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Regulating the growth black locust seedlings by inhibiting the sprouting roots; An examination of effectiveness of root-growth regulation plates

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Abstract

Black locust (*Robinia pseudoacacia* L.) is an alien species that is commonly planted in devastated lands, parks, residential gardens and is used as a nectar source for bees. However, the Japanese government has designated this species as an “Industrial exotic species under watch" because its rapid growth and reproductive capacity allows it to outcompete indigenous vegetation. Field surveys revealed that the back locust spreads through its sprouting roots and the elongation of its horizontal roots at soil depths between 0 and 10 cm. A practical method to regulate the spread of black locust would be to suppress the development of horizontal roots. Based on a nursery experiment, we propose the use of root-barrier panels to block and suppress the growth of horizontal roots. Monitoring the growth of roots in the field is labor-intensive, so we investigated the impact of root-barrier panels on shoot development by measuring the length of sprouting roots, the decrease in secondary flush, the increase in yellowed leaflets, and impact on plant height in a nursery setting. We concluded that root-barrier panels set at a depth of 30 cm effectively inhibited the growth of the horizontal roots of young black locust less than 3years old.

Key words: Black locust, root-barrier panel, root-shoot communication, soil nutrients, sprouting root.

Introduction

Invasive species often negatively impact on native vegetation by inducing plagiosere succession (Mooney 2005). Even though these species were introduced for their beneficial industrial uses, they often escape from their original locations and quickly spread to areas where they may have a harmful impact (D’Antonio and Mahall, 1991; Sakio, 2009; Yin et al., 2014).

The black locust (*Robinia pseudoacacia* L.), an invasive species which originated in North America, is a deciduous broad-leaved tree. It has a symbiotic relationship with nitrogen-fixing microbes (e.g., *Bradryrhizobium* sp.) that forms root nodules (Mooney and Hobbs, 2000; Murakami and Washitani, 2002; Fujita et al., 2020). This symbiotic relationship allows the black locust to grow at open sites with nutrient-poor soils (Schultze et al. 2005; Koike et al., 2009); this species is commonly used for afforestation of areas devastated by landslides, typhoons, forest fires, etc. (Maekawa and Nakagoshi, 1997a, b; Masaka 2013; Qiu et al., 2010; Yin et al., 2014).

Black locust, with its great reproductive capacity, often spreads from where it was originally planted site to other locations (Sakio 2009). Its reproductive capacity is amazingly due to its seed dispersal and their quick germination (Masaka and Yamada, 2009; Watanabe et al., 2014; Nicolescu et al., 2020), as well as the propagation of root sprouts developing from horizontal roots (Gyokusen 1991; Zhang et al., 2006; Yin et al., 2014). Usually, black locust suppresses the growth of other species and significantly modifies the native vegetation communities (Maekawa and Nakagoshi, 1997a; b; Muranaka et al., 2005; Jung et al., 2008, Nicolescu et al., 2020). Therefore, the Japanese Ministry of Environment (2015) designated it as a potentially invasive exotic plant, in the category of “Industrial exotic species under watch.” (Muranaka et al. 2005; Masaka and Yamada, 2009). However, a way to safely and efficiently manage black locust to prevent its spread into indigenous vegetation is still under discussion.

One of the problems hindering the development of a management system is the lack of information on their growth and reproductive strategies. Moreover, Sakio (2009) emphasized the need to establish practical methods to regulate the growth and spread of this species. In Lithuanian forestry, they successfully use native root-rot fungi isolated from the targeted area to regulate invasive black locust to avoid genetic contamination (Watanabe et al., 2014; Marozas et al., unpublished data).

To sustainably manage this species, it is crucial to find cheap and straightforward methods (Murakami et al., 2005; Sakio, 2009; Yin et al., 2014; Nicolescu et al., 2020). For example, roadsides are a common invasion route of this species (Morimoto et al., 2010), so it is necessary to protect them from black locust. However, more research needs to be done on the clonal growth

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habits of this species when invading disturbed areas such as forest edges, landslide slopes, and road construction areas (Sakio, 2009; Xu et al., 2009; Yin et al., 2014).

It has been reported that root-barrier panels have effectively prevented the invasion of plants with active elongation of horizontal roots or rhizomes. Using root-barrier panels, a seaside-plant conservation center in northern Japan aims to suppress the horizontal root growth of *Elymus mollis* (Ishikari-hama HP, 2009). Okayama Prefecture (2003), using research done by Ito and Hino (2007), is the inhibiting growth of subterranean stems of bamboo (*Phyllostachys pubescens*) with root-barrier panels Ito and Hino 2007). However, in the case of black locust, there is little known about the effect of root-barrier panels on the elongation of the horizontal roots (Sakio, 2009; Nicolescu et al., 2020).

