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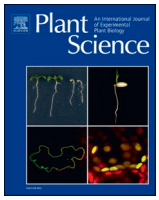

Research Article

Transcription-mediated tissue-specific lignification of vascular bundle causes trade-offs between growth and defence capacity during invasion of *Solidago canadensis*.

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ABSTRACT

Allocation of more resources to growth but less to defense causing growth vigor of invasive alien plant populations contributes to successful invasion. However, few studies has addressed to relationship between vascular development variation and this mechanism. In this study, a common garden experimentwas established to compare the growth and vascular bundle development between native and introduced populations of *Solidago canadensis*, which is a wide-distributed invasive species in China. Our results suggested that the rapid growth of introduced populations could be explained by the well-developed and highly lignified xylem; while native populations present more developed and highly lignified phloem, which contributed more resistance to the infection of *Sclerotiun rofsii* compared with introduced populations. This difference was resulted from tissue-specific tradeoff distribution of lignification related gene expression between xylem and phloem, which is regulated by upstream MYB transcription factors. Our study gives a novel insight of mechanism that explain invasion success: lignin-related gene transcription-mediated tissue-specific lignification of vascular bundle contributes tradeoffs in resource allocation between growth and defence capacity during successful invasion of *S. canadensis*.

1. Introduction

Invasion by alien plants is widely recognized as a serious threat to natural and managed ecosystems, causing consequences including the displacement of native species [1], modification of ecosystem primary functioning [2], and yield losses of agricultural production [3]. Therefore, the underlying mechanism of why alien species could successful invade into a certain ecosystem has drawn attention of many researchers for several decades [4]. Previous studies illustrated that successful invasive species tend to perform better in introduced range than in their native range [5], and several theories have been developed to explain such invasion success e.g., release from natural enemy [ERH] [6], evolution of increased competitive ability [EICA] [7]. Although results to test these hypotheses were controversial, the common ground of these theories indicated that once a plant is introduced into a new range, it may escape from the control of natural enemies and gradually evolve to reallocate their defences resources in a way that maximizes their fitness [7,8]. To date, such growth-defence tradeoffs have been tested for many invasive plant species [9–13]. Leger and Rice [10] found significant increases in size and fecundity of invasive populations that presented lower defence ability than plants from native populations. Siemann et al. [13] also found that *Sapium* from invasive populations had higher growth ability (40% faster) and lower defence against herbivores compared to those from native populations. These studies mostly focused on compared fitness (usually biomass) or resistance of offspring from native and invasive populations in same environment conditions. However, few studies have examined the occurrence of growth-defence trade-offs in a tissue perspective and the mechanism involved in.

Vascular tissue plays an essential role in the adaptation of plants to land. In typical angiosperm plants, the stem contains a large number of vascular bundles. The vascular bundles show a collateral pattern with xylem developing on the inside of the bundle and phloem on the outside [14]. Xylem has a direct role in the delivery of water and mineral nutrients from roots to aerial tissues, whereas phloem is involved in the transport of fixed carbon as well as other nutrients from photosynthetic to heterotrophic tissues [15]. Cell walls of some specific elements in the vascular tissue are impregnated with lignin [16], a phenylpropanoid compound that imparts water impermeability, including resistance

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against tensile forces of the water columns, and confers structural support and flexural stiffness to the aerial organs. It is generally thought that lignin plays an important role in enhancing the mechanical properties of cell walls by rigidifying the thickened wall, which provides essential support for the growth of plants [17]. On the other hand, the phenylpropanoid pathway is a crucial component of a plant's defence repertoire against abiotic and biotic stress factors [18]. Lignin can form defensive barriers to provide protection against herbivores or microbial degradation of cell walls [19,20]. Moreover, lignin synthesis is induced in response to mechanical damage or wounding, and many plants respond to invading pathogens with the deposition of lignin in vascular elements [21,22]. It can thus be suggested that the vascular tissue, and in particular, the lignification of vascular elements, play an essential role in plant adaptation to environmental abiotic and biotic stresses, which would help us to understand the mechanism controlling shifts in growth-defence capacity during invasion.

S. canadensis is an herbaceous perennial plant of the Asteraceae family. This plant is native to North America and has successfully invaded central and Western Europe, most of Asia, Australia, New Zealand and other locations. To date, the species has become one of the most destructive and widespread invasive species in China. Over the past three decades, a number of studies have provided strong evidence that compared to native populations, introduced populations of *S. canadensis* have greater fitness and higher competitive ability [23,24]. In addition, species from genus solidago have been proved to have increased growth ability and decreased defense ability in different invasion areas [25]. Thus, *S. canadensis* offers an excellent model system to study growth-defence trade-offs during the process of environmental adaptation of exotic plants. In our previous study [26], we have already strictly verified the increased growth ability of introduced population when invaded into china. In this context, we investigated the changes in defense ability and the mechanism which responsible for this trade-offs.

In common garden experiments, we investigated the variation for growth dynamics, development patterns of stem vascular elements and the tolerance to pathogen damage, in six introduced populations and six native populations of *S. canadensis*. To our knowledge, it's the first investigation of alternant in vascular elements development and lignification during invasion of *S. canadensis*, such a mechanism would result in introduced populations of *S. canadensis* presenting a relatively higher growth ability but weaker resistance to pathogen.

2. Materials and methods

2.1. Common garden experiments

Plants used in these experiments were grown under common garden experiments (Supplementary information) in glass house located in Pailou Experimental Base of Nanjing Agricultural University [Nanjing, Jiangsu, China (32°2′N, 118°50′E), in March 2015]. A total of 12 *S. canadensis* populations from two regions of six native (USA) and six introduced (China) populations (Table S1) were used in this experiment. The seeds were germinated in 5 cm diameter plastic cups containing potting mix. The seedlings were then transplanted into 13 cm diameter pots with 4 seedlings per pot. The seedlings were grown in the glasshouse with the temperature of 20-25 °C. Continuous sampling with plant growth was started from 90-day-old plants (growth stage 1). The next four stages were 120, 150, 180 and 210 days (growth stages 2, 3, 4 and 5, respectively). During each stage, randomly selected ten plants from each population were first measured for the length and diameter (measured in the middle of the stems, across the short axis) of the stems.

2.2. Biochemical analysis

To compare stem biomass allocation between native and introduced populations, during stem development, 3-cm-long stem sections from the middle of plants (ten individual plants per population) were chopped, oven dried (70 °C) and ground with a hammer to pass through a 1-mm screen. The cell wall residue (CWR) content was obtained after a two-stage extraction of the dry matter in ethanol and water [27]. The lignin content was estimated according to the Klason procedure [28] and was expressed as the weight percentage of Klason lignin (KL) in the CWR. Each experiment was repeated three times and was performed with ten plants for each replicate.

