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Effects of Phenolic Compounds on Seed Germination of Shirakamba Birch, *Betula platyphylla* var. *japonica*

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**Abstract**

Nine phenolic compounds, which were reported as the allelochemicals found in the soil beneath the trees of genus *Quercus* including mizunara oak, *Quercus mongolica* Fisch var. *groseserrata* Rehd. Wils., inhibited the seed germination of shirakamba birch, *Betula platyphylla* Sukatchev var. *japonica* Hara (60-100% inhibition) at a concentration of $5 \times 10^{-4}$ M. They were salicylic acid (1), $p$-hydroxybenzaldehyde (9), $p$-hydroxybenzoic acid (2), vanillic acid (4), $p$-coumaric acid (11), 3,4,5-trimethoxybenzoic acid (8), chlorogenic acid (14), 3,4-dimethoxybenzoic acid (7) and ferulic acid (13). The results supported the speculation by Shibuya et al. (1996) that *B. platyphylla* var. *japonica* could not regenerate beneath the trees of *Q. mongolica* var. *groseserrata*, even after the raking out of sasa bamboo, *Sasa* spp., in a forest stand of Hokkaido, northern Japan because of the effect by allelochemicals in the soil beneath the trees of *Q. mongolica* var. *groseserrata* and also support the speculative statement by Li et al. (1993-a) that the phenolic compounds found in the soil beneath the trees of *Q. mongolica* var. *groseserrata* might have important roles for the distribution of forest species. *B. platyphylla* var. *japonica* seeds seem to be more sensitive to these phenolic compounds on the inhibition of the germination than lettuce, *Lactuca sativa* L.

(Figures in parenthesis should refer to the appendix, structures of chemicals)

**Key words:** birch (*Betula*), oak (*Quercus*), lettuce (*Lactuca*), allelochemicals, inhibition of germination and growth

**Introduction**

Mizunara oak, *Quercus mongolica* Fisch var. *groseserrata* Rehd. Wils., distributes widely in cool temperate zones including Hokkaido, northern Japan. Shibuya *et al.* (1996) reported that shirakamba birch, *Betula platyphylla* Sukatchev var. *japonica* Hara, did not regenerate beneath the trees of *Q. mongolica* var. *groseserrata* even after the raking out of sasa bamboo, *Sasa* spp., in a forest stand in Hokkaido. The phenomenon is interesting because it is well known that raking out of *Sasa* spp. promoted the regeneration of *B. platyphylla* var. *japonica* in Hokkaido (Okumura *et al.* 1985). The phenomenon may be caused by environmental factors such as light, inter- and intra-specific competition or allelopathy (Lei 2000). However, there was sufficient light flux beneath the trees of *Q. mongolica* var. *groseserrata* and the competition could not explain the lack of seedlings of *B. platyphylla* var. *japonica* beneath the canopy of *Q. mongolica* var. *groseserrata*. Shibuya *et al.* (1996) speculated that the chemical effect of allelochemicals caused the phenomenon.

Several studies have examined the allelopathic effect caused by the trees of genus *Quercus*. Bell (1971) observed the growth of natural vegetation as well as the survival and growth of planted *Q. falcata* seedlings beneath the trees of *Q. falcata* and found that the plants grew slower beneath the crown of *Q. falcata* than those grown in other areas. He found that cold water extracts from the fresh leaves of *Q. falcata* contained a substance that inhibited the growth of sweetgum, *Liquidambar styraciflua* L., and it was later identified to be salicylic acid (1) (Figures in following parenthesis should refer to the appendix, structure of chemicals). Griessman (1978) reported that the extracts from green leaves and freshly fallen leaves of *Q. eugenofolia* were toxic to cucumber seedlings, and he assumed that phenolic compounds caused the effect. Lodhi (1976, 1978) found caffeic acid (12), ferulic acid (13), $p$-hydroxybenzoic acid (2), gallic acid (5), ellagic acid (18), scopoletin (16), and chlorogenic acid (14) in the fallen leaves and the soil beneath the trees of *Q. alba* and *Q. borealis*. Li *et al.* (1993-a) paid attention to the phenomenon that the wild grasses did not grow beneath the trees of mizunara oak, *Q. mongolica* var. *groseserrata*, grown in Hokkaido and confirmed that the soil beneath the trees of *Q. mongolica* var. *groseserrata* inhibited the growth of lettuce, green amaranth, wheat and timothy, and found that allelochemicals such as *p*-hydroxybenzaldehyde (9), *p*-hydroxybenzoic acid (2), vanillic acid (4), *p*-coumaric acid (11), ferulic acid (13) and kaempferol (17) existed in high contents in the soil beneath the trees of *Q. mongolica* var. *groseserrata*. Although they

