Leaf secretory ducts were located on top of the xylem of the branched vein, and were connected to the upper epidermis through transfer cells (Figs. 1e-1f). Hence, secreted substances could be transported to the leaf upper surface via limited short routes. In petioles, the secretory ducts were in the upper and lower parts of the vascular bundle, and the secreted substances could easily enter the conducting tissues via intercellular horizontal transport, to be delivered to other parts of the plant. It has been suggested that the leaf extract of *Ageratina adenophora*, an invasive plant, can affect the seed germination of other plants (Han & Feng, 2007). Similar findings in *S. canadensis* have also been reported. Studies have suggested its aboveground tissues contain large amounts of saponins and flavonoids (Fang *et al.*, 2004). However, whether these components are secreted by epithelial cells of the secretory ducts, and the function of these secreted substances, remains unclear. Structurally, the secretory structure in the leaves of *S. canadensis* and its products should be beneficial to its invasion, growth, competition, and spread.

In addition to trichomes, there were three other types of cells in the upper and lower epidermis: mesenchymal cells, bundle sheath cells, and guard cells (Figs. 1b-1c; Table 1). Generally, the thickness of the cuticle attached to the upper epidermis was approximately 3.0 μm, whereas that attached to the lower epidermis was approximately 2.4 μm. The stomatal arrangement was irregular, and their number was low. There was a difference in the number of stomata between the upper and lower epidermis, and their densities were 12.3% and 17.2%, respectively.

Tall trichomes, tall ridges on the leaf surface, and thick cuticles provide protection, which, together with low stomatal density, are typical features for heliophile plants and function in preventing water loss (Li & Zhang, 1983), the primary issue that needs to be solved for terrestrial plants. Heterogeneous environments affect the physiology and morphological structure of invasive plants, and invasive plants also respond to these effects (e.g., changes in physiology and structure). Morphology is determined by physiological functions, and physiological changes subsequently trigger changes in morphological structure (Xu, 1980). The structure of *S. canadensis* showed a certain degree of change under the influence of different environmental factors; for example, stomatal density was affected by water level, and cuticle thickness was affected by the amount of light, which have been proven in other invasive plant species (Lou & Shen, 2005).

