

Mycorrhiza Formation in Ash-Leaved Maple (*Acer negundo* L.) within the Urbanization Gradient

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Abstract—The development of *Acer negundo* L. mycorrhiza is analyzed on five sample plots in Yekaterinburg. The plots are organized in an urbanization gradient: from a forest park on natural soils to avenue plantings on sealed soils. It is found that *A. negundo* L. forms a typical arbuscular mycorrhiza in all habitats. However, as urbanization grows, the quantitative characteristics of mycorrhiza development decrease.

Keywords: *Acer negundo* L., invasive plants, mycorrhiza formation, arbuscular mycorrhiza, urbanization, sealed soils

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INTRODUCTION

The bioecological features of invasive plants were indicated by Mirkin and Naumova (2002) to include a set of traits associated primarily with the reproductive sphere, which is due to a worse knowledge of the properties of organization of vegetative organs in these species. However, with respect to invasive plants and plant invasions, there is at least one instrument for soil nutrition which is studied rather actively—the capability of mycorrhiza formation (Pendleton and Smith, 1983; Reinhart and Callaway, 2006; Stinson et al., 2006; Shah et al., 2009; Hempel et al., 2013). Plants with different types of mycorrhizae satisfy their needs in soil resources in diverse ways, so the ability of mycorrhiza formation is due to the functional properties of species, their positions in communities, and succession changes.

By analogy with ruderal species, the invasive plants that mainly adapt to disturbed habitats and show the features of R-strategists (Mirkin and Naumova, 2002) can be expected to have a decreased relationship with mycorrhizal fungi—namely, an increased share of nonmycorrhizal species (Pendleton and Smith, 1983; Stinson et al., 2006; Betekhtina and Veselkin, 2011; Hempel et al., 2013). We can also assume that invasive plants can have a strongly pronounced optionality of mycorrhizal interactions, i.e., the ability to enter into symbiosis with a varying closeness of interaction depending on external conditions. This property is known for many plants (Selivanov, 1981), and the optional mycorrhizal status is positively correlated

with the high activity of species at initial stages of successions (Veselkin, 2012a, 2012b).

A convenient object for checking the latter assumption is ash-leaved maple (*Acer negundo* L.), which is an invasive species and transformer in the Russian Federation (Vinogradova and Kuklina, 2012). Various biological properties of this species are actively studied (Efimova and Antonova, 2012; Antonova and Gnilyovskaya, 2013; Kostina et al., 2013). The species is known to form an arbuscular mycorrhiza in its native land in North America (Comas and Eissenstat, 2009; Zadworny and Eissenstat, 2011), in Bulgaria (Kovacs and Szigetvari, 2002), and within the territory of the Russian Federation (Lusnikova and Selivanov, 1974; Ivashkina and Loginova, 1981; Kryuger and Selivanov, 1989; Adamova, 2009). Meanwhile, ash-leaved maple was indicated for the territory of the former Soviet Union as a species with a variable capability of mycorrhiza formation, i.e., a species that is able to form arbuscular mycorrhizae or grow without them (Akhmetzhanova et al., 2012), like other species of the *Acer* genus, which are cited in the world summary of plants studied as regards the capability of mycorrhiza formation (Wang and Qiu, 2006).

The goal of the work is to study the properties of mycorrhiza formation in *Acer negundo* in the secondary range in the urbanization gradient. In order to evaluate the impact of changing environmental conditions on mycorrhizae of this species and, in particular, to test the hypothesis that it may form mycorrhizae optionally, we covered a wide range of habitats. The urbanization gradient means a series of habitats in



Fig. 1. Map of places where *Acer negundo* roots were sampled within the territory of Yekaterinburg (hexagons): (1) forest park; (2) intra-yard plantings; (3, 4) avenue plantings on artificially made grounds; (5) avenue plantings on urban grounds. The asterisk marks the historical center of Yekaterinburg.

which the degree of deviations of habitat conditions from the natural environment grows as a result of transformation under the impact of various human activities.

MATERIALS AND METHODS

Region. The samples of *A. negundo* roots were selected in the southern and southwestern parts of Yekaterinburg (Fig. 1), which is a large industrial city in the Middle Urals with an area of 50 000 ha and population of about 1.4 million residents. The territory of the city is strongly polluted (Sturman, 2008). In 2010–2012, atmospheric emissions amounted to 190 000–215 000 t, of which about 10 000 t were nitrogen compounds. The greatest contribution to atmospheric pollution (up to 85%) is made by motor vehicles (Sturman, 2008; *O sostoyanii...*, 2013). The average daily concentrations of NO_2 are up to 20–30 $\mu\text{g}/\text{m}^3$ in central regions and near major highways and 15–20 $\mu\text{g}/\text{m}^3$ in most of the residential areas (Antropov and Varaksin, 2011).

