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ORIGINAL PAPER

Regulation of the growth of sprouting roots of black locust seedlings using root barrier panels

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Abstract How can we regulate an invasive alien species of high commercial value? Black locust (*Robinia pseudoacacia* L.) has a unique capacity for seed dispersal and high germination. Field surveys indicate that black locust increases its growing area with sprouting roots and the elongation of horizontal roots at a soil depth of 10 cm. Therefore, a method to regulate the development of horizontal roots could be effective in slowing the invasiveness of black locust. In this study, root barrier panels were tested to inhibit the growth of horizontal roots. Since it is labor intensive to observe the growth of roots in the field, it was investigated in a nursery setting. The decrease in secondary flush, an increase in yellowed leaflets, and the height in the seedlings were measured. Installing root barrier panels to a depth of 30 cm effectively inhibit the growth of horizontal roots of young black locust.

Keywords Black locust · Horizontal roots · Nutrients · Root barrier panel · Sprouting roots

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Introduction

Alien or introduced species often induce a plagioclimax in native vegetation communities (Mooney et al. 2005). These species were intended for economic and/or ecological purposes and often have potential industrial uses. However, they often escape from their initial planting and quickly occupy unexpected locations (Sakio 2009; Yin et al. 2014). How can these alien species be controlled in the field? Black locust (*Robinia pseudoacacia* L.) is an alien, deciduous broad-leaved species originally from the United States. It has a symbiotic relationship with nitrogen-fixing microbes (*Bradyrhizobium* spp.) that form root nodules (Mooney and Hobbs 2000; Murakami et al. 2002; Fujita et al. 2020) and allows the species to flourish on infertile soils in open sites (Koike et al. 2009; Kitaoka et al. 2022). As a result, black locust is commonly used for the afforestation of degraded lands and for forest plantations and as shade trees in urban environments (Maekawa and Nakagoshi 1996; Qiu et al. 2010; Masaka et al. 2013; Yin et al. 2014; Schwärzel et al. 2018).

Black locust can invade other locations from where it was initially planted because of its robust root sprouting (D'Antonio and Mahall 1991). It also has wide seed dispersal, effective germination (Masaka and Yamada 2009; Karaki et al. 2012; Watanabe et al. 2014; Nicolescu et al.

2020), wide spread root sprouts that develop from horizontal roots (Gyokusen et al. 1991; Fukuda et al. 2005; Zhang et al. 2006), and vegetative reproduction from root pieces (Yin et al. 2014). With its rapid growth, black locust suppresses other species and crowds out native vegetation (Maekawa and Nakagoshi 1997a, b; Muranaka et al. 2005; Nicolescu et al. 2020). It has, therefore, been recognized as an invasive species under the Invasive Alien Species Act in Japan (Masaka and Yamada 2009; Ministry of Environment of Japan 2004). However, a strategy to control the encroachment of black locust into native vegetation has not been fully established, which is partly due to limited information on its growth and sprouting characteristics, and the lack of practical studies on techniques to stop its spread.

It is important to develop simple, low-cost methods to manage this species for sustainable use (Muranaka et al. 2005; Sakio 2009; Yin et al. 2014; Nicolescu et al. 2020). However, little is known about its growth habits in invading disturbed areas such as forest edges and slopes created by landslides or road construction (Sakio 2009; Xu et al. 2009; Yin et al. 2014). Roadsides are a common invasion route of this species (Morimoto et al. 2010), and its establishment negatively affects native vegetation (Mooney and Hobbs 2000; Nicolescu et al. 2020).

The use of root barrier panels can effectively prevent the spread of plants with elongated horizontal roots or rhizomes (Morgenroth 2008; Mullaney et al. 2015). Root barrier panels have also been effective in inhibiting root growth of subterranean stems of moso bamboo (*Phyllostachys pubescens* (Carrière) J. Houz.) (Okayama Prefecture 2003; Linvill et al., 2012), as suggested by Itô and Hino (2007). However, there is little information about the effect of root barrier panels in inhibiting sprouting and horizontal roots of woody species such as black locust (Sakio 2009; Eshel and Beeckman 2013; Nicolescu et al. 2020).

Although roots of woody species display various distribution patterns, they can be roughly classified into shallow-rooted and deep-rooted (Karizumi 2010; Eshel and Beeckman 2013; Hirano et al. 2020). Knowledge of a plant's root system is needed to determine the value of root barrier panels in inhibiting the elongation of horizontal roots. Therefore, in this study the effect of root barrier panels in both field observations and nursery experiments were investigated. After the panels were set, root growth dynamics were observed to determine their effect on root growth (Zhang et al. 2006; Eshel and Beeckman 2013; Masaka et al. 2013).