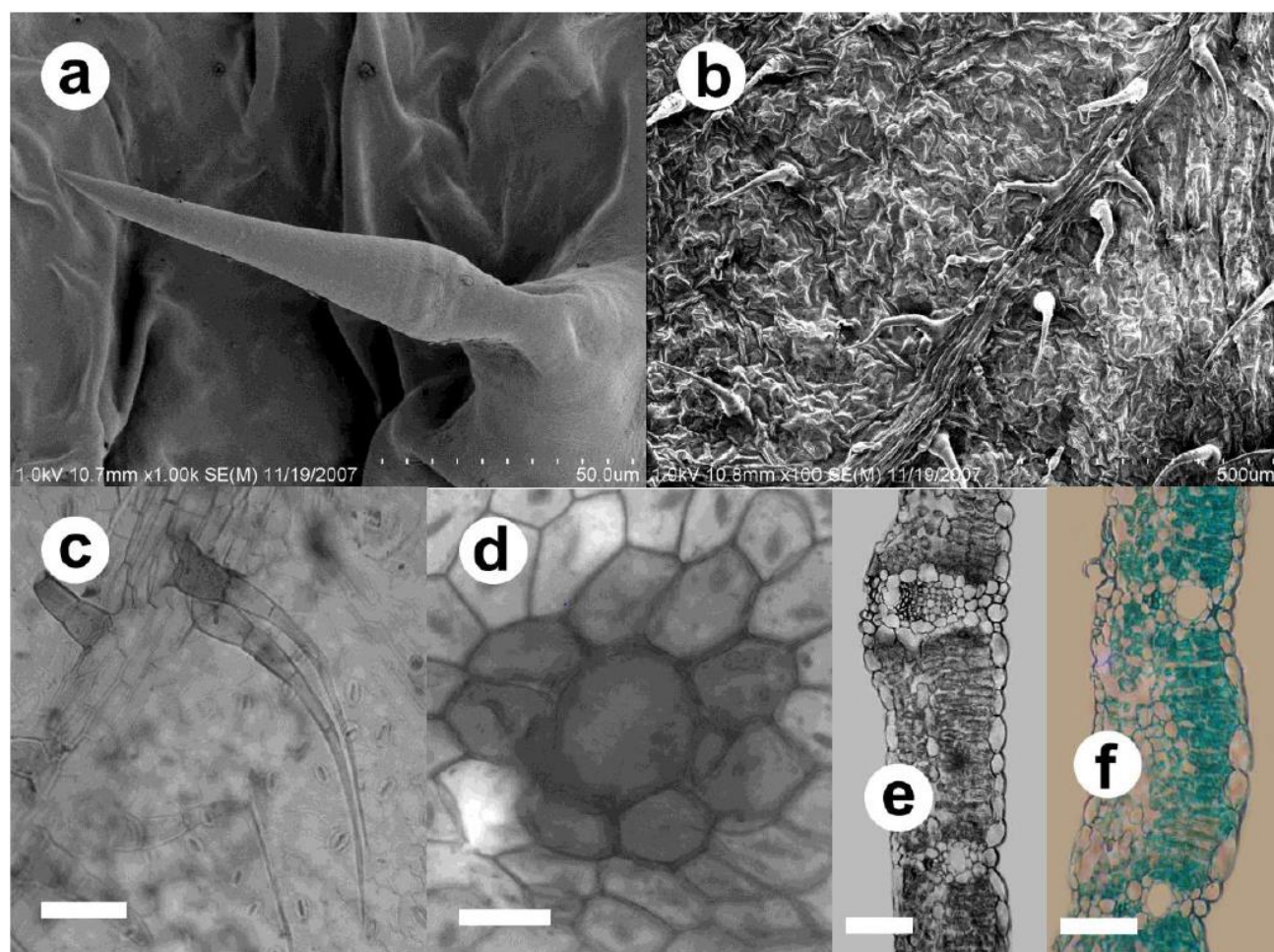


Fig. 1. Morphology and anatomy structure of the leaves.

a. Epidermal hair with inflexion (SEM); b. Epidermal hairs along the vein (SEM); c. Two types epithelium cell and epidermal hair (LM,  $\times 40$ , scale = 50  $\mu\text{m}$ ); d. Epidermal cells around the basal cell of the epidermal hair (LM,  $\times 100$ , scale = 10  $\mu\text{m}$ ); e. Secretory canal and epithelium in petiole; ⑤ Vascular bundle sheath and vascular bundle sheath extension (LM,  $\times 40$ , scale= 50  $\mu\text{m}$ ); f. Secretory cavity and secretory cells upon veinlet (LM,  $\times 40$ , scale= 50  $\mu\text{m}$ ).

**Table 1. Size of leaf epidermis cells (Average  $\pm$  S.E,  $\mu\text{m}$ ).**

Cell type	Upper epidermis ( $\mu\text{m}$ )	Lower epidermis ( $\mu\text{m}$ )
Vein islet	$(48.7 \pm 0.56) \times (16.7 \pm 0.45)$	$(18.2 \pm 0.20) \times (11.6 \pm 0.07)$
Out of vein	$(38.3 \pm 0.78) \times (32.5 \pm 0.52)$	$(51.2 \pm 0.62) \times (23.8 \pm 0.28)$
Guard cell	$(26.6 \pm 0.19) \times (9.90 \pm 0.06)$	$(27.0 \pm 0.15) \times (10.1 \pm 0.08)$

*S. canadensis* has bifacial leaves with a single layer of palisade cells. The thicknesses of leaves, palisade mesophyll, and spongy mesophyll were approximately 130  $\mu\text{m}$ , 67  $\mu\text{m}$ , and 51  $\mu\text{m}$ , respectively. The leaf midrib had thick branches, the bundle sheath was surrounded by parenchyma cells, and there were significant bundle sheath extensions (Figs. 1e-1f).

One of the evolutionary directions of plants is toward better food production (Xu, 1980). Only one layer of palisade cells was found in leaves of *S. canadensis*. The layer was well developed and its structure allowed chloroplasts to fully receive sunlight, maximizing light usage. The bundle sheath around the leaf vein expanded the contact area between the mesophyll cells and leaf vein, benefiting material exchange between the mesophyll and the vascular tissue. Leaf epidermal cells were connected to the vascular tissue via bundle sheath cells, and the bundle sheath extension consisted of parenchyma cells. The bundle sheath, bundle sheath extension, and the

connecting epidermal cells constitute an auxiliary transport system (Han & Feng, 2007), and the transport of certain substances through the bundle sheath was faster than through the vascular tissue. Plants that have bundle sheath extensions generally have fewer leaf veins (Liu, 2006). However, *S. canadensis* had dense and thick veins, which might be associated with the large nutrient requirement of rhizomes and inflorescences. Thick leaf veins and their dense distribution may enhance nutrient transport and utilization efficiency.