Sampling plots. Plots were selected so that they could characterize the urbanization gradient or gradient of growth in the total anthropogenic transformation of living conditions, including soil, ground cover,

and the atmosphere. In total, stands of trees at four conditionally distinguished urbanization stages were studied.

(1) The Southwestern Forest Park: the relief is the top of a flat slope; the soil is natural undisturbed sod-podzol or weakly disturbed (compacted); natural horizons are clearly tracked. The environment is a reed-bilberry pine forest; maple clumps have an artificial origin, but are actively naturally renewed.

(2) Intra-yard plantations: in courtyards of residential apartments; on a flat slope; the soil is deeply transformed anthropogenic (urbanozem) (the names of transformed soils are presented according to (Rysin, L.P. and Rysin, S.L., 2012) and strongly damaged by construction works; dense tree and shrub layers are absent; *A. negundo* plants were partially planted and partially are a result of natural renewal.

(3) Avenue roadside plantings on artificially made grounds on lawns, which separate the streets and sidewalks; two points are in the territory of the city. The relief is the middle parts of flat slopes; the soils are artificially made mineral grounds of different structure and a built-up highly fertile layer; shrubs and the living ground cover are fragmented.

(4) Avenue roadside plantings on screened grounds, i.e., soils sealed under asphalt or concrete pavement; the environment is a sidewalk and street; living ground cover is absent except for few *Plantago major* L. and *Taraxacum officinale* F.H. Wigg. near the bases of maple trunks. Samples of *A. negundo* roots were taken from the soil areas which were under asphalt for a long time and were uncovered when repairing a street.

Study of mycorrhiza. The samples of *A. negundo* roots were dug out in the first half of July 2012 from a depth of 5–15 cm from ten different places in each sampling plot. These samples or fragments formed ten independent sample groups. Only living roots of the two to three last orders were selected. The roots were fixed in 70% ethanol. The presence of mycorrhizae was established by light microscopy (Leica DM 5000B, Germany, $\times 100$ –200) after pre-maceration of roots in KOH and dyeing with blue aniline (Selivanov, 1981). Ten to 25 individual root fragments with a length of about 1 cm were analyzed in each sample (usually, 15 fragments). The abundance of mycorrhizal fungi was determined on a five-point scale (Selivanov, 1981).

Statistical analysis. The STATISTICA 6.0 program was used. The nonparametric criteria χ^2 and Kruskal–Wallis test (H) were used owing to the fact that the characteristics of the mycorrhiza formation activity (the parameters of the occurrence and abundance of mycorrhizal structures) were not originally expressed on quantitative scales. The work for the most part used individual one-centimeter root fragments as accounting units, in each of which the presence of aseptate hyphae, arbuscular mycorrhizae (specifically branching hyphae), and vesicles (blisters) was fixed. The calculation based on

the ratio between the amounts of microscope fields inhabited and not inhabited by fungi was additionally used to estimate the occurrence of mycorrhiza (the parameter F according to I.A. Selivanov, 1981) to ensure the comparability of original estimates with data of other authors.

RESULTS

In all the studied habitats, *A. negundo* forms arbuscular mycorrhizae, which do not differ from the descriptions presented for arbuscular mycorrhizae of other plants. Mycorrhizal roots usually have both the intracellular and extracellular mycelium. Arbuscular mycorrhizae, vesicles, and intracellular hyphae clusters are regularly found. Vesicles are quantitatively prevalent, but arbuscular mycorrhizae are represented in all habitats. The property of *A. negundo* is that fungal structures occur very seldom and are characterized by low abundance. In addition, the quantitative characteristics of mycorrhiza formation are strongly variable. Root samples within the same habitat, as well as roots in the same sample, are usually inhabited with fungi irregularly. Along with arbuscular mycorrhiza, roots were found to contain mycelium morphologically corresponding to dark septate endophytes.