It was hypothesized that the inhibiting effects of the panels on horizontal roots would suppress aboveground growth, such as slower shoot elongation and weak development of secondary flush, and lead to physiological imbalances in leaves by limiting nutrient and water absorption by the roots (Chapin III et al. 1990; Sato 1995; Qu et al. 2003; Choi et al. 2007). The objectives of the study were to determine the effects of root barrier panels on the growth of horizontal roots and shoots in black locust seedlings (Iwai et al. 1987). Two experiments were carried out: (1) field surveys for assessing the sprouting of roots in three field stations; and (2) nursery experiments to examine the effect of panels on the regulation of root sprouts under uniform conditions (light and soil).

Materials and methods

Plant material and study sites

The horizontal development of black locust root systems were studied in secondary forests at three spatially independent Hokkaido University Forests: (1) the nursery of the Sapporo Experimental Forest (SEF) (43°06′ N, 141°20′ E) with recent climatic data via Agathokleous et al. (2022a, b); (2) the forest compartment "Utanai No. 17" of the Nakagawa Experimental Forest (NEF) (44°52′ N, 42°04′ E); and 3) along the "Furusato Forest Road" in the Teshio Experimental Forest (TEF) (44°55′ N, 141°59′ E) of the Field Science Center for Northern Biosphere (FSC) of Hokkaido University.

The dominant vegetation in the SEF is oak (*Quercus mongolica* Fisch. ex Ledeb. var. *crispula* (Blume) H.Ohashi), with the ground vegetation mainly perennial herbs (*Helianthus tuberosus* L. and *Rudbeckia laciniata* L.). In the NEF, the dominant woody species are black locust and white birch (*Betula platyphylla* Sukaczev var. *japonica* (Miq.) H.Hara), mixed with other woody species such as cherry (*Prunus ssiori* F.Schmidt) and willow (*Salix bakko* Kimura). The ground vegetation and on the forest road sides is mainly dwarf bamboo (*Sasa senanensis* (Franch. et Sav.) Rehder). In the TEF, the dominant woody species is white birch mixed with oak and willow, and ground vegetation mainly a large butterbur (*Petasites japonicus* (Siebold et Zucc.) Maxim. subsp. *Ggiganteus* (G. Nicholson) Kitam.). The study sites of the NEF were not uniform because the forest is mountainous with few flat rocky areas. The root distribution of black locust saplings < 3 m in height were examined. Black locust seedlings at the TEF and NEF sites were planted to stop erosion (Morimoto et al. 2010).

The depth of root distribution was first examined by exposing the roots in the field using a hand trowel. A Yamanaka soil-hardness (YSH) tester (Fujiwara-ss Co. Ltd, Tokyo, Japan) was used to measure soil hardness at depths of 10, 20, and 30 cm. Our preliminary survey suggests that the horizontal roots of black locust cannot grow in hard soil layers that are 20 – 30 cm thick, such as heavy clay soil (Pseudogley or Stagnosol at the TEF and NEF sites) below a depth of 30 cm.

The Yamanaka soil-hardness (YSH) tester index is (Nakatsu et al. 2004): between 11 – 20 mm almost no root growth inhibited; 20 – 24 mm root growth of some plant species inhibited; 24 – 27 mm root growth of most species inhibited, and 27 mm > root growth totally inhibited. Three samples from different points were taken from the surface to between 30 – 40 cm under the litter layer at the TEF and NEF and 30 – 50 cm at the SEF to confirm soil physical traits.

Survey of root sprouts developing from horizontal roots

All roots originating from the seedling trunks were uncovered as far as possible using a hand trowel so as not to destroy horizontal roots. Although each sapling appeared to be a separate individual with a single trunk, it could also be a root sprout connected by horizontal roots to others. For clonal plants that use this strategy to propagate, each sapling is a ramet, and a group of ramets (= aboveground parts) connected by horizontal roots is a genet. The genet was considered an individual.

The vertical distribution of the horizontal roots was investigated. A horizontal root is defined as a root developing from the bottom part of the trunk at an angle of less than 20 degrees from the soil surface. The number of ramets of black locust saplings investigated was 38 in the SEF, 16 in the NEF, and 18 in the TEF. The size of the saplings was between 2 and 4 m in height (root collar diameter ranged from 4.6 to 5.8 cm). The number of ramets emerging from horizontal roots were counted in each soil layer from 0 to 10, 10 – 20, 20 – 30, and 30 – 50 cm. Three soil samples from different points at each site were taken from the surface to a depth between 30 and 40 cm beneath the litter layer at the TEF and NEF and 30 – 50 cm at the SEF to confirm soil physicochemical traits. At the TEF and NEF sites, surveys were not possible at depths below 30 – 40 cm because of the combination of heavy clay soil and serpentine soil. At these soil depths in the serpentine layer, almost no roots are found (Matsunami et al. 2009).