The root system of black locust is roughly classified as shallow, while other broad-leaved deciduous trees have root classified into a root type with deep tap roots with shallow horizontal roots (Karizumi 2010). Data on root distribution is needed to determine the effectiveness of root-barrier panels in blocking the spread of horizontal roots. In this study, we examined the effects of the root-barrier panels in both field observations and nursery experiments. We measured growth dynamics to determine the root-barrier panels' effect on root regulation (Masaka, 2013; Zhang et al., 2006).

We expect that installing root-barrier panels will inhibit horizontal root growth, which will slow shoot elongation and lead to the poor development of secondary flush. Suppressed horizontal root growth will also cause physiological imbalances in leaves because roots will not absorb as many nutrients and less water (Sato, 1995). To test our hypothesis, we examined the effects of root-barrier panels on the growth of horizontal roots and shoots in black locust seedlings from the viewpoint of root-shoot communication (Iwai et al., 1987). Two experiments were conducted; 1) field surveys to assess the sprouting of roots in three experimental forests, 2) a nursery experiment at Hokkaido University to observe the effect of root-barrier panels for the suppression of root sprouts growth. Based on the obtained results, we discuss the effectiveness of root-barrier panels in regulating horizontal roots.

Materials and Methods

Plant material and study sites

1)Field survey

To investigate the root systems of the black locust (*Robinia pseudoacacia* L.), we conducted field surveys at secondary forests of three university forests in Hokkaido, Japan; 1) Sapporo Experimental Forest (SEF) (43°06'N, 141°20'E), 2) Nakagawa Experimental forest (NEF) (44°52'N, 142°04'E), and 3) Teshio Experimental Forest (TEF) (44°55'N, 141°59'E) of the Field Science Center for Northern Biosphere (FSC) of Hokkaido University.

First, we removed the soil covering roots with a small shovel to measure the depth of the root distribution of the black locust seedlings. To confirm the physical traits of the soil, we took three soil samples from the surface to a depth of 30~40 cm under the litter layer. A soil hardness reading was taken at each depth (10, 20, and 30 cm) with a Yamanaka soil-hardness (YSH) tester (Fujiwara-SS, Co. Ltd, Tokyo, Japan). We used the criteria set by Nakatsu et al. (2004) for the YSH index [unit: mm]. A measurement of [11~20] indicates almost no inhibition in root growth; [20~24] some inhibition in root growth in certain plant species; [24~27] inhibition in root growth in most plant species; and [27>] no root can enter the soil.

At each site in the three experimental forests, we dug up by hand all the roots originating from the base of the black locust saplings as far as possible, trying not to destroy the horizontal roots. We recorded the vertical distribution of the horizontal roots of the black locust saplings. We defined a horizontal root as a root developing from the bottom part of the trunk at an angle of less than 25 degrees from the soil surface. The number of stems for each black locust sapling was 38 in the SEF, 16 in the NEF, and 18 in the TEF. Finally, the size of the target saplings was about 2-4 m in height, with a diameter at the bottom of the stems ranging between 4.6 cm and 5.8 cm.

We recorded the number of stems found in each soil layer (10 cm) from 0-30 cm. Almost no roots were found at a depth below 30-40 cm (Matsunami et al., 2005). To confirm physicochemical traits of the soil, we took three soil samples at each study site. At the TEF and NEF sites, the samples were taken from the surface to a depth of 30-40 beneath the litter layer and 30-50 cm at the SEF site.

Chemical analyses:

We analyzed the soil samples and the leaflets for the following elements: nitrogen (N), Calcium (Ca), Potassium (K), Magnesium (Mg), and Phosphorus (P). The elements were measured by inductively coupled plasma spectroscopy (IRIS, Jarrel ash, Franklin, MA, USA). We used nitric-chloric perchloric acid digestion (Association of Analytical Method of Plant Nutrients, 1990) to obtain the concentrations of Ca, K, and Mg. The Bray II method was used to analyze P (Kayama et al., 2002), and the N concentration in leaves was measured using an N-C analyzer (NC-900, Sumica, Osaka, Japan).