2.3. Anatomical analyses

For anatomical studies, 1-cm-long segment was sampled from the middle part of plants, and were immediately placed in 70% ethanol: water (v/v). For each segment, 50 serial stem cross-sections, 100 μm thick, were prepared with a cryostat (Leica CM1950).

Mäule staining was first used to characterize development pattern of vascular elements and to count the number of metaxylem vesseles in *S. canadensis*. Stem cross-sections were incubated for 7 min in a 0.5% potassium permanganate solution, then rinse 3-4 times with distilled water until the water solution stays clear. Discard the water. Quickly add 15% HCl until the deep brown colour is discharged from the sections, pipette out all the 15% HCl solution and immediately add concentrated ammonium hydroxide solution. After staining, sections were examined under Carl Zeiss microimaging GmbH (AX10) and were digitalized as color images with a resolution of 10 μm per pixel. Each image was thus analysed to count the number of metaxylem vessels per stem. Each experiment were repeated three times and performed with three individual plants for each replicate.

Fasga staining was used to assess lignification of vascular elements. Stem cross-sections were stained overnight using a Safranin and Alcian blue solution as previously reported [29]. After staining, sections were examined under a magnifying glass (OLYMPUS SZX7) and were digitalized as colour images with a resolution of 10 μm per pixel (3 sections per individual plant). Each image was numerically and automatically analysed using ImageJ software with the plugin presented by Zhang et al. [29] to segment and evaluate the red/blue intensity ratio relative to the distance to the outer epidermis for the five stages of development. Then, the evolution of the lignification rate for each region of the stem sections during stem development was assessed (Supplementary information). Each experiment were repeated three times and performed with three individual plants for each replicate.

2.4. Gene cloning and gene expression analyses

For gene cloning (partial-length of *ScMYB46*, *ScMYB58*, *ScPAL-1*, *ScCAD-1*, *ScLac4-2*, and *ScLac17-1* gene) and gene expression analyses, 3-cm-long stem were sampled. The phloem and xylem tissue of each stem were rapidly separated with a knife and immediately stored in liquid nitrogen. Total RNA was isolated using Biospin Plant Total RNA Extraction kit (BioFlux, San Fransisco, CA, USA) according to the manufacturer's protocol. Fifty micrograms of total RNA were purified and treated with RNase-free DNase using the plant RNeasy kit (Qiagen, Mississauga, ON, Canada). The final concentration was determined using an Eppendorf spectrophotometer (Eppendorf AG, Hamburg, Germany).

The template cDNA for cloning was prepared using MMLV reverse transcriptase (TaKaRa BIO, Shiga, Japan) following the manufacturer's protocol. Gene specific primers were designed according to the homologous gene sequences of *A. thaliana*, *Lactuca sativa* and *Chrysanthemum dichrum* (data from NCBI) using the DNAMAN 6.0 software (Table S2). All designed primers had efficiencies equal to or greater than 90% when tested using a five-fold dilution series of the template starting with 30 ng. Quantitative RT-PCR was performed in 20-μL reaction volumes containing 10 μL SYBR® Premix Ex TaqTM (2 ×) (TaKaRa BIO, Shiga, Japan), 3.8 μL dH2O, 0.5 μL of each gene-specific primer (10 μM) and 1 μL of cDNA. The relative expression levels of each gene were standardized with actin gene in *S. canadensis* (The *ScActin* gene was

determined to be stably expressed; Supplementary information, Table S2). The reaction conditions for qPCR were as follows: 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s, 55 °C for 15 s and then 68 °C for 20 s. Quantitative analysis was performed using the Eppendorf Mastercycler® ep Realplex2 (Eppendorf, Hamburg, Germany) and the data were calculated using the $2^{-\Delta\Delta CT}$ method [30] with data corrected for PCR efficiency and normalized against both reference genes. Each experiment were repeated three times and performed with three individual plants for each replicate. QUALITY-ONE software (Bio-Rad) was used for quantification.

2.5. Monitoring the injury of S. canadensis stems by Sclerotiun rofsii mycelia and gene expression analysis

The pathogen *S. rolfsii* isolate SC64 used in this study is a strong *S. canadensis* pathogen identified in our laboratory [31]. Pathogenicity assays of *S. rolfsii* were tested with mycelia blocks (diameter =2.5 mm) punched from the margins of actively growing colonies on PDA (potato extract, 20 g D-glucose, 15 g agar and water to make 1 L). SC64 were cultured on PDA for 4-5 days at 28 °C.

Inoculation experiments were performed at 90-day-old plants in the common garden experiment. For necrotic lesions studies five groups of 90-day-old plants (each consisting of ten individuals) were inoculated for 0, 18, 24, 30, and 36 h, respectively. An Imaging-PAM Chlorophyll Fluorometer (M Series, Heinz Walz GmbH, Germany) was used to measure photosynthesis after inoculating fungal mycelia onto *S. canadensis* stems as previously reported by Kang et al. [32]. Necrotic severity was evaluated by lesion size. The following recommended settings were applied in the study: pulse-modulated measuring light intensity (ML) ¼ 2; measuring light pulse frequency (MF) ¼ 1; Gain ¼ 2; Damping ¼ 2; actinic light (AL) ¼ 8; and saturating flashes ¼ 10.

In inoculation experiments, plants used for gene expression analyses were inoculated at a single site on the middle part of the main stem (inoculated). Control plants were treated with petals inoculated with a drop of deionized water (mock inoculated). At 0, 12, 24, 26, 28, 30, 32, 34 and 36 hours post-inoculation, the phloem and xylem tissue of