Chemical analyses

Nitrogen (N), calcium (Ca), potassium (K), magnesium (Mg), and phosphorus (P) were analyzed. For Ca, K, and Mg, the samples were dissolved in nitric-chloric perchloric acid (Japanese Society of Soil Science and Plant Nutrition 1990). The solutions were then combined with 50 ml deionized water measured by inductively coupled plasma spectroscopy (IRIS) (Jarrell-ash, Franklin, MA). Phosphorous was analyzed by the Bray II method (e.g., Kayama et al. 2002). Nitrogen in leaves was determined with a N–C analyzer (NC-900, Shimadzu, Kyoto, Japan).

Nursery experiments

Plant material and study site

To provide a uniform growth environment and minimize transplanting effects, thirty-six 3-year-old seedlings, cut to a uniform size (aboveground and roots 20 cm in length), were planted in the study site in early June 2007. Preliminary experiments found that black locust has extremely high recovering capacity after root cutting (Matsunami et al. 2009). Root collar diameter of the seedlings was (0.8 ± 0.1) cm. The seedlings were obtained from the Hokkaido Horti-Afforestation Center Co. Ltd., Sapporo City. The study site was located at the experimental nursery of the SEF (43°06′ N; 141°20′ E) of the FSC of Hokkaido University. Based on the FAO-UNESCO soil categorization, the soil type of the SEF site at 40 cm was Dystric Cambisols (brown forest soil (Japanese Society of Soil Science and Plant Nutrirtion 1997), mixed with heavy clay at 40 – 80 cm. The experiment was conducted in a nursery field where the soil was well tiled to uniform physical conditions.

Eight soil samples were taken from 30 – 50 cm at each site to confirm soil chemical traits. The seedlings were planted 3 m apart a depth of 10 cm in a 10-cm hole. A 10-cm guide pole was used to ensure uniform positioning of each seedling. The aboveground performance of the seedlings was recorded from late May 2008 to early October 2008 at one-month intervals during the growing season, and the roots were sampled in early November when all leaves were shed. At the end of the experiment, the tallest seedling was about 3.2 m with a basal diameter of 6.4 cm.

Placement depth of root barrier panels

The field survey showed that black locust produces wide-spreading, horizontal roots at a depth of 0 – 10 cm (see below for details). The panels, made of polyvinyl chloride (PVC) were placed around the seedlings in mid-June 2007 at depths of 10, 20, and 30 cm. Holes were dug with a pickaxe, and the panels installed to form a 90 cm × 90 cm square around the seedlings. The size of the enclosure to allow for healthy horizontal root growth was based on our field survey. The 90 cm × 90 cm control area had no panels installed, but the 10 cm depth intervals were maintained because few roots were previously found in deeper parts of the soil (Matsunami et al. 2009). Soil moisture content (fresh soil – dry soil / fresh soil %) among the panel treatments in late July were 36.8 ± 2.3%. There was no statistical difference between the control and the treatments (10 – 30 cm).

Treatments with different root panel depths were placed randomly in the nursery. The growth of individual seedlings was also investigated, discounting seedlings infected with powdery mildew (*Microsphaera alphitoides* Grifin

& Maubl.) and one broken by the weight of snow. Eight seedlings were studied for all the treatments. Most of the seedlings were difficult to dig up by hand. In the first year, two seedlings were infected by powdery mildew and were immediately eliminated. Branches infected by powdery mildew were also eliminated from the study, but the main stems were kept maintaining plant density.

Aboveground growth parameters

The effects of the root barrier panels on root growth were determined by monitoring the aboveground performance of the seedlings. The seasonal change in the length of the current shoot at 1.0 m height from the crown was recorded from late May to late September to offset any effects of transplanting. In each treatment, the number of individuals showing secondary flush from the current shoot and a yellowed leaflet near the bottom of the main stem was observed and measured with a SPAD-502 chlorophyll meter (Minolta, Tokyo, Japan). The pinnate compound leaves were sampled, including yellowed leaflets near the lower part of the main stem. A *yellowed leaflet* was defined as having more than 80% of the leaflet blade yellow.

In addition, the nutrient levels in the leaflets were examined to identify the status of the green and yellowed leaves. When the leaves started to change color from mid- to late-September, yellowed leaflets were collected for nutrient analysis. Green leaves were collected in mid-August. After these leaflets were dried at 70 °C for three days, they were powdered in a blender for chemical analyses. The concentrations of N, P, K, Ca, and Mg were calculated on a dry mass basis. No difference was found in nutrient conditions in green and yellow leaflets among treatments, therefore they were pooled (green vs. yellow) for nutrient analysis. The details of nutrient analyses were the same as in the chemical analyses section.