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Although they examined and confirmed the effects of these allelochemicals on lettuce, green amaranth, wheat and timothy, they did not unfortunately use the seeds and the seedlings of woody plants including _B. platyphylla var. japonica_.

The information (Li _et al_. 1993-a; Shibuya _et al_. 1996) lead us to examine the effects of the phenolic compounds which were reported as the allelochemicals of the trees of genus _Quercus_ including _Q. mongolica_ var. _grosseserrata_ (Bell 1971; Rodhi 1976, 1978; Li _et al_. 1993-a) on the seed germination and seedling growth of shirakamba birch, _B. platyphylla var. japonica_.

The experimental results obtained in this study using thirteen allelochemicals being purchasable will allow us to support the speculative statement by Li _et al_. (1993-a) that the allelochemicals might have important roles in the distribution of forest species and also enable us to explain that the phenomenon, that no regeneration of shirakamba birch, _B. platyphylla var. japonica_, beneath the trees of mizunara oak, _Q. mongolica_ var. _grosseserrata_ reported by Shibuya _et al_. (1996), is caused by allelochemicals in the soil beneath the trees of _Q. mongolica_ var. _grosseserrata_.

Materials and Methods

1. Chemicals

Thirteen phenolic compounds were used for germination tests and seedling growth tests. They were salicylic acid (1), _p_-hydroxybenzaldehyde (9), _p_-hydroxybenzoic acid (2), vanillic acid (4), gallic acid (5), 3,4-dimethoxy benzoic acid (7), 3,4,5-trimethoxybenzoic acid (8), _p_-coumaric acid (11), caffeic acid (12), ferulic acid (13), ellagic acid (18), chlorogenic acid (14) and kaempferol (17). The _p_-coumaric acid (11) was purchased from Merck & Co., Inc. (N.J., U.S.A.) and the other chemicals were from Wako Chemical Industries, Ltd. (Tokyo, Japan). Each chemical was used at the concentrations of 10⁻³ M, 10⁻⁴ M, and 5 x 10⁻⁴ M.

2. Seed germination tests and growth tests of seedlings.

2.1 _Betula platyphylla_ Sukatchev var. _japonica_

_Hara_: The seeds of shirakamba birch, _B. platyphylla var. japonica_, were obtained from the Nayoro Nursery Station in 1993 (collected from the Uryu Experiment Forest of the North Ccenter, Hokkaido University Forests). The seed germination and seedling growth tests were conducted on advantec No.3 filter paper in 9.0 cm diameter petri dishes with 3.2 ml test solutions containing 100 ppm Tween 80. Tween 80 aqueous solution (100 ppm) was used in all controls. For the seed germination test, 100 seeds for each test were added to the dishes containing the test solution and germinated in the dark at 20° C for 48 hours (Li _et al_. 1993-b). Each test was repeated three times. Elongations of hypocotyls and roots of the controls were 8.9 mm and 13.6 mm, respectively.

Germination percentage of the control averaged 71%.

For the seedling growth test, 2 day-old etiolated seedlings were used. For each test, six etiolated seedlings were planted in the petri dishes containing the test solutions. The lengths of hypocotyls and roots were measured on the final day after the seedlings were cultured under the condition of 5,300 lux (R/RL=1.0) at 20 °C for 10 days. Each test was repeated three times. Elongations of hypocotyls and roots of the controls were 23.5 mm and 5.5 mm, respectively.