**Stem:** Soon after the primary structure of the *S. canadensis* stem was differentiated, the secondary structure began to form, which included an obvious pith and well-developed secondary xylem. Macerated stem material included tubular molecules and parenchyma cells (ray parenchyma cells) (Figs. 2e, 2h). Tubular molecules, observed in 20 fields, were counted, and there were 78 xylem vessels. Types of vessels, types of perforation plates, and pitting patterns



were diverse (Figs. 2e-2j; Table 2). Vessels with alternate pitting accounted for 53.8%, and the vessel to tracheid (fiber) ratio was 1:9. Some vessels had vestured pits (Fig. 2a). There few separating fibers (Fig. 2g) and many storied tissues (Fig. 2b).

The goal of plant growth is to complete its life cycle (i.e., produce offspring and promote species propagation). For invasive plants, there are also issues such as adaptation to the heterogeneous ecological environment, settlement, and dispersal. Adaption to heterogeneous ecological environments requires corresponding structural basis, and its dispersal depends on its fruit. Invasive plants produce numerous fruits. *S. canadensis* inflorescences are mainly produced on the middle and

upper parts of the main stem. The production of these lateral inflorescence branches requires the stem to be strong and upright, to enhance the load-bearing ability for many inflorescences. Meanwhile, the large number of flowers also increases the dependence on nutrient transport through the stem. The tubular structure of the stem is material-saving and effective for exerting its function (Liu, 2006). The premature generation of secondary structure is beneficial to the stems' supporting function, especially for the large number of inflorescences post-flowering. It also meets the requirement of water and nutrient transport to the numerous leaves, and especially inflorescences, on the upper part of the plant.