Belowground: root growth parameters

To measure the elongation of horizontal roots of seedlings grown in the SEF nursery, all roots were dug up in early November after all leaves were shed. To quantify the effectiveness of the panels to inhibit the elongation of horizontal roots, the number of roots that had grown under the panels were used as the percentage of the total number of roots:

$$PIR(\%) = NIR/NAR \times 100 \quad (1)$$

where *PIR* means the percentage (%) of roots invading soil under root barrier panels, *NIR* indicates the number of roots invading soil under root barrier panels, and *NAR* means the number of all roots.

To further illustrate root morphology and function, the following characteristics were measured:

The number of horizontal roots (10 – 15 mm in diameter) which escaped from the root barrier panels at different depths and the same size as roots in the control;
The number of nodules per unit length (about 10 cm) of the 2nd order roots positioned between 3 and 13 cm (about 10 cm) from the tip of the horizontal root; and,
The maximum length of the horizontal root dug from the soil.

The dry weight (after drying at 65 °C for 7 d) of the roots which escaped from the 90 cm × 90 cm panel enclosure installed.

Statistical analysis

All statistical analyses were performed using the R language (R Core Team 2019). Differences between the three treatments were evaluated using the generalized linear model (GLM) from the "stat" package (Crawley 2005; R Core Team 2019). The response variables were the parameters of the aboveground and belowground growth. The explanatory variables were the depths of the panels. If there were any significant effects of the depth of the panels on shoot elongation, on the percentage of roots that grew under the root barrier panels, or leaf nutrient concentrations in the individuals with pale green and yellowed leaves, multiple pair-wise comparisons were conducted to see how the depth of each panel influenced shoot elongation, root elongation, and the nutrient concentration of leaves.

The *P*-values were adjusted using Holm's modified Bonferroni procedure. The significance level was set at $P < 0.05$. For the statistical analysis of soil characters (Table 1), multiple pair-wise comparisons were carried out within the same soil depth. The *P*-values were adjusted using Holm's modified Bonferroni procedure in the same way as the analysis of shoot and root growth.

Results

Presence of root sprouts developing from horizontal roots

Thirty-eight ramets were surveyed in the SEF from eight genets after digging up the aboveground portion of the seedling (Fig. 1). A total of 13 "apparent" stems (ramets) were surveyed. However, three genets had three ramets each, and three "apparent" stems were unconnected by a horizontal root in the NEF. In the TEF, 17 ramets originated from six genets along the Furusato Forest Road.

Table 1 Soil nutrient concentration (mg g^{-1}), C/N ratio and Yamanaka soil-hardness (YSH) tester index (mm) at each depth on three study sites

Site	Depth (cm) or item (unit)													
	10 cm							*20 cm*						
	N (mg g^{-1})	P*10^{-3} (mg g^{-1})	K *10^{-1} (mg g^{-1})	Ca (mg g^{-1})	Mg*10^{-1} (mg g^{-1})	C/N*10	YSH*10 (mm)	N (mg g^{-1})	P*10^{-3} (mg g^{-1})	K *10^{-1} (mg g^{-1})	Ca (mg g^{-1})	Mg*10^{-1} (mg g^{-1})	C/N*10	YSH*10 (mm)
TEF	1.6 ± 0.2b	2.3 ± 0.1a	1.5 ± 0.1b	3.2 ± 0.2b	2.3 ± 0.2b	1.3 ± 0.1a	1.6 ± 0.2a	2.2 ± 0.1a	2.3 ± 0.1a	1.6 ± 0.1ab	3.1 ± 0.2a	2.3 ± 0.2a	1.2 ± 0.1b	1.9 ± 0.1a
NEF	1.6 ± 0.1b	2.2 ± 0.3a	1.86 ± 0.1a	3.5 ± 0.1a	2.9 ± 0.1a	1.3 ± 0.1a	1.8 ± 0.2a	2.2 ± 0.1a	2.2 ± 0.1a	1.4 ± 0.1b	3.3 ± 0.1a	2.6 ± 0.1a	1.4 ± 0.1a	1.3 ± 0.1b
NEF	2.2 ± 0.1a	2.4 ± 0.1a	1.8 ± 0.1a	3.1 ± 0.1b	2.2 ± 0.1b	1.2 ± 0.1a	1.3 ± 0.1a	2.2 ± 0.2a	2.3 ± 0.1a	1.7 ± 0.1a	3.3 ± 0.1a	2.1 ± 0.1b	1.3 ± 0.1b	1.3 ± 0.1b

