

## Conference Paper

# Absorbing Roots of Invasive Woody Plants Apparently Have a Thicker Cortex Parenchyma Compared to Native Species

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## Abstract

Invasive plants are usually fast-growing species with a high rate of physiological processes. However, the relative growth rate has not been estimated for many invasive species, including *Acer negundo* L. (*Sapindaceae*) and *Amelanchier spicata* (Lam.) K. Koch (*Rosaceae*); this hampers the understanding of the reasons for the success of their invasion. The authors compared the absorbing roots of *A. negundo* and *A. spicata* with respect to mycorrhizal colonization with two native woody species of the same families, *Acer platanoides* L. and *Sorbus aucuparia* L. The samples were collected from several sites in the Central Urals and analysed using standard morphological and anatomical methods. The abundance of arbuscular mycorrhiza, dark septate endophytes and root hairs did not differ between the invasive and native species. Nevertheless, the roots of the invasive species were shown to have a thicker cortex parenchyma formed by larger cells. We suggest that this could contribute to invasion success, but a higher growth rate of the roots of invasive species has not yet been proven.

**Keywords:** *Acer negundo*, *Amelanchier spicata*, invasive plants, absorbing roots, arbuscular mycorrhiza, root cortex, parenchyma

## 1. Introduction

Invasive species have morphological and functional traits allowing them to successfully invade new regions and communities; their analysis is important to better understand the general mechanisms of plant invasion and is thus of considerable applied relevance. Invasive plants have been shown to have a high growth rate, a high rate of physiological processes and a preferential allocation of biomass and nutrients to leaves and shoots [1]. However, the relative growth rate has not been estimated for many

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invasive species, including *Acer negundo* L. and *Amelanchier spicata* (Lam.) K. Koch. It was important for us to reveal the morphological traits associated with the suggested high growth rate. Such traits can be found in both above- and below-ground plant organs [2, 3].

As far as below-ground organs are concerned, the strategic characteristics of plants are associated mainly with the root cortex [4], more specifically with the most metabolically active tissue such as cortex parenchyma [5]. Usually, thicker roots with a greater number of cortex parenchyma cell layers and higher tissue density require more energy and nutrients, grow slower and live longer [4, 6]. In contrast, the roots of fast-growing species have a high specific root length (SRL) and a small number of cell layers; therefore, they are able to grow faster and intercept resources [7]. Invasive plants have been shown to have a high SRL as well [8], which means that their roots are energetically cheap to produce [4]. Although SRL strongly correlates with root diameter [9], it is usually calculated for all roots less than 1 mm in diameter which are functionally divergent: this does allow for an accurate comparison. The data on fine root diameter are limited; it has been shown to be the same [10, 11], smaller [12] or greater [14, 15] in invasive plants than in native species. Such a difference between studies could be due to the analysis of either taxonomically or ecologically distinct plant species.

The aim of the present study was to compare several morphological and anatomical traits between (i) functionally homogeneous absorbing roots with primary development and (ii) taxonomically close invasive and native species growing in the same conditions. This will reduce methodological, taxonomic and ecological effects.

## 2. Methods

### 2.1. Plant species

We selected two invasive species, box elder (*Acer negundo* L.) and low juneberry (*Amelanchier spicata* L.), shown to be invasive in Europe [16], Russia [17] and the Central Urals [18]. For comparison, two native species were studied: Norway maple (*Acer platanoides* L.) and rowan (*Sorbus aucuparia* L.). Both species are rather common in the Central Urals, where the samples were collected.

## 2.2. Study sites

The roots were collected in Ekaterinburg, a city in the south taiga zone with a temperate continental climate. To measure stable morphological and anatomical characteristics, two sites were chosen; an additional sampling from a third site was performed to analyse arbuscular mycorrhizal (AM) colonization as a more variable feature. In all sites, invasive and native species grew together or in close proximity to each other.

## 2.3. Sampling and morphological analysis

Root fragments were sampled during June and July in 2015, from 5–15 cm deep for five plants of each species at each sampling site. Only the live roots of two–three distal orders were selected and fixed in 70% ethanol. For each plant, 5–10 intact apical roots were transversally cut into sections 15–20  $\mu\text{m}$  in depth on a freezing microtome. The following parameters were then determined: root diameter, central cylinder diameter, the thickness of the cortex parenchyma, number of cortex parenchyma cell layers and parenchyma cell diameter. Only parenchyma cells without any cell wall thickening were analysed. Additionally, the abundance of AM fungi was assessed in 15 random root segments 1 cm in length and isolated from the roots of two distal orders. These segments were macerated in KOH for 1 hour, then coloured with aniline blue and used to make squash preparations. For each segment, five fields of view were inspected with a Leica DM 5000 microscope (Leica, Germany) at 100x magnification to measure the proportion of the field of view covered by (1) AM hyphae; (2) arbuscles; (3) vesicles; (4) dark septate endophytes (DSE); and (5) root hairs.