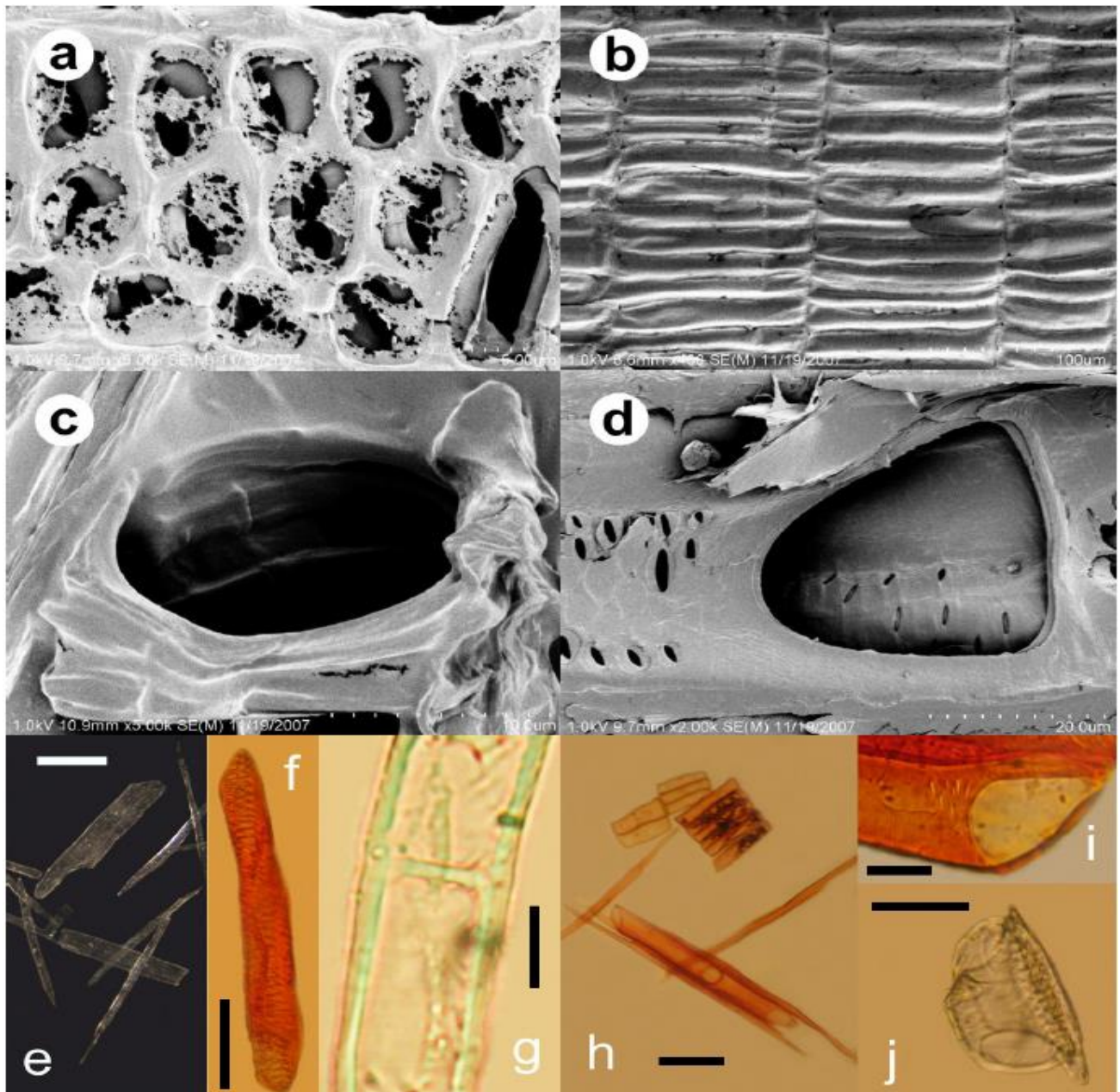


Fig. 2. Structure and components of stem

a. Vestured pits and Alternate pitting of vessel (SEM); b. Stratified tissue (SEM); c. Simple perforation vessel with plane perforation plate (SEM); d. Vessel with middle perforation (SEM); e. Xylem eductant of stem (DGM,  $\times 100$ , scale = 50  $\mu\text{m}$ ); f. Scalariform perforation vessel with lean perforation plate (LM,  $\times 100$ , scale=50  $\mu\text{m}$ ); g. Septate fiber (LM,  $\times 1000$ , scale=5  $\mu\text{m}$ ); h. Vessel with middle perforation (LM,  $\times 100$ , scale = 50  $\mu\text{m}$ ); i. Simple perforation vessel with lean perforation plate (LM,  $\times 1000$ , scale = 10  $\mu\text{m}$ ); j. The shortest vessel with mucronate (LM,  $\times 400$ , scale = 20  $\mu\text{m}$ ).

**Table 2. Vessel characters of stem.**

Code	Size (µm)	Perforation plate type	Pitting	Vessel type	Quantity
1	580×18	Simple, Lean, Flat	Opposite	Reticulate	4
2	410×48	Simple, Plane Flat, Flat	Opposite	Spiral	5
3*	350×40	Simple, Lean, Lean, Middle	Opposite	Spiral	1
4	340×15	Simple, Lean, Lean	Alternate	Pitted	3
5	300×14	Simple, Lean, Lean	Opposite	Reticulate	4
6	230×47	Simple, Flat, Flat	Alternate	Pitted	10
7	185×29	Simple, Lean, Lean	Opposite	Spiral	8
8	185×25	Simple, Lean, Lean	Opposite	Reticulate	5
9	185×23	Compound, Lean, Lean	Opposite	Reticulate	4
10	110×28	Simple, Lean, Lean	Opposite	Spiral-Scalariform	5
11	100×28	Simple, Flat, Flat	Alternate	Pitted	12
12	84×43	Simple, Flat, Flat	Alternate	Pitted	11
13	58×40	Simple, Lean, Lean	Alternate	Pitted	6

Note: \* Vessel with three perforations

**Table 3. Perforation plate of stem.**

	Flat perforation	Lean perforation	Total
Simple perforation plate	80	69	149
Percentage	51%	43.9%	94.9%
Multiple perforation plate	0	8	8
Percentage	0	5.1%	5.1%
Total	51%	49%	

The conducting tissue is the core of the plant, and its structure and composition determine its supporting and transport functions. *S. canadensis* has highly differentiated secondary xylem. Vessel plates with simple perforation accounted for the majority (94.9%) and vessels with alternate pits accounted for 53.8%. The vessel perforation plate showed highly evolved characteristics (Figs. 2c-2j; Table 3). Some vessels had vestured pits (Fig. 2a), and there few highly differentiated separating fibers (Fig. 2g), which were very highly efficient structural characteristics for support and transport. The presence of a larger percentage of fibers directly enhanced support, and separating fibers are considered the most advanced supporting fiber structure. On the walls of the thick and short vessels, there were alternate pits and vestured pits, and there were also simple perforation plates and the scalariform perforation plates, which were the most efficient structure for transporting water and nutrients (Figs. 2c-2j; Table 3). Vessels with middle perforation (Figs. 2d, 2h) suggested the diverse connection between vessels: connection at the ends and in the middle. The stem structure of *S. canadensis* meets the needs of both support and transport. At the same time, the combination of these structures provided a reliable structural basis for its invasion, settlement, and adaption to different ecological environments.

**Root:** Like the stem, the developed secondary structure also appeared early in roots. The tip (0.3-0.5 mm) of the main root and the center of the lateral root, determined by the transverse section, were almost completely occupied

by the secondary xylem. The maximum diameter of the xylem vessel reached 60 µm.