2)Nursery experiments

Plant material and study site:

To have uniform-sized plant material we planted, 36 black locust (3-year-old) seedlings in May 2007. Each seedling had a stem length of about 20 cm (without branches) and the longest root possible. We obtained the seedlings from the Hokkaido Horti-Afforestation Center Co. Ltd., located near Sapporo, Hokkaido. The seedlings had a base diameter of 0.80 ± 0.08 cm. The Experimental Nursery of the SEF (43°06'N, 141°20'E) of the FSC of Hokkaido University was selected as the study site to provide a uniform growth environment. The soil type of the SEF site, based on the FAO-UNESCO categorization, to a depth of around 40 cm was brown forest soil.

We conducted the chemical analyses of soil and leaf samples using the same methods described in the chemical analyses section for the field data (Association of Analytical Method of Plant Nutrients, 1990). At approximately one-month intervals, we observed the aboveground performance of the seedlings from late May 2008 to early October 2008. We dug up the roots in early November when all leaves had been shed

Treatments:

We set the root-barrier panels around the seedlings in mid-June 2007, at three depths: 10, 20, and 30 cm. The 90-cm square root-barrier panels were made from polyvinyl chloride (PVC). We used the 10 cm depth intervals because Matsunami et al. (2005) found few roots in deeper parts of the soil. The 90 cm x 90 cm enclosure was selected based on the field survey to ensure that the seedlings had enough horizontal roots to grow. We did not place any root-barrier panels around the control, but the research area was defined as 90 cm x 90 cm. One individual stem was located in the center of each root-barrier panel square. The different depth treatments (10, 20, and 30 cm) were placed at random in the nursery. Powdery mildew infected two seedlings in the first year, and we immediately removed the infected shoots. We left the main stem in place for plant density.

Aboveground:

The effects of the root-barrier panels on root growth were studied indirectly by examining the aboveground reaction of the black locust seedlings to the different treatments. We monitored the following aboveground growth parameters:

--The seasonal change in the length of the current shoot at around 1.0 m from the crown top from late May to late September in 2008 to avoid effects of the planting.

Figure 1. Leaf color of the control (left) and the 30-cm treatment (right)

--The number of individuals showing “secondary flush from the current shoot” and “yellowed leaflets near the bottom of the main stem” using a SPAD-502 (Minolta, Tokyo, Japan).

--Nutrient concentrations were analyzed in the leaflets to identify the nutrient traits of the yellowing leaves (Figure 1). The leaves were dried at 70ºC for 3 days, then powdered for chemical analyses. They were analyzed using the same methods as described in the chemical analysis section.

Belowground-Roots:

We carefully dug up all the black locust seedlings with a small front-end loader in early November, after all leaves had been shed, to measure the elongation of the horizontal roots. (Figure 2). We quantified the root-barrier panels’ ability to inhibit the elongation of horizontal roots by counting the number of roots that had burrowed into the soil under the root-barrier panels and dividing that number by the total number of roots in the 90 cm square and multiplying that number by 100 to get the percentage of roots invading soil under root-barrier panels. Percentage (%) of roots invading soil under root-barrier panels = (Number of roots invading soil under root-barrier panels / Number of all roots) x 100.

Figure 2. Dig up and transfer of saplings of black locust with a front-end loader (upper) and the root size with 20-cm barrier plate treatment (lower).

Statistical analysis:

We performed all statistical analyses using the R language (R Development Core Team, 2016) with the generalized linear model (GLM) using the “stat” package. We used aboveground and root growth parameters as the response variables. The depths of the root-barrier panels were the explanatory variables. We employed multiple pair-wise comparisons when we found that the root-barrier panel depth had any significant effect on shoot elongation, on the percentage of roots that burrowed into the soil under the root-barrier panels, or the leaf nutrient concentrations in the individuals with pale green and yellowed leaves. We adjusted the P-values using Holm’s modified Bonferroni procedure (sig., $^{**}P<0.01$ and $^{*}P<0.05$).

Results and discussion

From the results of our field survey and nursery experiment we believe we have found an effective method to regulate the horizontal root growth of black locust.

1)Field surveys

At soil depths of 30-50 cm, no statistical difference was detected in YSH soil hardness; the YSH value was about 27 mm for all three sites at that soil depth. Some tree species cannot develop roots when the YSH soil hardness value is between 20 mm and 24 mm, and most species cannot grow when it is over 25 mm (Nakatsu et al., 2004).