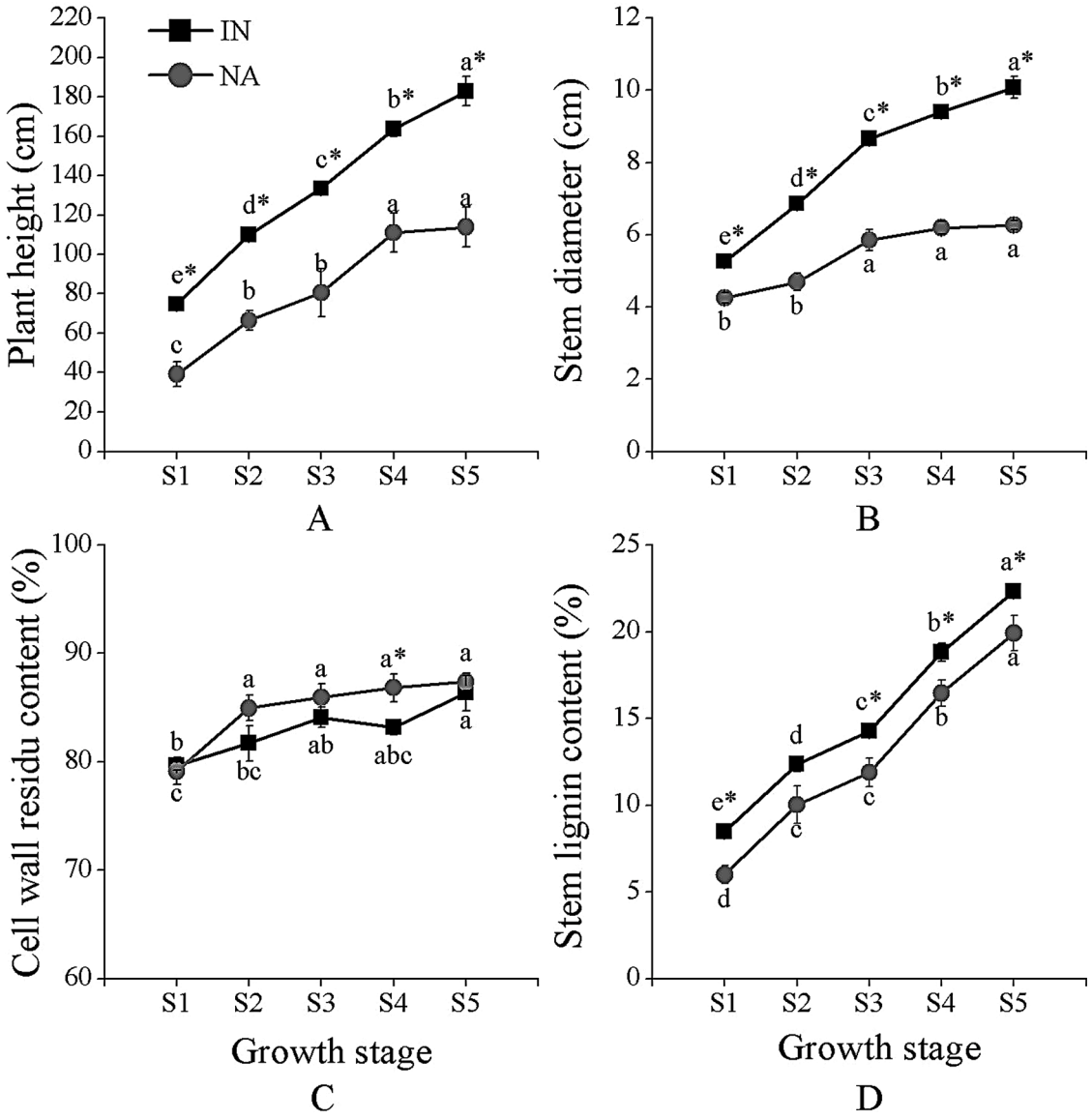


Fig. 1. Comparison of stem growth between native (NA) and introduced (IN) populations of *S. canadensis* during different growth stage. (A) Plant height (cm). (B) Stem diameter (cm). (C) Cell wall residue content (%) of stem. (D) Stem Klason lignin content (%) of cell wall residue. For each experiemnt, three independent replicates were performed, and ten individual plants per population were used for each replication. Data represent means ± SD (n = 6 populations). Different lowercase letters indicate significant differences among growth stages ($P < 0.05$). Asterisks indicate significant differences between IN and NA genotype populations in each growth stage ($P < 0.05$).

inoculated and mock-inoculated plants were rapidly separated with a knife and immediately stored in liquid nitrogen for future quantitative RT-PCR following the same steps as in the part "Gene cloning and gene expression analyses". For each detected time, the value of gene expression was calculated by relative expression of inoculated minus mock-inoculated. Each experiment were repeated three times and performed with three individual plants for each replicate.

2.6. Statistical analysis

Statistical analysis was conducted using SPSS software (IBM SPSS Statistics 20, Chicago, Illinois, USA). An independent sample t-test was used to assess differences between IN populations and NA populations at same stem growth stage, and an ANOVA with Duncan's test was used to assess differences between different stem growth stage within the populations from same range and significance was determined at $P < 0.05$. The correlation analyses between different parameters were analyzed using SPSS software (IBM SPSS Statistics 20, Chicago, IL, USA). Drawing with Origin software version 8.0 (Origin Lab Corporation, Northampton, MA 01060, USA).

3. Results

3.1. IN population had superior growth ability

We observed a significant difference in the stem growth rate between introduced (IN) and native (NA) populations (Fig. 1A, B). During stem development, the plant height and stem diameter of the IN populations displayed strained growth until stage 5, while the NA populations ceased growth before stage 4. Compared to NA populations, the plant height and stem diameter of the IN populations were significantly higher throughout the entire experiment. The stem dry matter content did not increase during growth and did not significantly differ between the two populations (Fig. 1C). In contrast, both IN and NA populations showed a significant increase in the stem lignin content during stem development (Fig. 1D). Additionally, the stem lignin content was significantly higher in the IN populations than in the NA populations throughout the entire experiment.

3.2. IN population had developed xylem vessels

Mäule staining is known to impart a red coloration to S lignin, which was present in the secondary cell wall of metaxylem vessels (MV), while the primary cell walls of the protoxylem vessels (PV) had a negative reaction (Fig. 2A-10) [33]. The Mäule staining results of stem cross-sections from CN25 (IN) and US07 (NA) are shown in Fig. 2A to represent vascular element development for IN and NA populations, respectively. At the elongation stage (S1), all of the vessel elements of the IN populations reacted positively with the Maüle reagent (Fig. 2A-4), corresponding to a typical secondary cell wall that presented only mature vessel elements (MV), while most of the primary cell walls of the protoxylem vessels (PV) of the NA populations reacted negatively and had noticeably thinner cells; a small number of vessel elements (MV) located in the xylem fibres (XF) exhibited a positive reaction (Fig. 2A-10). This result suggests that the NA populations contained both protoxylem vessel elements and metaxylem vessel elements. Additionally, during this stage the numbers of metaxylem vessels were significantly higher in IN populations (Fig. 2B). The stained vascular tissue region, including both phloem and xylem, significantly increased for all populations between each growth stage (Fig. 2A), which represents a significant development pattern in vascular tissue during stem growth. Within the xylem, the number of metaxylem vessels (MV) significantly increased in both IN and NA populations (Fig. 2B). Moreover, there was a marked development in xylem fibres (XF) in the NA populations. Thus, at the senesced stage (S5), the xylem of the NA populations contained more fibres (Fig. 2A-12), and the xylem of the IN populations contained significantly more metaxylem vessels (Fig. 2B).