Using random sampling, the underground tissues and the reproductive tissues (including both sexual reproduction and vegetative propagation structures) of six *S. canadensis* plants were observed and measured (Table 4). The results showed that lateral root branching was scarce, fine roots were lacking, and root hairs were few. At the base of the main roots, there were varying numbers of rhizomes and adventitious roots, and there numerous adventitious roots at the rhizome nodes.

The nutrient absorption ability (absorption efficiency) of the root system is the prerequisite for nutrient usage and transformation, and successful invasion requires the invasive plant to adapt to diverse habitats and compete effectively for environmental resources. *S. canadensis* is both an invasive plant and a weed. Successful invasion and settlement are bound to result in competition with native species for resources and space. The underground competition between plants is far greater than the aboveground competition (Qiang *et al.*, 2021). The underground competition includes two aspects: competition for water and nutrients. Plants with a strong competitive ability generally have an advanced root system. Studies have shown that, compared with other exotic weeds in the family Asteraceae, *S. canadensis* has the largest values for underground indicators such as length, surface area, and volume. It also has extensive underground adventitious structures, which include rhizomes, adventitious roots grown from rhizomes, main roots, and adventitious roots grown from lateral roots. The premature cessation of main root development in *S. canadensis* is beneficial. First, the termination of main root development promotes the development of adventitious roots and rhizomes, and the area of lateral expansion of rhizomes is much larger than that of the main roots and lateral roots. This creates a larger surface area for water and nutrient absorption. Second, rhizomes are a vegetative propagule, and therefore spreading of rhizomes means spreading of clonal plants, which is beneficial to invasion and settlement.

**Table 4. The parts underground.**

Index	Code						Average
	1	2	3	4	5	6	
High (cm)	71	119	151	148	166	85	123.3 ± 6.4
Axial root length (cm)	10.6	10.5	12.7	12.2	14.1	9.8	11.7 ± 0.27
Lateral root quantity	8	6	9	12	11	6	8.7 ± 0.42
Rhizome quantity	6	11	10	14	13	8	10.3 ± 0.50
Total length of rhizome (cm)	104	166	184	328	277	142	200.2 ± 14-2

**Reproductive organs:** The plant life cycle begins with seeds, and producing a new generation of individuals is the target. Invasive plants have features such as strong fecundity and spreading ability, and they enter into the invaded land via migration and settlement. For an exotic plant to invade into a new environment and adapt to the heterogeneous habitat, it must face the pressure posed by the new environment. Generally, invasive plants can quickly adapt to the new environment and overcome this pressure, and have inherent structural and evolutionary bases for adaptation. In addition, these bases enhance the clustering of the same invasive plant species and their ability to compete with native plants.

**Flower and inflorescence:** *S. canadensis* inflorescences are generally considered a panicle. Field observation revealed that 10-18 tubular flowers form a capitulum inflorescence and 3-5 capitulum inflorescences gather to form a cymose inflorescence that resembled a scorpion's tail. Cymose inflorescences grow and hang from the axil of the leaf on the lateral branch. Multiple lateral branches, growing from a central axis, form a large panicle with many small capitula (<3 mm). This type of inflorescence, composed of uniaxial florets, is very advanced and efficient for pollination (Xu, 1980). However, this does not mean that during the migration and initial settlement of *S. canadensis*, this structural evolutionary advantage could be transformed into a competitive advantage. *S. canadensis* has inconsistent flowering times among regions in China and around the world (Zhang, 2015), which is one characteristic of weeds. For example, near the Huaihe River (the demarcation line between north and south China), *S. canadensis* flowers in late October, and the fruits mature from November to mid-December. Generally, *S. canadensis* has indefinite inflorescences, and the capitulum inflorescence maturation time was different among different plant parts. Affected by the weather in late fall and early winter, some tubular flowers may die before maturation, thereby failing to reproduce. Of the randomly selected six *S. canadensis* plants, in theory, an average of 32,000 achenes can be produced by each plant, markedly different from the reported 20,000 achenes (Huang *et al.*, 2007). The heterogeneous climate in the invaded land might be one of the reasons causing this discrepancy.

*S. canadensis* is a typical self-sterile plant and its inflorescence structure is highly adaptive to insect pollination. Therefore, *S. canadensis* requires insect pollination vectors. *S. canadensis* is native to North America (latitude 26°-45°) (Gleason, 1963; Weber, 1998). Its tubular florets have both nectar and pollen, making it an important nectar plant in its place of origin and resulting in a fixed pollination relationship with bees (Yin *et al.*, 2004). Near the Huaihe River, the flowering season of *S. canadensis* is not ideal for insect pollination, especially for

the capitulum inflorescences that are at the end of the branch and bloom late. Just as the natural enemies of *S. canadensis* have not been found in China (Dong *et al.*, 2006), *S. canadensis* has not established a fixed visiting relationship with local insects. From the evolutionary point of angiosperms, fixed pollinator visitation is important for species propagation (Xu, 1980). Therefore, the fact that some pistils could not be fertilized, and that substantial pollen were wasted, might be another reason for the discrepancy in achene numbers.