Statistical analysis was carried out using the package STATISTICA 8.0 with three-factors ANOVA. In all cases, individual plants were used as experimental units.

## 3. Results

In this study, we analysed the influence of three main factors: invasive status (invasive/native), plant family (Sapindaceae/Rosaceae) and sampling site. The common feature of all absorbing roots of the studied species was a high mean proportion of the cortex, 95–96%, and a small mean diameter of the central cylinder, 60–76  $\mu\text{m}$ . A high proportion of the cortex is probably needed for the interaction with fungal symbionts since all the species were mycorrhizal.

The number of cortex parenchyma cell layers depended on plant family: the roots of *Rosaceae* plants usually had two layers of parenchyma cells while the roots of *Acer* sp. had two or three layers. It is worth noting that the roots of *A. negundo* had three cell layers significantly more frequently, but not more than 8% overall.

The abundance of AM fungi, DSE and root hairs differed depending on the site; root hair abundance in *Sapindaceae* species was significantly higher, which could be a family-specific trait. No clear association between invasive status and AM abundance was found, except for a higher abundance of AM hyphae, vesicles and arbuscles in the roots of the invasive species *A. negundo* (15–75% in different sites) compared with the native *A. platanoides* (11–35%). *A. spicata* and *S. aucuparia* had a similar AM abundance, but in *A. spicata* this feature differed more strongly between sampling sites. The fact that the abundance of AM fungi depends on the site rather than other factors is in line with the current conception of symbiosis as a tool that allows the fine tuning of absorbing roots to function in specific soil conditions [15].

Root diameter varied depending on the site and plant family but was greater in the species of *Sapindaceae* (Figure 1). Most importantly, the roots of invasive species were thicker by 10%, mainly due to larger cortex parenchyma cells (Figure 1), and partly due to a larger number of cell layers in *A. negundo*. The ANOVA interactions of the 'invasive status' factor together with 'plant family' or 'sampling site' were not significant for this parameter (Table 1).

Thus, the absorbing roots of two invasive and two native species that are both taxonomically and ecologically related differed in root diameter, the thickness of the root cortex parenchyma and parenchyma cell diameter. Invasive *A. negundo* and *A. spicata* had thicker roots with a thicker parenchyma formed by larger cells. These results are supported by the fact that *A. negundo* has thicker roots compared to the native *Acer* species in the Southern Urals [15]; a similar pattern has been shown for the invasive species *Heracleum sosnowskyi* [14, 15]. However, our results do not correspond with the previous studies showing the same or thinner roots [10–12] or a higher SRL [8] in invasive plants. We found that roots with more parenchyma cell layers (three vs two) were significantly more frequent in invasive *A. negundo* than in native *A. platanoides*: however, since this frequency did not exceed 8%, we cannot claim that invasive and native plants significantly differ in the metabolic cost of root growth. Although we did our best to reduce all the effects of the choice of samples and methods, the data are still insufficient to make any clear conclusion.

The question is how a thicker cortex parenchyma with larger cells would contribute to invasion success. We think that large parenchyma cells with a large central vacuole

TABLE 1: ANOVA  $F$ -values for several parameters of absorbing roots calculated for the three main factors and their interactions.

Parameter	Factor			Factor Interactions			$R^2_{adj}$
	Invasive Status (dF = 1) [1]	Plant Family (dF = 1) [2]	Sampling Site [3]	[1] × [2]	[1] × [3]	[2] × [3]	
Root diameter <sup>§</sup>	7.65**	12.76***	17.96***	0.41	0.30	15.00***	0.54
Central cylinder diameter <sup>§</sup>	3.84	0.06	1.97	0.05	0.01	4.44*	0.10
Cortex parenchyma thickness <sup>§</sup>	13.65***	6.46*	14.05***	0.05	0.27	4.11	0.44
Number of cell layers <sup>§</sup>	7.49*	60.28***	6.85*	1.79	0.11	6.05*	0.66
Cell diameter <sup>§</sup>	5.51*	2.49	5.95*	1.51	0.16	0.71	0.24
Abundance of AM hyphae <sup>§§</sup>	3.66	2.50	41.60***	1.93	2.39	2.99	0.61
Arbuscles <sup>§§</sup>	0.39	5.84*	37.24***	0.44	1.33	4.20	0.59
Vesicles <sup>§§</sup>	2.58	0.35	8.94***	0.87	4.55*	0.01	0.28
DSE <sup>§§</sup>	1.49	0.04	7.33**	0.01	3.80*	4.79*	0.39
Root hairs <sup>§§</sup>	1.81	15.88***	3.88*	0.79	1.06	2.30	0.36