3.3. IN population preferably deposited lignin to xylem

The stem cross-sections were subsequently stained with the Fasga reagent, and we quantified the corrected lignification of vascular elements using the Quantifasga method (Fig. S2) [29]. The average lignification ratio in the phloem and xylem during stem growth of the IN and NA populations is shown in Fig. 2C. From stage 1 to stage 5, the lignification ratio in both phloem and xylem significantly ($P < 0.05$) increased in the IN and NA populations. These observations demonstrate that significant lignin deposition occurred following the stem development of *S. canadensis*. At all growth stages, the IN populations presented a significantly lower lignification ratio in the phloem compared with the NA populations; however, the lignification ratio in the xylem was relatively higher in the IN populations, and the difference was significant only during growth stage 1 and 5.

3.4. IN population had weaker resistance to pathogen's infection

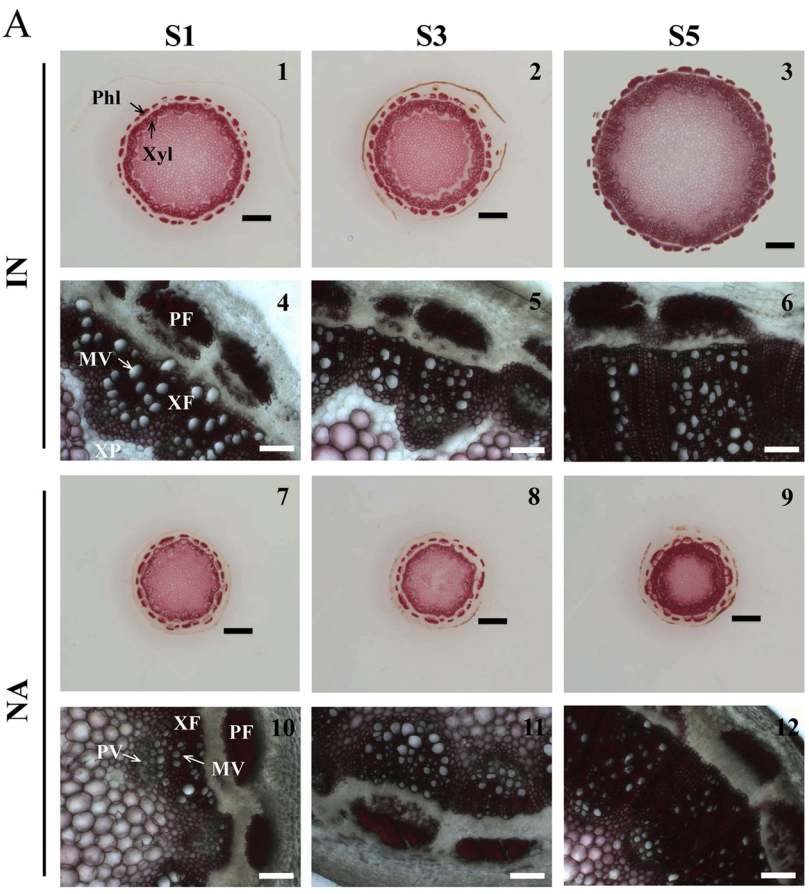
In order to simulate the attack of natural enemies and compared the defence responses of the IN and NA populations, 90-d-old plants of the two populations were infected with *S. rolfsii*. Necrotic lesions were studied from 18 to 36 h post-inoculation using unwounded stems of IN and NA populations inoculated with *S. rolfsii* (Fig. 3A). A rapid fungal attack and serious damage were observed in the IN populations, with lesions first occurring at 18 hpi in the fluorescence images (Fig. 3A), and most of the IN populations presented leaf wilting at 36 hpi (Fig. S1A). In contrast, disease progression was slower in the NA populations; lesions remained confined to the outer cortex of the stem, and no wilting occurred at 36 hpi (Fig. S1B). From 18 to 36 hpi, lesions were significantly ($P < 0.05$) smaller in the NA populations than in the IN populations, and the NA populations showed lesions that were less than half the size of lesions in the IN populations (Fig. 3B).

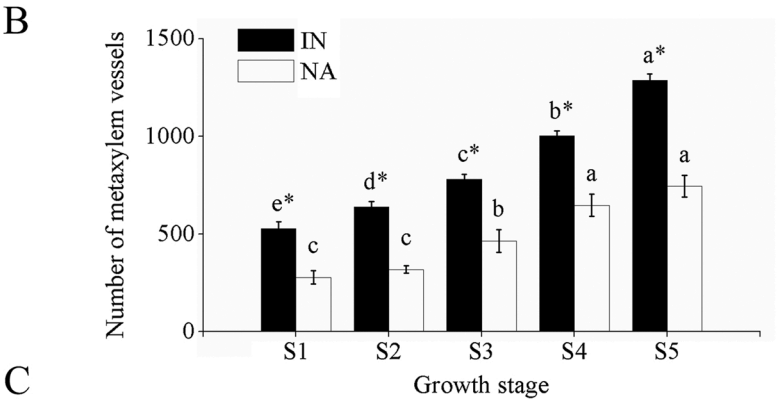
3.5. Genes regulated tissue-specific lignin allocation between phloem and xylem

The expression profiles of six key genes involved in lignification pathway from both contrasting populations were analysed by qRT-PCR. Analyses were performed from S1 to S5 using separated phloem and xylem tissue. In both populations, the expression levels of all six genes investigated in the phloem and xylem increased during stem development and reached highest levels at stage 3 (Fig. 4), which decreased from stage 3 to stage 5. In the phloem, all six genes displayed a significantly lower expression level in IN populations than in NA populations; however, no significant difference in the gene expression level in the xylem was observed between two populations during all growth stages.

3.6. Genes mediated disease resistance through lignin allocation difference between phloem and xylem

The expression profiles of six key genes involved in lignification pathway were analysed by qRT-PCR. Analyses were performed using peripheral stem tissue surrounding the inoculation site. In the phloem, all six genes examined were induced after *S. rolfsii* inoculation in both IN and NA populations (Fig. 5). Moreover, the expression peak appeared from 12 hpi to 26 hpi, depending on the gene. The transcription level of *ScMYB46* and *ScMYB58*, which is a transcription factor involved in monolignol production, was pronounced following inoculation, and the highest level were reached at 12 hpi. *ScPAL-1* and *ScCAD-1*, which encode enzymes involved in the phenylpropanoid pathway, were induced following inoculation and reached the highest level at 24 hpi. The expression levels of *ScLac4-2* and *ScLac17-1*, which lead to the assemblage of monolignol in the cell wall, were induced from 12 hpi, and a progressive increase was observed during inoculation, with the highest transcript level found at 26 hpi. Remarkably, transcript levels of all six genes increased to a significantly greater extern in NA populations