Site	Depth (cm) or item (unit)						
	30 cm						
	N (mg g^{-1})	P*10^{-3} (mg g^{-1})	K *10^{-1} (mg g^{-1})	Ca (mg g^{-1})	Mg*10^{-1} (mg g^{-1})	C/N*10	YSH*10 (mm)
TEF	1.4 ± 0.1b	1.8 ± 0.1a	1.5 ± 0.1a	3.2 ± 0.1a	3.7 ± 0.2a	1.6 ± 0.1a	2.3 ± 0.4a
NEF	1.3 ± 0.1b	1.7 ± 0.1a	1.6 ± 0.1a	3.4 ± 0.1a	3.7 ± 0.1a	1.6 ± 0.1a	2.4 ± 0.3a
NEF	2.3 ± 0.1a	1.7 ± 0.1a	1.4 ± 0.1b	3.3 ± 0.1a	2.2 ± 0.1b	1.5 ± 0.2a	1.9 ± 0.1a

The abbreviations indicate TEF: Teshio Experimental Forests, NEF: Nakagawa Experimental Forests, SEF: Sapporo Experimental Forests. All of the values indicate means ± standard deviation (SD), and the different alphabetical small letters (a, b) indicate the significant differences ($p < 0.05$) following the Holm's modified Bonferroni procedure tests which applied at the same depth among three sites

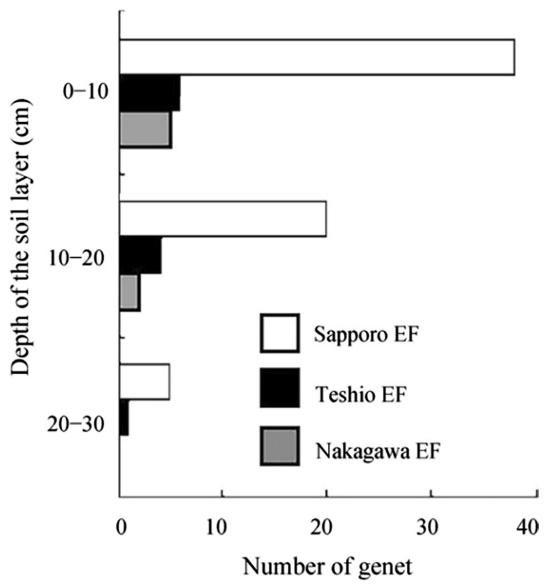

Fig. 1 The number of genets in each soil depth that horizontal roots of Black locust distribute. Vertical distribution of horizontal roots of black locust grown in the three study sites (Teshio Experimental Forest: TEF, Nakagawa Experiment Forest: NEF, Sapporo Experimental Forest: SEF). The number of gents was referred to in the main text

Field surveys: soil condition and vertical distribution of roots

Except for P, soil nutrients and physical properties (YSH) were significantly different among the sites at each soil depth (Table 1). SEF showed significantly higher N levels at both 10 cm ($P<0.05$) and 30 cm ($P<0.05$) depths. K concentration varied at each depth, and SEF had the lowest K at 30 cm ($P<0.01$). There were significant differences in Ca levels at the 10-cm depth in NEF but no differences at both 20- cm and 30- cm depths. For Mg, except at the 10- cm depth, SEF showed significantly lower values in both 20 cm and 30 cm depths. Significant differences among the sites for C/N were observed only at 20- cm depth; there were no differences in both 10 cm and 30 cm depths. No statistical difference was detected in soil hardness except in the 10 – 20 cm layer. Between 10 – 20 cm, soil hardness was significantly smaller at the NEF sites than at the TEF sites ($P<0.05$, Table1). The YSH of the SEF site was slightly smaller compared to the NEF and TEF sites at both 0 – 10 cm and 20 – 30 cm depths, but not significantly. For all three sites at 30 – 50 cm, the YSH was 27 mm, which suggests that root growth would be difficult.

Nursery experiments

Aboveground growth

Mean shoot length (cm) at the end of the growing season was 96.2 ± 18.2, 92.0 ± 21.0, 92.3 ± 23.1, and 60.1 ± 4.2 at panel depths of 0 (control), 10, 20, and 30 cm, respectively. Shoot growth started in late May and varied among the treatments by mid-June (Fig. 2A). At the end of the experiment, growth was largest with the 10- and 20-cm panels. Shoot growth of saplings grown with the 20-cm panel sharply increased in late July to mid-August and reached the level of the 10-cm panel treatment. After late August, shoot growth markedly decreased in individuals within the 30-cm panel. By late September, shoot elongation in the 30-cm panel treatment was significantly less than the other two treatments and the control, at the end of September, it was the lowest among the three treatments (Fig. 2A, $P < 0.05$).