2.2 Lettuce (_Lactuca sativa_ L., wearhead form):

The seeds of lettuce were purchased from Sapporo Koumouen Co. Ltd., harvested in 1997 (U.S.A.). The seed germination and seedling growth tests were conducted on advantec No.3 filter paper in 4.5 cm diameter petri dishes with 1.6 ml of the test solution containing 100 ppm Tween 80. Tween 80 aqueous solution (100 ppm) was used in all controls. For the seed germination tests, fifty seeds for each test were added to the dishes containing the test solutions and germinated in the dark at 20° C (Li _et al_. 1993-b). The number of germinated seeds was counted after 48 hours, when no more germination was observed. The germination percentage of the control averaged 67%.

For the seedling growth test, 2 day-old etiolated seedlings were used. For each test, six etiolated seedlings were planted in the petri dishes containing the test solutions. The lengths of hypocotyls and roots were measured on the final day after the seedlings were cultured in the dark at 20° C for 48 hours (Li _et al_. 1993-b). Each test was repeated three times. Elongations of hypocotyls and roots of the controls were 8.9 mm and 13.6 mm, respectively.

Results

1. Effects of phenolic compounds on seed germination

1.1 _Betula platyphylla_ Sukatchev var. _japonica_

_Hara_: Among the thirteen compounds used for germination tests, nine compounds strongly inhibited the seed germination of shirakamba birch, _B. platyphylla var. japonica_, (60-100% inhibition) at a concentration of 5x10⁻⁴ M. They were salicylic acid (1), _p_-hydroxybenzaldehyde (9), _p_-hydroxybenzoic acid (2), vanillic acid (4), _p_-coumaric acid (11), 3,4,5-trimethoxybenzoic acid (8), chlorogenic acid (14), 3,4-dimethoxybenzoic acid (7) and ferulic acid (13) (Fig.1).

The thirteen compounds examined could be classified into three groups (A, B, and C) according to their inhibitory behaviors. Group A contained six compounds that severely inhibited germination (90-100% inhibition) at a concentration of 5x10⁻⁴ M: salicylic acid (1), _p_-hydroxybenzaldehyde (9), _p_-hydroxybenzoic acid (2), _p_-coumaric acid (11), vanillic acid (4), and 3,4,5-trimethoxybenzoic acid (8) (Fig.2). Group B contained three compounds that caused fairly strongly inhibit germination (60-85%...
Inhibition of regeneration of white birch under oak

Inhibition) at a concentration of $5 \times 10^{-4} \text{ M}$: chlorogenic acid (14), 3,4-dimethoxybenzoic acid (7), and ferulic acid (13). Group C contained three compounds that slightly inhibited germination (10-50% inhibition) at the same concentration of $5 \times 10^{-4} \text{ M}$: gallic acid (5), ellagic acid (18), and kaempferol (17). Caffeic acid (12) was the only compound that promoted seed germination (10% promotion), even at the highest concentration of $5 \times 10^{-4} \text{ M}$. At the lowest concentration of $10^{-5} \text{ M}$, only 3,4,5-trimethoxybenzoic acid (8) inhibited seed germination, while ferulic acid (13) and salicylic acid (1) promoted seed germination (10-45% promotion, Fig. 2). All the other compounds had no effect on the germination at the lowest concentration of $10^{-5} \text{ M}$.

The group A compounds could be further classified

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Fig. 1 The results of the seed germination inhibition of shirakamba birch, *Betula platyphylla* var. *japonica*, by phenolic compounds. Expressed as percentage of the number of the seeds germinated on that of the control. Average seed germination percentage of the control (without phenolic compounds) was 31.5%.

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Fig. 2 Phenolic compounds classified by the inhibition behaviors on the seed germination of shirakamba birch, *Betula platyphylla* var. *japonica*. Average seed germination percentage of the control (without phenolic compound) was 31.5%.
into A1 and A2 according to their inhibitory behavior on the germination at a lower concentration of $10^{-4} M$.

The group A1 contained $p$-coumaric acid (11), vanillic acid (4), and 3,4,5-trimethoxybenzoic acid (8), which inhibited the seed germination (10-30% inhibition) at a concentration of $10^{-4} M$, while salicylic acid (1), $p$-hydroxybenzaldehyde (9), and $p$-hydroxybenzoic acid (2), which promoted the seed germination (10-45% promotion) at the same concentration of $10^{-4} M$ belonged to group A2 (Fig. 2). It is noticed that the same compounds inhibited germination at a concentration of $5 \times 10^{-4} M$ and promoted it at the lower concentration of $10^{-4} M$.