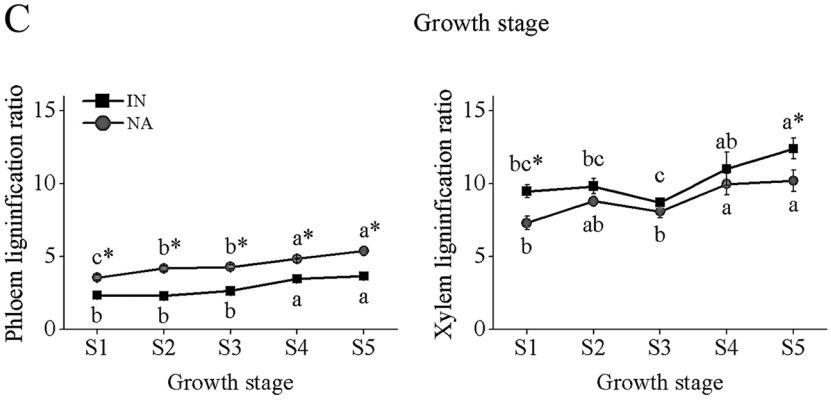


Fig. 2. Comparison of vascular elements development between native (NA) and introduced (IN) populations of *S. canadensis* during different growth stage. (A) Maüle staining of stems cross-sections. Note enhancement of tracheary elements in IN populations during stem development. S1-elongation, S3-inflorescence emergence, and S5-senesced stem internode transverse cross-sections. Scale bars correspond to 1 mm (1,2,3,7,8,9) and 200 μm (4,5,6,10,11,12). Phl: phloem, Xyl: xylem, PF: phloem fibres, MV: metaxylem vessels, PV: protoxylem vessels, XF: xylem fibres, XP: xylem parenchyma. (B) Number of metaxylem vessels following stem growth. (C) Variation of the average lignification ratio in stained sections of the phloem (a) and xylem (b) during stem growth. S1-S5 on the x-axis refers to the five growth stages. Three independent replicates were performed, and three individual plants per population were used for each replication. Data represent means ± SD (n = 6 populations). Different lower-case letters indicate significant differences among growth stages ($P < 0.05$). Asterisks indicate significant differences between IN and NA populations in each growth stage ($P < 0.05$).

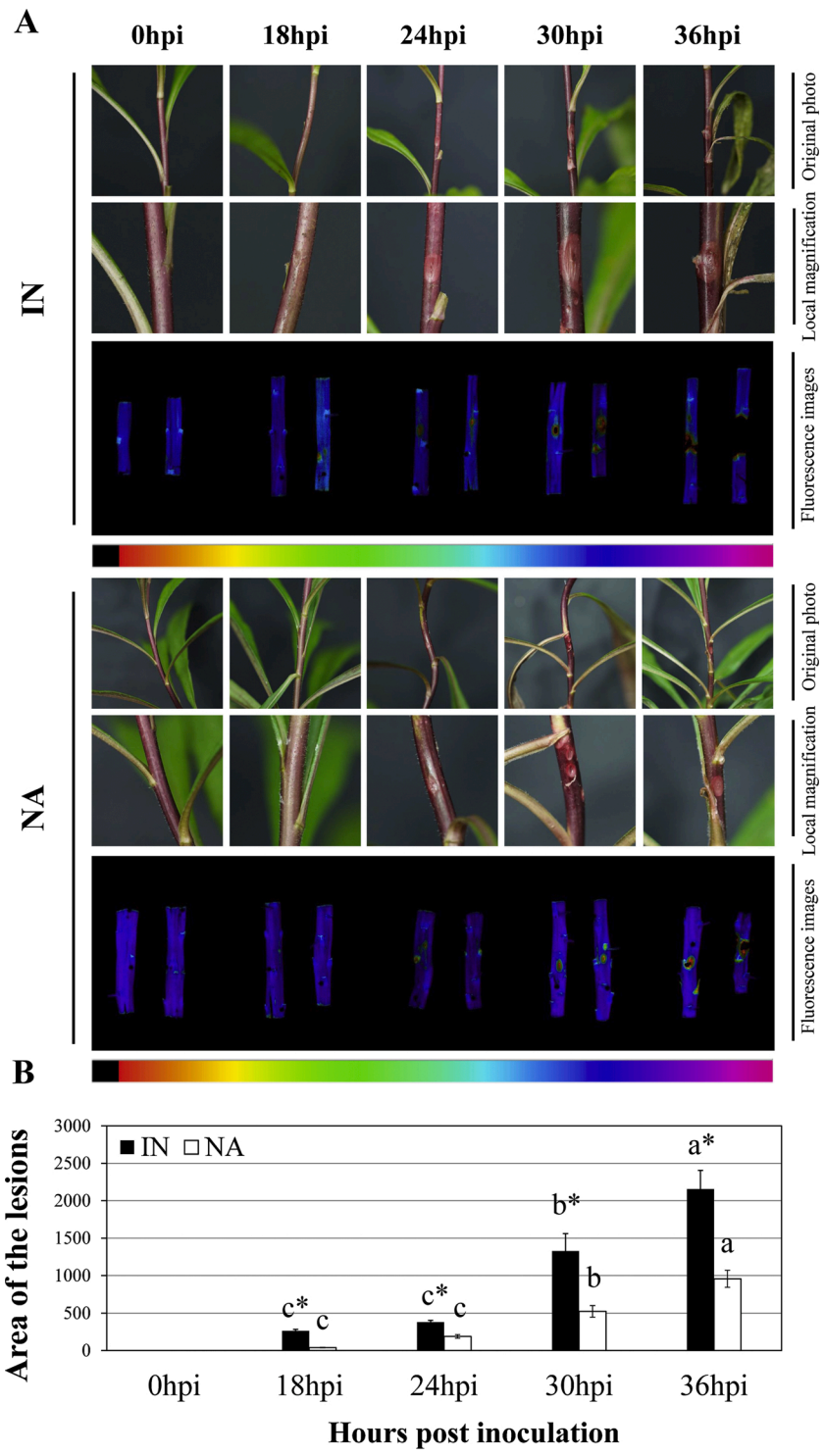


Fig. 3. IN populations of *S. canadensis* exhibit weakened resistance to *S. rolfsii*. (A) Comparison of necrotic lesions on stems between native (NA) and introduced (IN) populations. 90-day-old plants were harvested at 0 h, 18 h, 24 h, 30 h and 36 hours post *S. rolfsii* inoculation. (B) Resistance response of native (NA) and introduced (IN) populations. Lesion size was measured from 90-d-old plants at 0 h, 18 h, 24 h, 30 h and 36 h post *S. rolfsii* inoculation. Stems from 10 plants each of the 12 population were used for each experiment. Data represent average area of the lesion (pixels) ± SD (n = 6 populations). Different lower-case letters indicate significant differences among growth stages. Asterisks indicate significant differences between IN and NA populations in each growth stage ($P < 0.05$).

than in IN populations. The differences in transcript levels between IN populations and NA populations were most pronounced from 12 to 26 hpi. NA populations showed expression peaks that were more than 2-3 times for the MYB58 and CAD genes in comparison to the IN populations. In the xylem, a gradual down-regulation of all genes was observed following inoculation with *S. rolfsii* in both the IN and NA populations, and no significant difference in expression levels was observed between two populations.