Our study found the vertical distribution of soil nutrient concentrations were similar in the three study sites, except for Mg. The Mg concentration was lower at a soil depth below 20 cm at the SEF site than at the TEF and NEF sites. The N, P, and K concentrations were higher as the soil depths increased 10 cm, 20 cm, and 30 cm. We found no difference in vertical concentrations of Mg, Ca, and K in soils above 20 cm. P in soil plays an essential role in increasing the activities of the symbiotic microbe as researchers have found in the gully region of the Loess Plateau in China (Röhm and Werner 1991; Qiu et al., 2010). We found no statistical differences in nodule formation. We found the most roots at the three sites in 0-10 cm soil depth, suggesting that the black locust is a shallow-root type deciduous tree. Also, very few roots were found below 20 cm at the TEF and NEF sites. Finally, the yellowed leaves might result from restricted root elongation and development within the restricted underground space.

2)Nursery studies

Aboveground:

At the end of the growing season, the mean tree height was 4.1 ± 0.6 m, 3.5±0.8 m, 3.1±0.6 m, and 2.5±0.2 m for the control, root-barrier panel depths of 10, 20, and 30 cm, respectively. Shoot growth of the saplings grown with the 20-cm treatment sharply increased in late July to mid-August and reached the same levels as the control and the 10-cm treatments. The rate of shoot growth decreased with the 30-cm treatment after late August. Shoot elongation in the 30-cm treatment was significantly the smallest in late September.

The percentage of individuals forming secondary flush ranged between 40% and 80%; the largest was for the control, and the smallest was for the 30-cm treatment. At the end of September, shoot growth with the 30-cm treatment was the lowest among the four treatments ($P<0.01$). The SPAD value was almost constant at about 41 in mid-July and sharply declined to about 36 around mid-September. The SPAD values differed significantly between treatments in early October; it was 37 for the control and the 20-cm treatment, and 21 for the 30-cm treatment ($P<0.01$). At late September, leaf nutrients from the three different depths and the control were shown as; no difference was found in the concentrations of P and Ca of leaves with the root-barrier panel treatments. Yellow leaves had significantly smaller N (22 mg g^{-1}), K (9.2 mg g^{-1}), and Mg (0.6 mg g^{-1}) values.

Belowground-Root elongation:

The number of bent roots at the root-barrier panel of the 10, 20, and 30-cm treatments was 6.8, 14.5, and 19.2, respectively (Figure 3). At a depth of around, 10 cm many tree species develop a well-spread horizontal root system (Gyokusen et al., 1991; Sakio, 2003; Hoshino, 2006). The YSH soil hardness values in the three study sites at depths between 0-10 cm were 13-15 mm, allowing root growth and development of many species (Nakatsu et al., 2004).

Figure 3. The bent tip of horizontal root with the 30-cm treatment. The arrow indicates where the root-barrier panel was set.

Roots spread widely in the soil to obtain the nutrients and water the tree needs, but they also require oxygen for respiration (Reader et al., 1993: Schulze et al., 2019), which is more readily available at shallow depths.

The 30-cm treatment showed significantly shorter horizontal roots than the control ($P<0.01$). The number of horizontal roots (mean ± SD) with the different treatments (10, 20, and 30 cm) and the control was 10.7 ±1.7, 7.6±1.3, 3.4±1.0, and 5.1±0.9, respectively. We found no clear trend in the formation of root nodules. The smallest dry mass was found in roots that escaped from the 30-cm treatment ($P<0.01$). We found no difference between the control and the 10-cm treatment or the 10- and 20-cm treatments.

Once the horizontal roots reached the root-barrier panels, they grew back on themselves in a coil shape and turned in different directions (Figure 3). Moreover, the number of horizontal roots also increased from the main stem when enclosed with root-barrier panels, which may be attributed to the roots compensating for the restricted development (Matsunami et al. 2005). We believe that the restricted space created by the root-barrier panels caused a high density of roots, which led to root wrapping (Figure 4). Under these conditions, the seedlings exhausted the nutrients in the restricted space (Koike et al., 2009).

From examining the aboveground growth of the black locust saplings, we may conclude that the root-barrier panels are an acceptable way to suppress root-shoot performance. Because of the high plasticity of roots (Reader et al., 1993), setting the root-barrier panels deeper than 30 cm in depth is an effective way to regulate the growth of black locust saplings.

Figure 4. The final size (over 172 cm) of a control black locust sapling (left) and the clay soil layer (arrow) (right).

The person in the figures is top author of this study (height 175cm)

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