The root areas that do not contain the mycelium of arbuscular fungi are prevalent in all habitats (Fig. 2a; in this case, a microscope field is an accounting unit). However, the share of visual fields with fungal structures decreases in the urbanization gradient from 32% in forest parks to 23–31% in intra-yard and roadside plantings on open grounds and to 19% on sealed grounds. The distinctions between a forest park and planting on sealed ground are statistically significant ($n_1 = n_2 = 750$; $dF = 1$; $\chi^2 = 37.99$; $P \ll 0.001$). The same regularity is also clear when individual one-centimeter root fragments are used as an accounting unit (Fig. 2b). In the forest park and intra-yard plantings, 69–71% of fragments are inhabited with fungi, and in the case of strong urbanization, the share of such roots decreases to 50–61%. The distinctions between the extreme variants (the forest park and plantings on sealed grounds) are insignificant ($n_1 = 157$; $n_2 = 110$; $dF = 1$; $\chi^2 = 2.79$; $P = 0.095$), but the distinctions between the forest park and avenue plantings on artificially made grounds are significant ($n_1 = 157$; $n_2 = 160–163$; $dF = 1$; $\chi^2 = 6.44–14.18$; $P = 0.002–0.011$).

The abundance of fungal structures (the sum of hyphae, arbuscular mycorrhiza, and vesicles) changes in the same direction as their occurrence: as the degree of urbanization grows, the abundance of mycorrhizal fungi decreases (Fig. 3a). This conclusion is statistically reliable ($H_{(4; N=777)} = 24.73$; $P < 0.001$). The medians of fungal abundance in all habitats are 0.1–0.4 points, but the ranges are high (from 0 to 2–4 points). In other words, fungi were encountered in some root areas in 25–75% of bark cells, but on average only in a few

cells. The abundance of fungi was particularly low in avenue plantings on artificial and sealed grounds.

The total decline in the abundance of mycorrhizal fungi mycelium in proportion to urbanization growth either is mainly due to the decreased occurrence of fungi, i.e., the presence of “empty samples” (root fragments which are not inhabited with fungi at all), or is caused by the strictly lower activity of the formation of fungal structures in salinized root fragments. In order to estimate the significance of these causes, we analyzed the values of the “local abundance” of fungi, i.e., the abundance of fungal structures in the root areas in which a fungus was represented at least in some form (Fig. 3b). The total regularity of the decrease in the “local abundance” of mycorrhizal structures in proportion to the growth in artificiality of conditions is also significant: $H_{(4; N=480)} = 29.08$; $P < 0.001$. The mean values of “local abundance” of fungi in all habitats are 2 points, with the exception of an avenue planting on sealed ground, where the mean value is 1 point. The upper thresholds of “local abundance” are 4–5 points. Consequently, the decrease in average abundance of fungal structures in roots of ash-leaved maple in proportion to urbanization growth is not a reaction that depends only on whether “empty samples” are taken into account or not.

DISCUSSION

The presented estimates indicate that arbuscular mycorrhizae in *A. negundo* are formed in the city of Yekaterinburg even in the extreme conditions of artificial soils that are imported sand-gravel ground. It is significant that, in any conditions, there were no samples, i.e., individual root areas, in which maple roots did not have mycorrhizal structures—vegetative hyphae, vesicles, and at least a few arbuscular mycorrhizae were discovered in each sample. As far as we can judge from our knowledge of the literature, the presented data for the first time demonstrate the possibility of mycorrhiza formation in ash-leaved maple in sealed grounds, i.e., in grounds which were sealed for a long time with almost full mutual isolation of the aboveground and underground spheres of urban ecosystems. Consequently, neither the transformed environment of abiotic factors, nor the isolation of roots from the flow of arbuscular fungal diaspores, nor the low content of organic substances in soil, nor the absence of most of typical soil biota components lead to the complete suppression of mycorrhizal formation. This permits one to reject the assumption about the possibility of optional mycorrhiza formation in *A. negundo* in the generative state. On the other hand, if the level of urbanization is high, mycorrhiza formation of the considered species is less successful in comparison with the relatively favorable conditions of a forest park. The trend toward the lower activity of mycorrhiza formation in the urbanization gradient is evident in the case of different accounting units being used.