Researchers believe that successful invasion of a species depends on the invasibility of the land, which is the basis of invasion ecology. The formation of new interspecies relationships, especially the establishment of mutualistic symbiosis between an invasive species and other species is the key (Huang *et al.*, 2003). The invasibility of a recipient habitat includes climatic factors. For *S. canadensis*, the establishment of the relationship between the pollination vector (insects) and the invasive plant could be considered a new interspecies relationship, which is significant for sexual reproduction, invasion, and dispersal. The adaptation to heterogeneous habitats and the establishment of the relationship with native species are fundamental issues that need to be addressed for successful invasion.

Certainly, the ecological consequences resulting from this relationship should be acknowledged. It has been suggested that in Europe, when exotic invaders are frequently visited by certain specialized insects, it causes considerable competition with native flowering plants, worsening invasion damage (Doyle & Lehmann, 2003). The inflorescence structure of *S. canadensis* has detrimental effects on the prevention of invasion, spread, and weed management. Flower maturation time was inconsistent between, and within, inflorescences; therefore, fruit maturation time varied greatly. After the fruit spreads and undergoes a period of dormancy, the germination time also becomes variable, thus affecting herbicide, and overall management, efficiency.

**Fruit:** The thousand-grain weight of *S. canadensis* fruit was only 0.045-0.050 mg, and the seed germination rate under natural conditions was approximately 30%. However, germination rate fluctuates with environmental conditions (Zhang, 2015). Therefore, a mature plant, in theory, can produce about more than 5,000 seedlings. In addition, its achenes have a pappus, suitable for long-distance dispersal, and its range of expansion and number are very alarming. Strong fecundity and spreading ability are common features of invasive plants and weeds, and fecundity is positively correlated with invasiveness. In this regard, the suppression of the sexual reproduction of *S. canadensis* is a fundamental and effective way to prevent its invasion.