The SPAD value was almost constant at 40 – 42 (green) from early June to late August but sharply decreased to 35 – 37 mid-September (Fig. 2B). The percentage of individuals with yellowed leaflets in the lower parts of the crown decreased with increasing depth of the root barrier panel. Differences in SPAD values of the treatments were detected in timing of the change in leaf color. SPAD value mid-late September was found 28 – 32 for the control and the 10 cm treatment, 28 – 30 (yellow green) for the 20 cm treatment, and 19 – 23 (yellow) for the 30 cm treatment. From late May to early June, the SPAD value could not be obtained because the leaves were too soft for measurement.

Nutrient conditions of the leaves from the three treatments mid- to late- September, when visibly distinguishable color in the lower crown was seen, are shown in Table 2. There was no difference in N, C/N, K between pale green and yellow leaflets. In contrast, concentrations of P and Mg were higher in green leaflets than in yellow ones ($P<0.05$). Ca concentration of green leaves was lower than that of yellow ones ($P<0.01$).

The percentage of individuals forming a secondary leaf flush was, on average, (100%, 60% and 20% for control–no root barrier panel, 10- and 20-, and 30-cm treatments), respectively (Fig. 3). It was 60% in the 10 and 20-cm treatment while it was 40% in the 30-cm treatment.

Effectiveness of the root barrier panels

The number of roots growing under the panels was 7.3 ± 0.7, 9.2 ± 5.1, 7.4 ± 2.6, and 3.6 ± 1.5 for the 10-, 20-, and 30-cm treatments, respectively (Fig. 4). The 30-cm root barrier panel resulted in significantly shorter horizontal roots than the 10- and 20 cm panels ($P<0.05$).

Discussion

The growth and physiological activities of black locust demonstrate a clear link between above- and belowground growth (Figs. 2, 3 and 4). Before the nursery experiments, the target depth for panel placement was determined by a

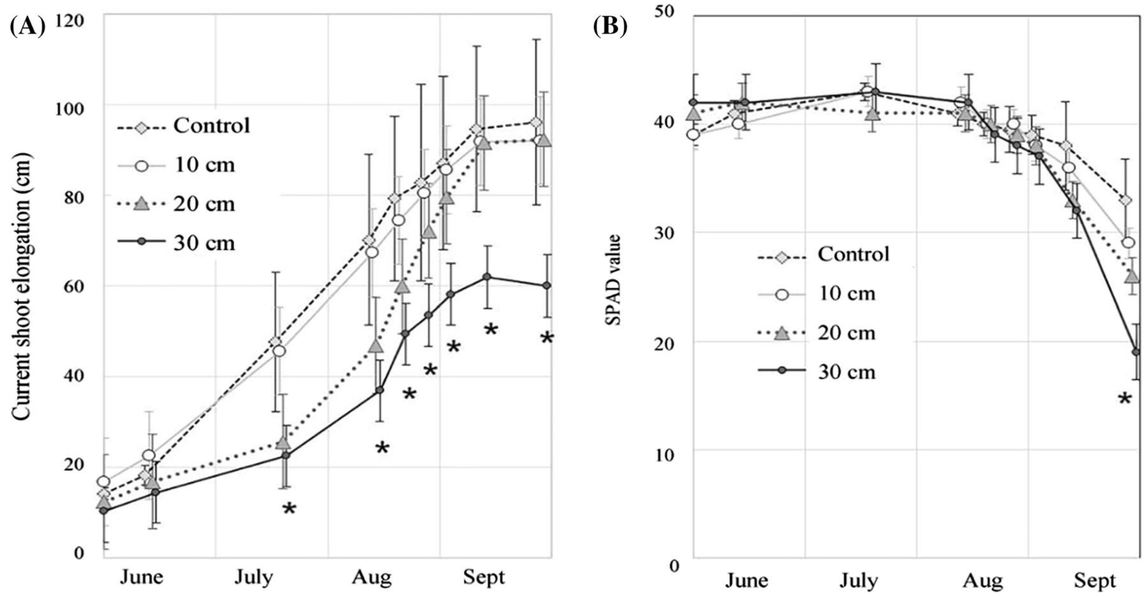

Fig. 2 **A** Seasonal change of average of current shoot elongation under each treatment by the different depth of root barrier panel on nursery experiments. **B** Seasonal changes in the SPAD value. Error bar shows standard deviation and asterisks on the 30 cm treatments indicate the significant difference ($P < 0.05$) from the control, and the absences of asterisks at the 30 cm treatments indicate the difference from control were non-significant

Table 2 Leaf nutrient concentrations and C/N ratios in seedlings with pale green or yellowed leaves