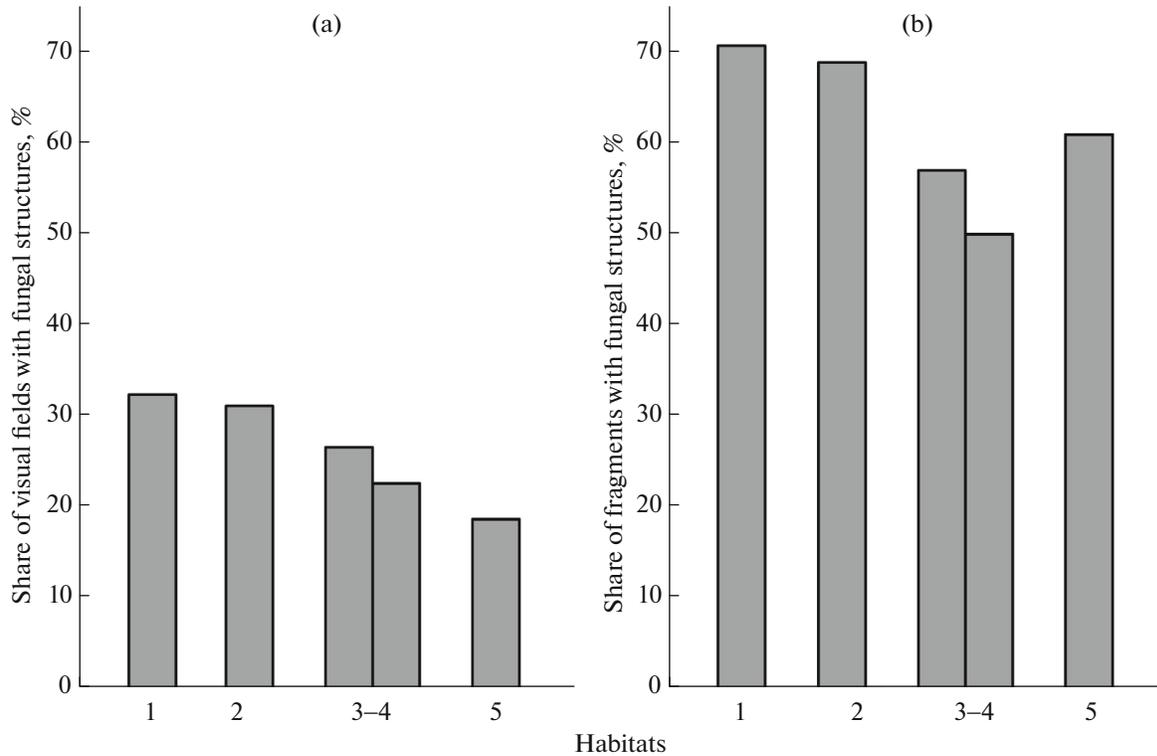


Fig. 2. Share of visual fields (a) and share of one-centimeter root fragments (b) with fungal structures in *Acer negundo* in the urbanization gradient. Here and in Fig. 3: in forest park (1), in intra-yard (2) and avenue plantings on artificially made grounds (3–4) and sealed grounds (5).

It is also clear that the decreased occurrence and abundance of fungal structures are reactions of the same type that indicate the decreased intensity of mycorrhiza formation in proportion to urbanization growth.

Consequently, the regulation of mycorrhiza formation in ash-leaved maple under the conditions of urbanization is manifested in the decreased quantitative parameters of mycorrhiza development, but breakdown of the formation of symbiosis does not take place.

In general, mycorrhizae of invasive plants and plants growing in urban conditions have not been studied enough. However, the weakening of mycorrhiza formation in *A. negundo* in proportion to urbanization growth which we have revealed seems to be a reaction common to many tree species (Bainard et al., 2011). It should be noted that herbaceous species are used in environmental research as model species of endomycorrhizal plants more often than tree species. They are known to have a decreased abundance of fungi in roots under different man-made stresses (Vosatka and Dodd, 1998; Trubina, 2002; Betekhtina and Kondratkov, 2003; Glazyrina et al., 2007; Betekhtina and Veselkin, 2011).

It is possible that the ability of *A. negundo* to regulate mycorrhiza formation within certain limits without abandoning it in extreme conditions is one of the functional properties that permits the species to com-

pete with local plants and be integrated into autochthonous ecosystems. This is supported by the general idea about the positive association of mycorrhiza formation and competitiveness in plants (Betekhtina and Veselkin, 2011). However, it should be emphasized that we have found no evidence that ectomycorrhizae can be formed in *A. negundo*. This corresponds to the published data on its mycorrhizal range, although ectomycorrhizae can be formed in other representatives of the *Acer* genus along with arbuscular mycorrhizae (Wang and Qiu, 2006). Consequently, *A. negundo* is similar in the range of symbiotic relationships to shrubs with a low competitiveness, such as *Lonicera*, *Ribes*, *Rosa*, *Rubus*, and *Viburnum*, for which ectomycorrhizae are not immanent. Competitively stronger shrubs (*Crataegus*, *Padus*, *Sorbus*) and trees (*Alnus*, *Salix*, *Populus*, and *Tilia*) feature ectomycorrhizae to a varied extent. This does not permit the specificity of symbiotic relationships in *A. negundo* to be directly attributed to its status of an invasive species.