4. Discussion

Numerous studies report that invasive plant species in a new range can be larger, can grow faster, and can produce more abundant populations [5,10,34]. In our common garden experiment, IN populations of *S. canadensis* were taller and had a larger stem diameter during all five growth stages and also tended to grow faster than NA populations. Previous studies have demonstrated that the higher competitive ability of *S. canadensis* from the introduced range may be associated with their greater aboveground size and faster growth ability compared to those

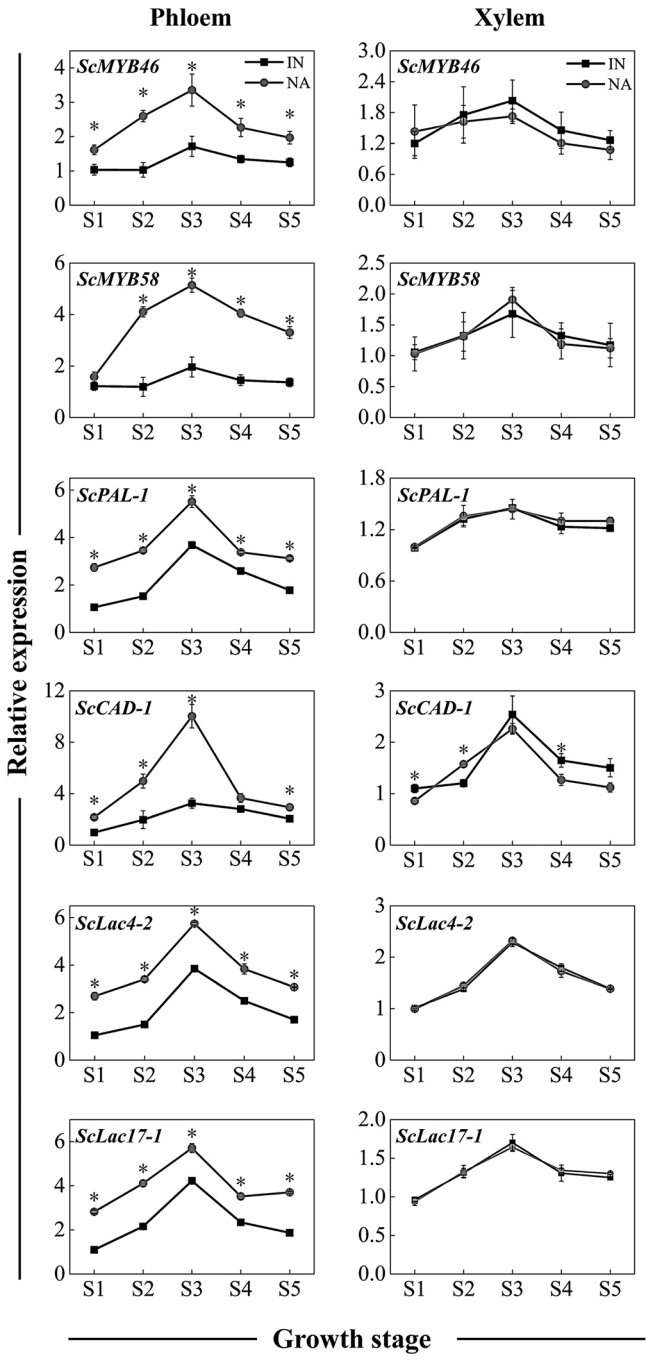


Fig. 4. Comparison of transcript levels of the Sc*MYB46, SCMYB58, ScPAL-1, ScCAD-1, ScLac4-2*, and Sc*Lac17-1* genes in the phloem and xylem of *S. canadensis* during stem growths as determined by qRT-PC. Black and white columns refer to introduced (IN) and native (NA) populations, respectively. *PAL*, phenylalanine ammonia-lyase; *F5H*, ferulate 5-hydroxylase; *Lac4*, Laccase 4; *Lac17*, Laccase 17. S1-S5 on the x-axis refers to the five growth stages. Three independent replicates were performed, and three individual plants per population were used for each replication. Data represent means ± SD (n = 6 populations). Asterisks indicate significant differences between IN and NA populations in each growth stage ($P < 0.05$).