Nutrition (mg g^{-1})	Green leaf	Yellow leaf	Statistical test
N	45.8 ± 4.2	40.9 ± 3.8	n.s
C/N	11.2 ± 0.9	12.1 ± 0.3	n.s
P	2.6 ± 0.3	0.2 ± 0.3	$P < 0.05$
K	12.8 ± 2.4	12.1 ± 4.1	n.s
Ca	21.9 ± 5.0	38.9 ± 4.5	$P < 0.01$
Mg	13.9 ± 0.5	9.2 ± 0.5	$P < 0.05$

All the values indicate means ± standard deviation (SD); significant differences ($P < 0.05$) applied to between green and yellowed leaves

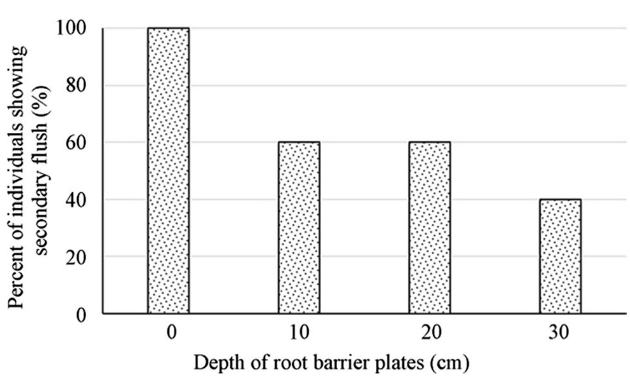

Fig. 3 The percentage of individuals detecting secondary flush. The replications are as follow; control ($n = 6$), 10 cm treatment ($n = 5$), 20 cm treatment ($n = 6$), 30 cm treatment ($n = 8$)

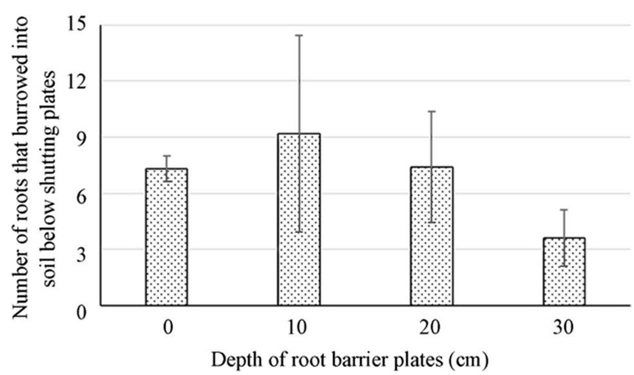

Fig. 4 The percentage of individuals detecting yellowed leaflets. The replications are as follow; Control (no panel: 0 cm, $n = 6$), 10 cm treatment ($n = 5$), 20 cm treatment ($n = 6$), 30 cm treatment ($n = 8$). Vertical bars in figure mean the standard deviation and different alphabets indicate statistical significance at $P < 0.05$

field survey. Most horizontal roots are found in the upper 10 cm soil layer (Fig. 1). The same pattern has been reported for black locust throughout Japan, i.e., the species develops shallow, wide-spread horizontal root networks to a 10 cm depth (Gyokusen et al. 1991; Sakio 2003; Hoshino 2006). Based on these findings, root barrier panels are an effective method to regulate horizontal root growth of black locust.

Soil hardness in the upper10 cm in the three study sites was 13 – 15 mm on the YSH index, which allows root growth and development of many species (Nakatsu et al. 2004). This may be attributed to the abundance of decomposed organic matter (e.g., from litterfall) by microbes that prefer shallow soil depths (Paul 2015). Black locust, with its predominantly shallow roots, efficiently acquires nutrition and can grow under nitrogen-poor conditions because nitrogen-fixing microbes in the root nodules prefer shallow soil (de Kroon and Visser 2003), as long as P is not limiting (Röhm and Werner 1991).

Consideration should also be given to root distribution patterns by examining soil physicochemical conditions (Table 1 and Fig. 1). Some tree species cannot develop a deep root system when soil hardness is 20–24 mm, and most species cannot develop adequate roots if the YSH value is > 25 mm (Nakatsu et al. 2004). The YSH was > 27 mm at the TEF and NEF sites, and at the SEF site at 30-cm depth, which indicated that most roots could not invade the hard soil layers.