1.2 Lettuce, Lactuca sativa L.: Fig. 3 also

![Fig. 3](image-url)

Fig. 3 The results of the seed germination inhibition of lettuce, Lactuca sativa L. by phenolic compounds. Expressed as percentage of the number of the seeds germinated on that of the control. Average seed germination percentage of the control (without phenolic compounds) was 71%.

![Fig. 4](image-url)

Fig. 4 Phenolic compounds classified by the inhibition behaviors on the seed germination of lettuce, Lactuca sativa L. Average seed germination percentage of the control (without phenolic compound) was 71%.
shows the effect of the thirteen phenolic compounds on lettuce seed germination at a concentration of $5 \times 10^{-4} M$. Lettuce was used as a reference plant from the light demanding plants, in which *B. platyphylla* var. *japonica* is belonged. The inhibitions on the germination by the phenolic compounds on *B. platyphylla* var. *japonica* (Fig. 1) were stronger than on lettuce, *Lactuca sativa* L. (Fig. 3).

Fig. 4 shows the effect of the concentration of phenolic compounds on the germination of lettuce seeds. Inhibition behaviors on the germination of lettuce seeds by the phenolic compounds were different from those of *B. platyphylla* var. *japonica* (Fig. 2). Namely, four compounds such as salicylic acid (1), *p*-hydroxybenzaldehyde (9), *p*-hydroxybenzoic acid (2) and vanillic acid (4) were found to strongly inhibit seed germination (80-100% inhibition) (Group D). 3,4-Dimethoxybenzoic acid (7), 3,4,5-trimethoxybenzoic acid (8), and *p*-coumaric acid (11) also acted as inhibitors, though to a lesser extent (40-80% inhibition, Group E), and ferulic acid (13), ellagic acid (18) and kaempferol (17) only slightly inhibited germination (10-20% inhibition, Group F). Chlorogenic acid (14), gallic acid (5) and caffeic acid (12) promoted the seed germination of lettuce, *Lactuca sativa* L., even in the highest concentration of $5 \times 10^{-4} M$ (Group G).

2. Effects of phenolic compounds on the seedling growth

2.1 *Betula platyphylla* Sukatchev var. *japonica*

*Hara*: Fig. 5 shows the effects of the phenolic *japonica*. Since some of the seedlings decayed at the final stage of the growth test, the data is not considered to be completely accurate. However, our observation showed that inhibition occurred more

![Graph of seedling growth inhibition](image)

Fig. 5 The results of the seedling growth inhibition of shirakamba birch, *Betula platyphylla* var. *japonica*, by phenolic compounds. Expressed as percentage of the length of hypocotyls and roots on those of the control. Average length of hypocotyls of the control (without phenolic compound) was 23.5 mm. Average length of roots of the control (without phenolic compound) was 5.5 mm.
strongly in the hypocotyls than in the roots. Salicylic acid (1), p-hydroxybenzaldehyde (9), and vanillic acid (4) inhibited the growth of hypocotyls at a concentration of $5 \times 10^{-6} M$.

2.2 Lettuce (*Lactuca sativa* L.) All the compounds, except for ellagic (18) and kaempferol (17), inhibited both hypocotyl and root elongation at a concentration of $5 \times 10^{-4} M$. Growth inhibition of the roots was stronger than that of the hypocotyls (Fig. 6).

Discussion

1. Seed germination affected by phenolic compounds

Phenolic compounds such as salicylic acid (1), p-hydroxybenzaldehyde (9) and p-hydroxybenzoic acid (2) (group A1), p-coumaric acid (11), vanillic acid (4) and 3,4,5-trimethoxybenzoic acid (8) (group A2), chlorogenic acid (14), 3,4-dimethoxybenzoic acid (7), and ferulic acid (13) (group B) inhibited the seed germination of shirakamba birch, *Betula platyphylla* Sukatchev var. *japonica* Hara, (more than 60% inhibition) at a concentration of $5 \times 10^{-4} M$.