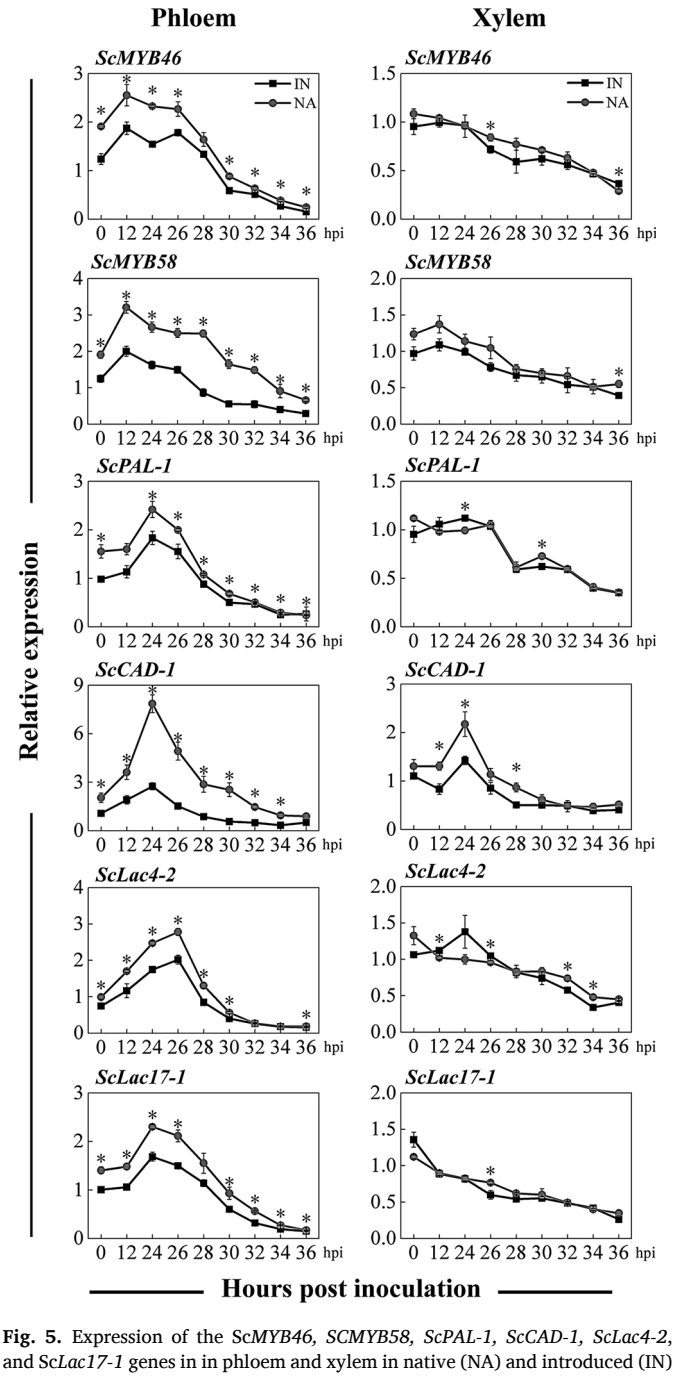


Fig. 5. Expression of the Sc*MYB46, SCMYB58, ScPAL-1, ScCAD-1, ScLac4-2*, and Sc*Lac17-1* genes in in phloem and xylem in native (NA) and introduced (IN) populations of *S. canadensis* following stem inoculation (0-36 h) with *S. rolfsii*. Stems were harvested at 0, 12, 24, 26, 28, 30, 32, 34 and 36 hours post-inoculation. Transcript levels were determined by qRT-PCR and were normalized to actin. Three independent replicates were performed, and three individual plants per population were used for each replication. Expression levels are presented as means ± SD (n = 6 populations). Asterisks indicate significant differences between IN and NA populations in each growth stage ($P < 0.05$).

from the native range [24]. To date, many studies have reported an enhanced growth ability of plants from the introduced range, which is a key index for the successful colonization of introduced plants [5,23]. Such growth advantage of the introduced population leads to greater fitness, and more competitive ability with other plants (for light, water, nutrients) [13,35,36]. Results of our study suggest different pattern of lignin allocation between invasive and native populations of *S. canadensis*. During all five growth stage, although there was no significant difference between stem dry matter content, IN populations seams to deposit more stem lignin content than NA populations. The importance of plant cell wall lignification ranges from its fundamental roles in the evolution of land plants, to the practical of plant growth and development [37]. Over the last few years, it has become evident that the lignification of cell wall may be a crucial event in the biotic and abiotic stress resistance of plants [38–40]. It is therefore possible that

variation of lignin metabolism is likely to be involved in the response of *S. canadensis* to novel environment biotic and abiotic stresses in the invasive range.

One interesting finding of this study is that during stem development, IN populations had more vascular bundles and well-developed metaxylem vessels, but fewer fibres as well as intrafascicular fibres and phloem fibres, all underlining a preferential development of vessels with a fundamental defect in fibre formation. Fasga-stained stem cross-sections were then analysed using the colour quantification method to assess lignin distribution among tissues. As observed in previous studies [29,41], our method can reveal differences in lignification levels in vascular tissues at different growth stages. During each growth stage, compared to NA populations, IN populations displayed slightly higher lignin levels in the xylem and severely reduced lignin levels in the phloem. Taken together, the above results suggest that during invasion, in addition to the evolution of vascular tissue development, the lignification of vascular tissue was also affected in IN populations. Such evolution led to the presence of well-developed metaxylem vessels in the xylem of the IN populations, while the lignification of phloem fibres decreased significantly. Mature vascular tissues consist of highly specialized cell types that are generally used to perform different functions. Tracheary elements, which facilitate water and solute transport between organs, and fibres, which provide structural support for the plant, possess thick secondary cell walls. Lignification of such cellulosic secondary cell walls makes a major contribution to the functionality of these cells [15]. This phenolic polymer imparts both increased structural stability and water impermeability to the cell wall [42]. In this study, a significant enhancement of tracheary elements of IN populations revealed the evolution of vascular elements development after invasion, which may suggested that the improvement of growth ability after invasion is likely to be related to the higher water impermeability capacity of vascular tissue.

It has established, in many successful invasive species, growth advantage of the introduced population caused by a compensatory release of limited resources utilized for defence, the invasive populations present thus lower resistance to herbivore [13] or to pathogen's infection [43]. In this study, after inoculation with *S. rolfsii*, the stems of IN populations presented significantly larger necrotic lesions than those of NA populations, indicating that IN populations were less defended than NA populations. IN populations have been shown to have a defect in phloem fibre formation and organization, which may have a defensive function. The presence of fibres acted as a constitutive physical barrier against pathogen infection for plant cells. A strong relationship between phloem development and plant defence ability has been reported in the literature [44,45]. It is possible that changes in the lignification of phloem fibres of *S. canadensis* were responsible for the difference in resistance to *S. rolfsii* infection after introduced in China.

To further elucidate the mechanism that led to the difference in the lignification of vascular elements between IN and NA populations, the variation in the expression of key genes with a putative function in lignification during stem development was analysed. These genes cover each of the steps in the lignification pathway. MYB46 and MYB58 are two MYB transcription factors that act as master switches regulating lignin biosynthesis, Phenylalanine ammonia-lyase (PAL) catalyzes the first step of the phenylpropanoid pathway specifically committed to the synthesis of monolignols. cinnamyl alcohol dehydrogenase (CAD) that catalyses the last step of the phenylpropanoid pathway that conversion of hydroxycinnamaldehydes into the corresponding hydroxycinnamyl alcohols (monolignols) [42]; Laccase are the main enzymes involved in the process of monolignol assembly that leads to lignin polymerization. Where Lac4 is necessary for lignification of fibre cell walls; And Lac17 is important for the lignification of tracheary elements [46]. As expected, the expression levels of all six examined genes in the phloem and xylem increased during stem development. These results corroborate the suggestion that, as observed in other species [42], these lignification pathway genes may perform important functions during vascular tissue development of *S. canadensis*. It is noteworthy that, compared to the NA populations, the genes displayed a relatively lower expression level in the phloem of the IN populations; however, no significant difference in the gene expression level in the xylem was observed between IN and NA genotype populations. The results are in agreement with the previous histological analysis, which suggests that the difference in the constitutive lignin content in the phloem was caused exclusively by a decrease in the expression level of genes involved in the lignification pathway in the IN populations.