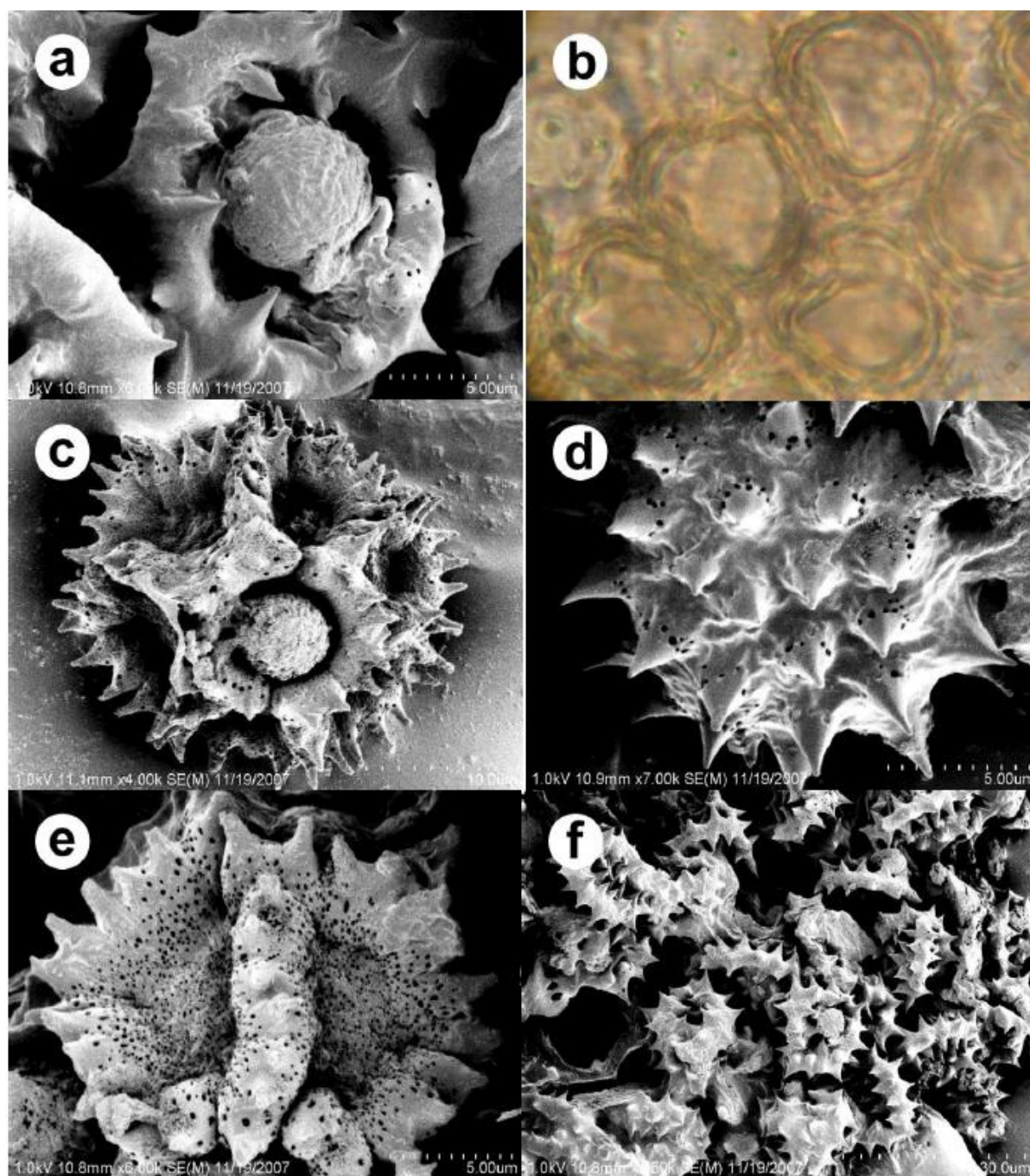


Fig. 3. Pollen grain morphological characters of 4 species in Compositae

a. Pollen of Canada goldenrod (SEM); b. Pollen grain enveloped by callose (LM, x 1000); c. Pollen of *Y. japonica* (SEM); d. Pollen of *E. annuus* (SEM); e. Pollen of *A. tataricus* (SEM); f. Pollen of Canada goldenrod (SEM).

**Pollen:** The pollen of *S. canadensis* are triaperturate, and the width and depth of the furrows were approximately 3.3  $\mu\text{m}$  and 3.5  $\mu\text{m}$ , respectively. The furrows were parallel to the plane of the equator. The pollen wall thickness was approximately 3.5  $\mu\text{m}$  (average of 20 pollen grains). The tetrad is the simultaneous type, and the pollen grain size was 17.6  $\mu\text{m}$   $\times$  21.4  $\mu\text{m}$  (polar axis (P)  $\times$  equatorial axis (E), Table 5). There were 14-24 spines, of which the size was 2.5  $\mu\text{m}$   $\times$  2.5  $\mu\text{m}$  (Fig. 3a). Pollen grains were enclosed by callose and even after maturation, pollen grains were still enclosed by a common callose structure. After soaking in the glacial acetic acid for 48 h, ground, and

treated with the acetic anhydride-sulfuric acid solution for 15 min, some pollen grains were still not separated (Fig. 3b). The presence of callose has a positive effect on pollen development. When megasporocytes undergo meiosis, the surrounding callose wall provides protection, or meiosis might go awry and give rise to abnormal pollen (Xi, 1986). Moreover, the clustered pollen may be more beneficial to insect feeding and spreading, thus compensating for the fewer insects in late autumn and early winter. It may also offset the lack of a fixed relationship with local insects, thereby improving pollination efficiency and increasing the chances of fertilization and fruit dispersal.

Table 5. Pollens contrast of 12 species in Compositae.

Species	Size( $\mu\text{m}$ )		P/E	Species	Size( $\mu\text{m}$ )		P/E
	P	E			P	E	
<i>A. tataricus</i>	26.1	28.7	0.91	<i>H. bowerii</i>	25.0	25.0	1.00
<i>E. annuus</i>	20.1	21.8	0.92	<i>H. crenatifolius</i>	23.7	25.0	0.95
<i>S. canadensis</i>	17.6	21.4	0.82	<i>H. gouldii</i>	25.4	23.7	1.07
<i>S. vorge-aurea</i>	20.0	22.5	0.89	<i>H. meyendorffii</i>	28.7	27.5	1.15
<i>Y. japonica</i>	20.5	21.7	0.94	<i>H. semiprostratus</i>	25.0	27.5	0.91
<i>H. altaicus</i> var. <i>millefolius</i>	22.5	25.0	0.90	<i>H. tataricus</i>	22.5	23.7	0.94

Note: P means polar axis, E means equatorial axis

Light microscopic and electron microscopic analyses were also performed on other weeds in the family Asteraceae that also bloom in late autumn and early winter: *Youngia japonica*, *Erigeron annuus*, and *Aster tataricus* (Figs. 3c-3f); *S. decur* (Zhang & Wang, 1965); and seven species of *Heteropappus* (Shao & Zhang, 2002) (*H. altaicus* var. *millefolius*, *H. bowerii*, *H. crenatifolius*, *H. gouldii*, *H. meyendorffii*, *H. semiprostratus*, and *H. tataricus*). The results showed that the *S. canadensis* pollen appeared oblate, the *H. meyendorffii* pollen were prolate, and the others were nearly spheroidal (Table 5).