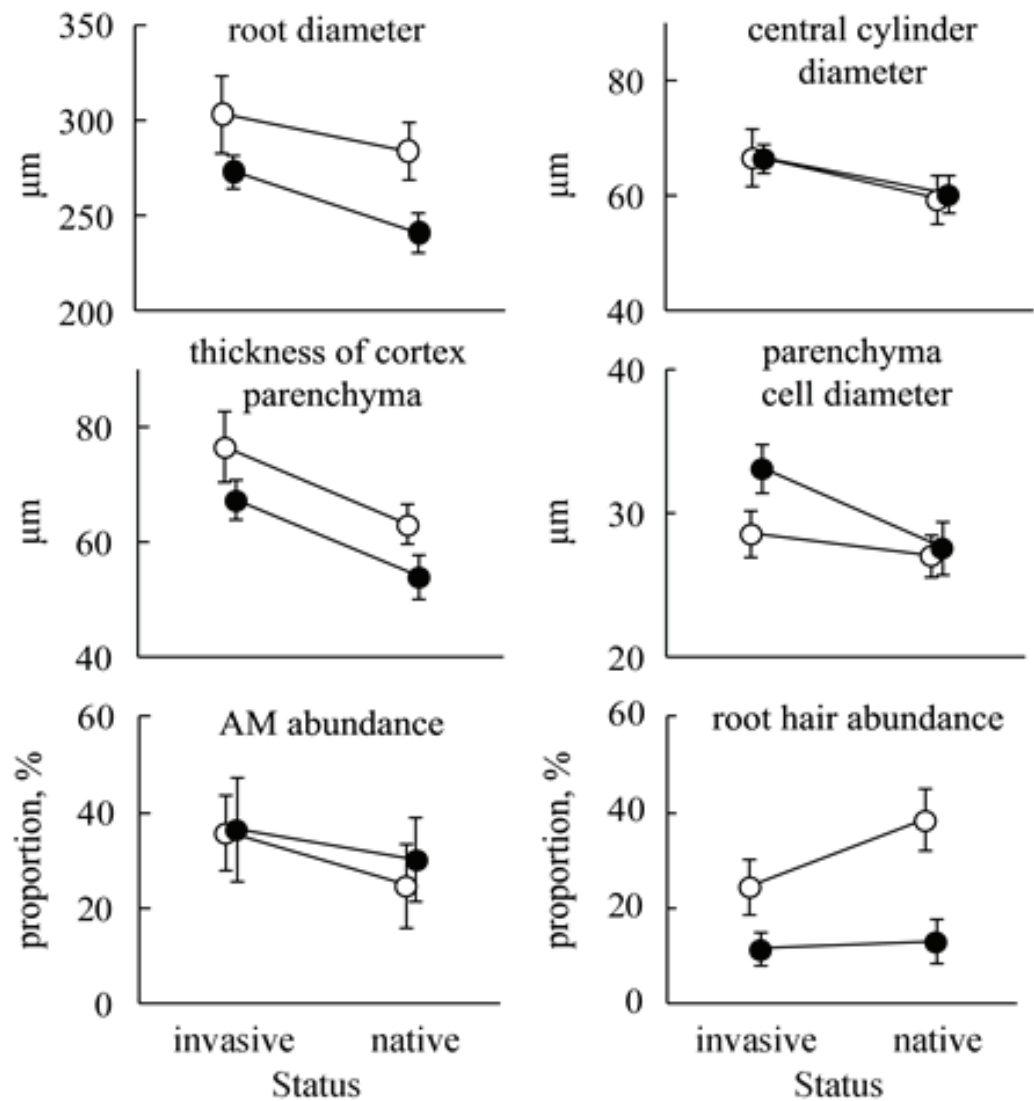
Source: Authors' own work.

Note: # dF = 1 for root diameter, central cylinder diameter and parameters of the cortex parenchyma; dF = 2 for the other parameters. The following transformations were made for ANOVA: <sup>§</sup> $x' = \ln(x + 1)$ ; <sup>§§</sup> $x' = \text{asin}(\text{sqrt}(x))$ . Statistical significance of  $F$ -values: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .  $F$ -values for the interaction of all three factors are not shown.  $R^2_{adj}$  –  $R^2$  adjusted for the full ANOVA model.

could optimize their water and nutrient uptake by substantially reducing cell respiration [5]. Moreover, the presence of large intercellular spaces in such parenchyma tissue could be an additional factor minimizing root respiration. The positive correlation between the size of parenchyma cells and growth rate seems to be universal, as it has been shown for photosynthetic tissues of fast-growing plants as well [2].

## 4. Conclusion

Our results suggest that the same or even lower thickness of the absorbing roots of invasive plants, shown previously might be inferred while comparing an integral value such as SRL; also, the plants studied differed not only in invasive status but also in their taxonomic position or habitat. When we compared both taxonomically and ecologically close species using a directly measured value, the diameter of absorbing roots with primary development, the roots of invasive plants turned out to have a thicker cortex parenchyma with larger cells. We suggest that this could contribute to invasion success by reducing root respiration, but data proving a higher growth rate in the roots of invasive species are still insufficient.



**Figure 1:** Comparison of the studied features of absorbing roots between invasive and native species of *Sapindaceae* (○) and *Rosaceae* (●) families. Mean values and standard errors are shown. **Source:** Authors' own work.

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