Black locust has a symbiotic relationship with *Rhizobia* spp., nitrogen-fixing microbes. Nitrogen plays an essential role in photosynthesis and increases root absorption of Mg and Ca (Larcher 2003; Kitaoka et al. 2022). High rates of photosynthesis are also supported by phosphorous with regulating the microbes (Röhm and Werner 1991; Choi et al. 2017; Fujita et al. 2020). Roots spread widely in the soil to obtain nutrients and water and require oxygen for respiration (Reader et al. 1993; Mao et al. 2014; Schulze et al. 2019), which are more readily available at shallow depths. A similar situation was also found in harsh environmental conditions with shallow soils of the Loess Plateau in China, where the essential role of P was for increasing activities of symbiotic microbes (Qiu et al. 2010; Yin et al. 2014).

The 30 cm root barrier panel suppressed above- and belowground growth of black locust seedlings by the end of the experiment. With increasing depth of the panels, there was an apparent reduction in aboveground growth 40 days after planting, demonstrated by the length of the current shoot (Fig. 2A), and the small number of shoots with a second flush (Fig. 3). The suppression of root barrier panels on shoot development also occurred in the 20- and 30-cm treatments. On the other hand, horizontal roots went under the root barrier panels with the second significant growth of the current shoot 90 days after planting in the 20 cm treatment (Fig. 2A).

As expected from field surveys, the percentage of roots that grew under the panels was smaller with the 30 cm treatment (Fig. 4). The restriction of root growth was significant with the 30 cm panel for the number and weight of the roots not blocked and the maximum root length.

Once the horizontal roots reached the panels, they coiled and turned in different directions. Moreover, the number of horizontal roots increased from the main stem in the area enclosed within the panels, which may be attributed to the roots compensating for lack of development (Eshel and Beeckman 2013). As a result, the number of roots that grew beyond the 30 cm panel was likely smaller than that of the 10- and 20-cm panel treatments.

Based on these results, it is speculated that the root barrier panels suppress the elongation of horizontal roots, resulting in insufficient shoot growth and lower chlorophyll causing leaf yellowing (Fig. 2). Further, it is possible that restricting horizontal root growth interfered with the absorption of Mg (Table 2), a key element in chlorophyll production (Larcher 2003; Schulze et al. 2019). Yellowed leaves might also show suppression of shoot development due to the restriction of root system. A reason why the black locust seedlings lacked sufficient nutrients for healthy growth may be the high density of roots in a restricted space causing root-wrapping and depleting the space's nutrients (Koike et al. 2009) by the panels. In addition, re-translocation of N was very limited while that of P was high, which may be due to the symbioses with N-fixing microbes (Schulze et al. 2019).

Moreover, roots extending beyond the root barrier panels might absorb nutrients from inherently poor soil if the concentration of nutrients decreases with increasing depth. These results suggest that the growth conditions of the shoot are a good indicator of the effects of the panels in inhibiting the elongation of horizontal roots. As the result of source-sink balance, the reduction in aboveground growth would be strongly correlated with the reduction of root growth (Chapin III et al. 1990; Zhang et al. 2006).

Field observations revealed that most horizontal roots were distributed in the upper 10 cm soil layer (Fig. 1). Setting root barrier panels to a depth of 30 cm reduced horizontal root elongation by 85%. However, black locust can extend horizontal roots beneath the panels at < 30 cm and can elongate roots vertically in response to the panels. Therefore, to better inhibit horizontal roots, it is necessary to insert the panels deeper than 30 cm, as suggested by (Sato 1995) for several tree species. Further studies will be needed to examine a larger number of specimens of black locust with deeper root barrier panels to attain more information on root development patterns related to depth.

Soil physical characteristics, such as hardness, moisture content, and water flow also affect root growth. However, this study focused on the effects of different depths of the root barrier panel on shoot and root growth. Further study of the seasonal changes of soil moisture content and with the installation of barrier panels is needed.

From an example of bamboo plantation, mini-bulldozers and other heavy machinery were used to install root barrier panels over a large area. The installation of the panels disturbs the forest ecosystem will affect the root growth of other local species. Considering these ecological aspects, installing root barrier panels at the time of planting would be labor saving.

Conclusion

This study results in the following conclusions: (1) aboveground growth and development of black locust seedlings with root barrier panels to restrict their roots is a valid way to study the growth of roots; (2) aboveground growth was suppressed by the root barrier panels; and, (3) given the high plasticity of roots, the root barrier panels are a valuable way to regulate black locust seedlings in forest soils.

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Authors contribution Dr. Satoshi Kitaoka wrote the main elements of the paper. Mr. Shiro Matsunami conducted the field, nursery, and chemical experiments and worked on a draft of the paper. Dr. Makoto Kobayashi conducted the field studies and edited the paper. Dr. Saki Fujita conducted the literature research, Dr. Toshiyuki Hirata provided nitrogen data and technical assistance, and Dr. Takayoshi Koike supervised the research plan and nursery studies with Mr. S. Matsunami. Dr. M. Kobayashi secured funding for the study.

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