Comparing the longest axes, the lengths of E and P of *S. canadensis* were both smaller than those of the other pollen grains, and the longest axis was also shortest. Its pollen grain was also the smallest and considered a small pollen grain, which is more evolutionarily advanced (Walker, 1976). Covas & Schnack (1935) believed that the relative reduction in pollen grain size is an evolutionary trend, because higher plants have higher metabolic rates; therefore, pollen grains only need to store a small amount of nutrients to supply the energy required for pollen tube growth. In addition, the evolutionary trend of pollen shape is from spheroidal to prolate (Walker, 1974). Among the above 12 pollens studied, only *S. canadensis* had oblate pollen. If only evaluating this particular trait, the pollen of *S. canadensis* is most primitive, which can be explained by asynchronous evolution.

**Vegetative reproductive organ:** Dispersal is an important study focus in plant ecology and plant geography. Any part of the plant that can grow into a new plant, under suitable conditions after separation from the parent plant, is called a diaspore (Ma & Li, 2002). In addition to using fruit for dispersal, *S. canadensis* uses another diaspore, which is an asexual propagule — rhizomes. The two reproduction methods play different roles in *S. canadensis* invasion: sexual reproduction is responsible for long-distance dispersal and the initial population establishment, and asexual reproduction is more responsible for the survival and growth of the local population (Dong *et al.*, 2006).

*S. canadensis* rhizomes are centered at the plant and show radial extension and growth. The rhizome length is generally 5-12 cm, and the longest can reach 1 m (Yin *et al.*, 2004). According to our observation and measurements, each plant generally had 7-15 rhizomes, and the mean internode length was approximately 1 cm. Each node had a lateral bud, and every rhizome had an apical bud. Under general conditions, the lateral bud at

the node is latent, and the apical bud is always at the growth stage during the active season. Hence, the underground parts of every *S. canadensis* plant have hundreds of buds, and in theory, every node is a potential asexual propagule (diaspore). There could be hundreds of these asexual propagules. Statistical data suggested that the density of clonal ramets could reach 64-208 ramets/m<sup>2</sup> (Bradbury, 1981). The nodes of rhizomes had adventitious roots of varying numbers, which had the absorption function. Chemical analysis of rhizomes revealed that it contained more inulin, suggesting the underground parts are equipped with materials and the structural basis for dispersal and for becoming an independent diaspore. Under normal conditions, the apical bud grows in the second year, forming an independent aboveground part. By using method of expansion, within 2-3 years, a small community could be formed from a single *S. canadensis* plant. In the event of mechanical damage or weeding activities, where rhizomes were cut off, dormant lateral buds enter the active stage early and become true asexual diaspores. These diaspores then form the aboveground part and rapidly spread, reproduce, and grow in a large swath. This is the fundamental reason why weeds that have asexual reproduction, such as *S. canadensis*, are difficult to remove.

## Conclusions

The most significant feature of an invasive exotic species, which is different from that of an ordinary exotic species, is its negative impacts on the structure and function of the native ecosystem, including ecological, social, cultural, and economic impacts (Blossey & Notzold, 1995). Therefore, the prevention and control of invasive species is particularly important. *S. canadensis* leaves have some typical characteristics in terms of protection, assimilation, and transport. Its stem has efficient structure for complete support, conductance, and resource allocation. Its belowground part is suitable for material storage and the production of asexual propagules. Its flower, inflorescence, and microspore exhibit high-degree evolutionary characteristics, which are also the structural basis for its invasion and dispersal. Because invasive plants display features such as rapid adaptability to heterogeneous environments and rapid evolution (Blossey & Notzold, 1995), it can be expected that the same species would have different structures in different habitats and regions, as described above. The root cause of *S. canadensis* being difficult to eradicate is its asexual propagules. Moreover, an effective means to curb its spreading is to strengthen the



control of its sexual reproduction. The mechanisms of adaptation and pollination, establishment of a fixed relationship with insect pollinators, and types of chemical substances produced and their functions may be closely associated with *S. canadensis* invasion, settlement, and dispersal. Therefore, these factors need to be further studied. Moreover, such chemical substances in *S. canadensis*'s body may have certain medicinal properties, but there is little research on it and rarely reported so far (Xu *et al.*, 2011).

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(Received for publication 3 June 2020)