Ferulic acid (13), p-coumaric acid (11) and chlorogenic acid (14) did not strongly inhibit lettuce seed germination in this study, but it has been reported (Li et al. 1993-b) that these acids inhibited germination of plants at a higher concentration of $5 \times 10^{-3} M$ (more than 50% inhibition) and that caffeic acid (13) completely inhibited seed germination of lettuce at a concentration of $5 \times 10^{-4} M$. In our study, although gallic acid (5), caffeic acid (12), ellagic acid (18) and kaempferol (17) (group C) had little effect on the seed germination of *B. platyphylla* var. *japonica* and lettuce, *Lactuca sativa* L., at a concentration of $5 \times 10^{-4} M$, they may inhibit

![Graph showing seedling growth inhibition of lettuce by phenolic compounds.](image)

**Fig. 6** The results of the seedling growth inhibition of lettuce, *Lactuca sativa* L., by phenolic compounds. Expressed as percentage of the length of hypocotyls and roots on those of the controls. Average length of hypocotyls of the control (without phenolic compound) was 8.9 mm. Average length of roots of the control (without phenolic compound) was 13.6 mm.
In our study, although caffeic acid (12) and ferulic acid (13) had additive inhibitory effects, both on seedling growth and seed germination. The inhibitory effect on lettuce was reversed by caffeic acid (10) and ferulic acid (13) at concentrations lower than $10^{-3} M$ except for the inhibition of germination by coumarin (19). In our study, although caffeic acid (12) promoted the seed germination of *B. platyphylla* var. *japonica* at the highest concentration of $5 \times 10^{-4} M$, *p*-coumaric acid (11), ferulic acid (13) and chlorogenic acid (14) inhibited seed germination at the same concentration of $5 \times 10^{-4} M$ (Fig. 1, 2).

The contents of *p*-hydroxycinnamic acids in the soil beneath the trees of *Q. mongolica* var. *grossexerrata* in Hokkaido were very high (Li *et al.* 1993-a): the contents of the six main phenolic compounds per 100 g soil were *p*-coumaric acid (11) (13,382 $\mu$ g), ferulic acid (13) (3,542 $\mu$ g), vanillic acid (4) (2,952 $\mu$ g), *p*-hydroxybenzoic acid (2) (2,164 $\mu$ g), *p*-hydroxybenzaldehyde (9) (1,378 $\mu$ g) and kaempferol (17) (990 $\mu$ g). Although the contents of 3,4-dimethoxybenzoyl acid (7) and 3,4,5-trimethoxybenzoyl acid (8) were not reported, their contents seem to be high enough being same order as those of *p*-hydroxybenzoic acid and vanillic acid from the HPLC chart reported (Li *et al.* 1993-a). In our study, 3,4-dimethoxybenzoyl acid (7) in the group B and 3,4,5-trimethoxybenzoyl acid (8) in the group A2 strongly inhibited seed germination of *B. platyphylla* var. *japonica* (Figs. 1 and 2).

The contents of the five main phenolic compounds except kaempferol (17) were higher than those in the soils where Sasa *kalilensis*, *Picea* *jezoensis*, rice, corn, potato, carrot, soybean and beet were growing, but lower than in the soil beneath Japanese red pine, *Pinus densiflora* Sieb. et Zucc. (Li *et al.* 1993-a).

In our study, salicylic acid (1), *p*-hydroxybenzaldehyde (9), *p*-hydroxybenzoic acid (2), *p*-coumaric acid (11) and vanillic acid (4) strongly inhibited seed germination of *B. platyphylla* var. *japonica* (Fig. 1) and vanillic acid (4), *p*-hydroxybenzoic acid (2) and salicylic acid (1) fairly inhibited the hypocotyl growth and 3,4-dimethoxybenzoyl acid inhibited root growth of shirakamba birch. Therefore, at present, it is difficult to pinpoint whether these phenolic acids interfered with hormones or whether they directly inhibited seed germination.