Instead of a conclusion, it seems justified to formulate two questions. (1) Does the registered abundance of fungi differ in invasive and local species? It is desirable that a response be received with exclusion of possible obscuring effects related to geographical and ecological variability. (2) Does the level of interaction with mycorrhizal fungi change in the ontogeny of invasive plants? In other words, can the properties of mycorrhiza formation (for example,

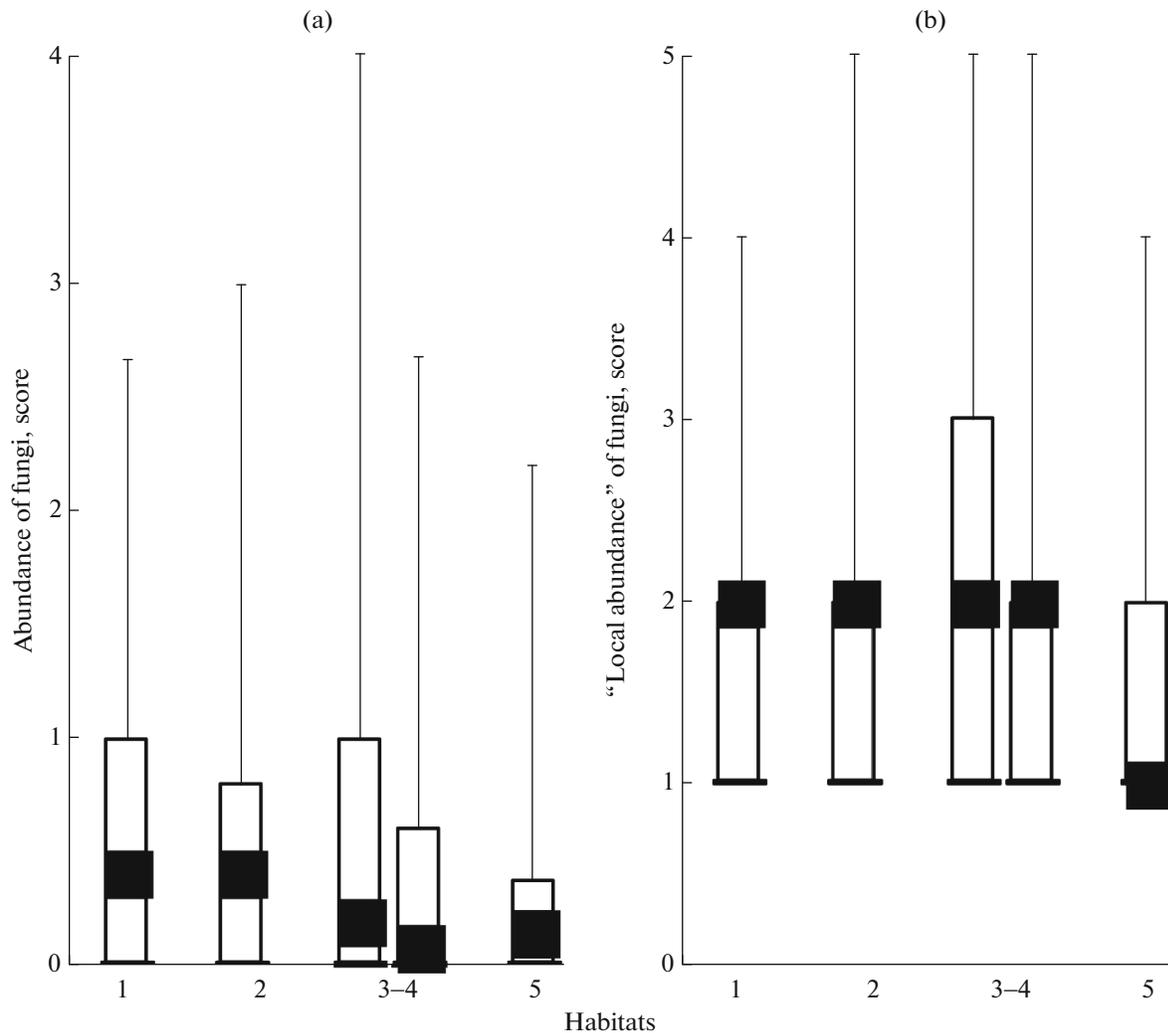


Fig. 3. Abundance (a) and “local abundance” (b) of fungal structures in *Acer negundo* roots in the urbanization gradient. Square is the median; rectangle is the upper and lower quartiles; lines show the absolute range.

optionality in the early ontogeny) be attracted to explain the success of a species in penetration and invasion into local communities or to explain the success of its settlement? An answer will help in understanding with what component of the invasive strategy mycorrhiza formation is connected more strongly: whether it is the ability of expansion or the level of competitiveness.

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