Previous studies have shown that lignin synthesis is induced in response to mechanical damage or wounding, and many plants respond to invading pathogens with the deposition of lignin and lignin-like material [20,21,47]. As expected, the expression levels of all six examined key genes with a putative function in lignification up-regulated in the phloem of both populations in response to infection. Interestingly, although studies have shown that the infected hyphae of *S. rolfsii* could colonize xylem [31,48], gene expression was not induced in the xylem, indicating that only phloem lignification was induced during the fungal defence mechanism of *S. canadensis*. This result, together with the vascular elements development and lignification analysis, suggested that phloem lignification may act as a constitutive resistance component in the resistance of *S. canadensis* to *S. rolfsii* and may also play an important role as an induced mechanism. Many defence responses, including the accumulation of polyphenols, formation of necrophylactic periderm and formation of traumatic resin canals, are induced by mechanical wounding and infection caused by fungi and insects in the phloem [49]. It has been proposed that such phloem-based defence (PBD) mechanisms are regulated by the phytohormone ethylene and MYB transcription factors [50]. In the current study, after inoculation with *S. rolfsii*, lower pathogen-induced gene expression levels in the phloem were associated with a lower defence ability of the IN populations. The reduction in the resistance ability of the IN populations may therefore be partly associated with the transcription variation of two MYB transcription factors upstream of lignification pathway (*ScMYB46*, *ScMYB58*), and thus the lowered activation of monolignol biosynthesis led to the observed onset of rapid fungal attack and serious damage.

Based on the data of twelve populations, we conducted a linear regression analysis on the lignification ratio in both phloem and xylem (Fig. 6A). The results showed a significant negative relationship between levels of lignin in phloem and xylem, this may support the hypothesis that trade-offs mechanism occurred to regulate lignin distribution in these two tissues. More importantly, during stem development, populations from the introduced range had more vascular bundles and well-lignified metaxylem vessels but less fibre lignification than populations from the native range. The correlation analyses showed that the number of metaxylem vessels and the levels of lignin in xylem were both positively correlated with plant height (Fig. 6B and C). It seems that the priority development of vessels may contribute to the increased growth ability of the populations from the introduced range. We also found a negative correlation between levels of lignin in phloem and pathogen susceptibility (Area of the liesions after *S. rolfsii* infection) (Fig. 6D); indicated populations from introduced range have lower defence ability during pathogen infection.

5. Conclusion

In conclusion, all these results suggest that lignin distribution among vascular elements plays a vital role in the mechanism which control shifts in growth-defence capacity involved in the invasiveness of *S. canadensis*. The empirical findings in this study provide a new understanding of the mechanism that explains invasion success. A further study could assess the mechanism controlling lignin distribution among vascular elements during plant adaptation to environments that involved escape from natural enemies, and the ecological significance can be evaluated.

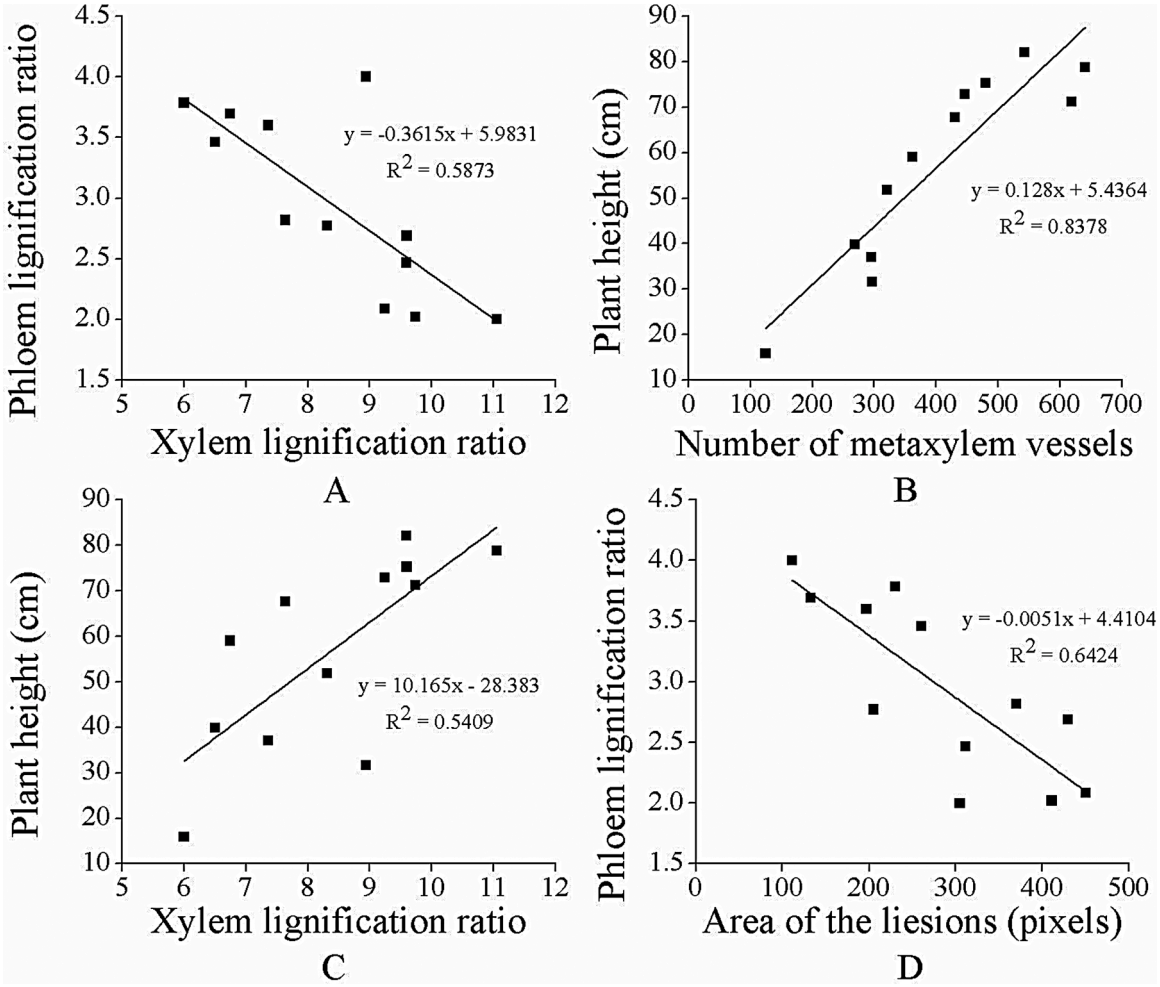


Fig. 6. Correlation analysis to examine trade-offs in growth-defence capacity during *S. canadensis* invasion. A. Relationship between the lignification ratio in the phloem and xylem; B. Relationship between number of metaxylem vessels and plant height [cm] of *S.canadensis*; C. Relationship between xylem lignification ratio and plant height of *S.canadensis*; D. Relationship between levels of lignin in phloem and area of the liesions [pixels] after *S. rolfsii* infection. A significant negative correlation between the lignification ratio in the phloem and xylem would indicate a tradeoff associated with growth and defence capacity. Depicted are raw data points (12 populations) and a fitted line.

Author contributions

S. Qiang conceived the ideas; S. Qiang and Y. Zhang designed the methodology; L.J. Xu collected and analysed the data; Y. Zhang and S.G. Cheng led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Declaration of Competing Interest

The authors report no declarations of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.plantsci.2020.110638.

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