### 3. Bioconversion of phenolic compounds including glucosides into biological active forms

Inoue *et al.* (1992) isolated and identified anthraquinone compounds, emodin (20) and physcion (21) from the rhizomes and roots of *Polygonum sachalinense* Fr. Schm. and found that they inhibited the seedling growth of lettuce, green amaranth and timothy grass. He also isolated glucosides from *P. sachalinense*, emodin-O- $\beta$ -D-glucoside (22) and physcion-O- $\beta$ -D-glucose side (23). These glucosides showed no phytotoxic activity on lettuce seedling growth. The concentration of emodin (20) and physcion (21) was relatively high in the rhizomes with roots and fallen leaves, and the effective concentration of emodin (20) was maintained in the soil of this plant community in the fall. He proposed that glucosides or aglycons are released from the roots and then glucosides are decomposed to active...
forms. In regards to the allelopathy of \textit{Q. mongolica} var. \textit{grosseserrata}, similar events might have occurred. Phenolics, including their glycosides, in the green leaves of \textit{Q. mongolica} var. \textit{grosseserrata} must be studied in detail, focusing on their phytotoxic activity and bio-conversion of the intact substances in the leaves into phytotoxic substances during the decay of the fallen leaves on the ground under the trees (Lei 2000). The existence of 3,4-dimethoxybenzoic acid (7) and 3,4,5-trimethoxybenzoic acid (8) in the soil (Li et al. 1993-a) implies that they were formed by methylation of \textit{p}-hydroxylphenyl derivatives during the decay of the fallen leaves by soil bacteria, because the existence of \textit{p}-methoxy-phenyl derivatives in the decay of \textit{B. platyphylla} leaves. In addition to the inhibitory effect on seed germination, the inhibitory effect of the phenolic compounds on the growth of the seedlings of \textit{B. platyphylla} var. \textit{japonica} is due to allelopathy, which is caused by allelochemicals in the soil beneath the trees of \textit{Q. mongolica} var. \textit{grosseserrata} has not been reported so far.

4. No regeneration of \textit{B. platyphylla} var. \textit{japonica} beneath the trees of \textit{Q. mongolica} var. \textit{grosseserrata}

Though \textit{B. platyphylla} var. \textit{japonica} seeds do manage to germinate beneath the trees of \textit{Q. mongolica} var. \textit{grosseserrata}, the task is made difficult and their very existence is threatened by the phenolic compounds produced from the decaying leaves. In addition to the inhibitory effect on seed germination, the inhibitory effect of the phenolic compounds on the growth of the seedlings of \textit{B. platyphylla} var. \textit{japonica} leads us to suppose that the failure of \textit{B. platyphylla} var. \textit{japonica} to regenerate beneath the trees of \textit{Q. mongolica} var. \textit{grosseserrata} observed by Shibuya et al. (1996), is due to allelopathy, which is caused by allelochemicals produced by \textit{Q. mongolica} var. \textit{grosseserrata} and partly bio-converted by soil micro organisms.

The actual contents of the phenolic acids in the soil beneath the trees of \textit{Q. mongolica} var. \textit{grosseserrata}, where no regeneration of \textit{B. platyphylla} var. \textit{japonica} was observed (Shibuya et al. 1996), should be analyzed and clarified whether the phenolic acid contents in the soil are high enough to prevent the regeneration and seedling growth of \textit{B. platyphylla} var. \textit{japonica} or not, for getting the conclusion that the phenomenon is caused by allelopathy. However, the results obtained in this preliminary laboratory study strongly imply the effect of the phenolic acids on seed germination and seedling growth of \textit{B. platyphylla} var. \textit{japonica} at the actual site. The speculative statement (Li et al. 1993-a) that the phenolic compounds found in the soil beneath the trees of \textit{Q. mongolica} var. \textit{grosseserrata} might have important roles on the distribution of forest species is also supported by our experimental results.

It is interesting to examine in future the relationship among the contents of each phenolic compound and their effects on the regeneration of \textit{B. platyphylla} var. \textit{japonica} on the soil beneath the trees of \textit{Q. mongolica} var. \textit{grosseserrata} at the actual site.

References


Distributional interrelationship between tree species in natural mixed-species forests birches rarely appear under oak crowns in scarified sites.