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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. *grosseserrata* Rehd. Wils., inhibited the seed germination of shirakamba birch, *Betula platyphylla* Sukatchev var. *japonica* Hara (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). 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. *grosseserrata*, 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. *grosseserrata* 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. *grosseserrata* 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. *grosseserrata* Rehd. Wils., distributes widely in cool temperate zones including Hokkaido, northern Japan. Shibuya *et al.* (1996) reported that shirakamba birch, *Betula platy phylla* Sukatchev var. *japonica* Hara, did not regenerate beneath the trees of *Q. mongolica* var. *grosseserrata* 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. *grosseserrata* and the competition could not explain the lack of seedlings of *B. platyphylla* var. *japonica* beneath the canopy of *Q. mongolica*. var. *grosseserrata*. 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. falcate* and found that the plants grew slower beneath the crown of *Q. falcate* 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). Gliessman (1978) reported that the extracts from green leaves and freshly fallen leaves of *Q. eugenefolia* 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. boreadis*. Li *et al.* (1993-a) paied attention to the phenomenon that the wild grasses did not grow beneath the trees of mizunara oak, *Q. mongolica* var. *grosseserrata*, grown in Hokkaido and confirmed that the soil beneath the trees of *Q. mongolica* var. *grosseserrata* inhibited the growth of lettuce, green amaranth, wheat and timothy, and found that allelochemicals such as *p*-hydroxybenzaldehyde (9), *p*-hy-droxy-benzo-ic 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. *grosseserrata*. 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-dimehtoxy 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 $10^{-5} M$, $10^{-4} M$, and $5 \times 10^{-4} M$.

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 under the condition of 5,300 lux (R/fR=1.0) at 20 ℃. The number of germinated seeds was counted after 10 days, when no more germination was observed. Germination percentage of the control averaged 31.5%.

For seedling growth tests, 7 day-old seedlings were used. for each test. Ten seedlings were planted in the petri dishes containing the test solutions. The length of roots and hypocotyls were measured on the final day after the seedlings were cultured under the condition of 5,300 lux (R/fR=1.0) at 20 ℃ 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 Kounouen 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℃ (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 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 in the dark at 20℃ 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 $5 \times 10^{-4} M$. 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) (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 $5 \times 10^{-4} M$: 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) at a concentration of 5x10^{-4} *M.*: chloro-genic acid (14), 3,4-dimethoxybenzoic acid (7), and ferulic acid (13). Group C contained three com-pounds that slightly inhibited germination (10-50% inhibition) at the same concentration of 5x10^{-4}*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 5x10^{-4}*M*. At the lowest concentration of 10^{-5}*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 com-pounds had no effect on the germination at the lowest concentration of 10^{-5} *M*.

The group A compounds could be further classified

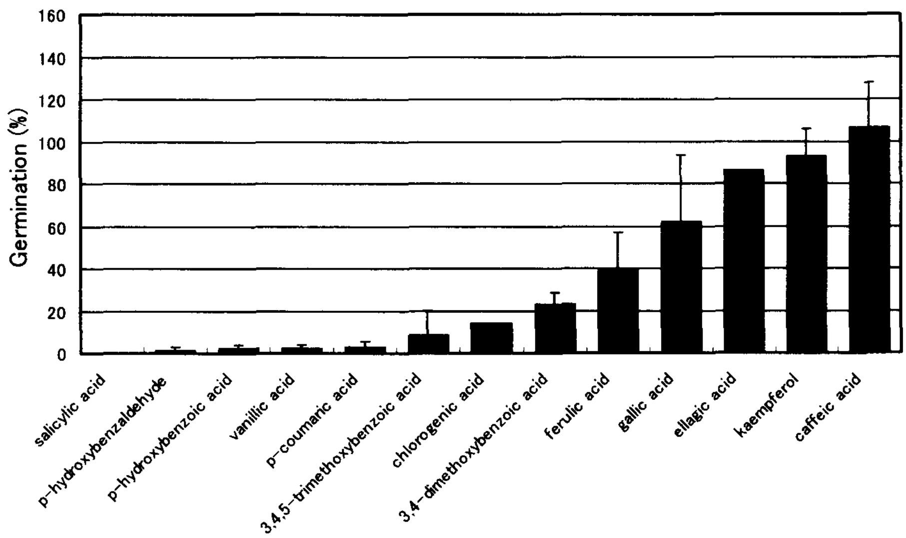


Fig. 1 The results of the seed gernination inhibition of shirakamba birch, *Betula platyphylla* var. *japonica*, by phenolic compounds.
Expressed as percentage of the number of the seeds germinated on that of the control. Average seed germination percentage of the control (without phenolic compounds)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

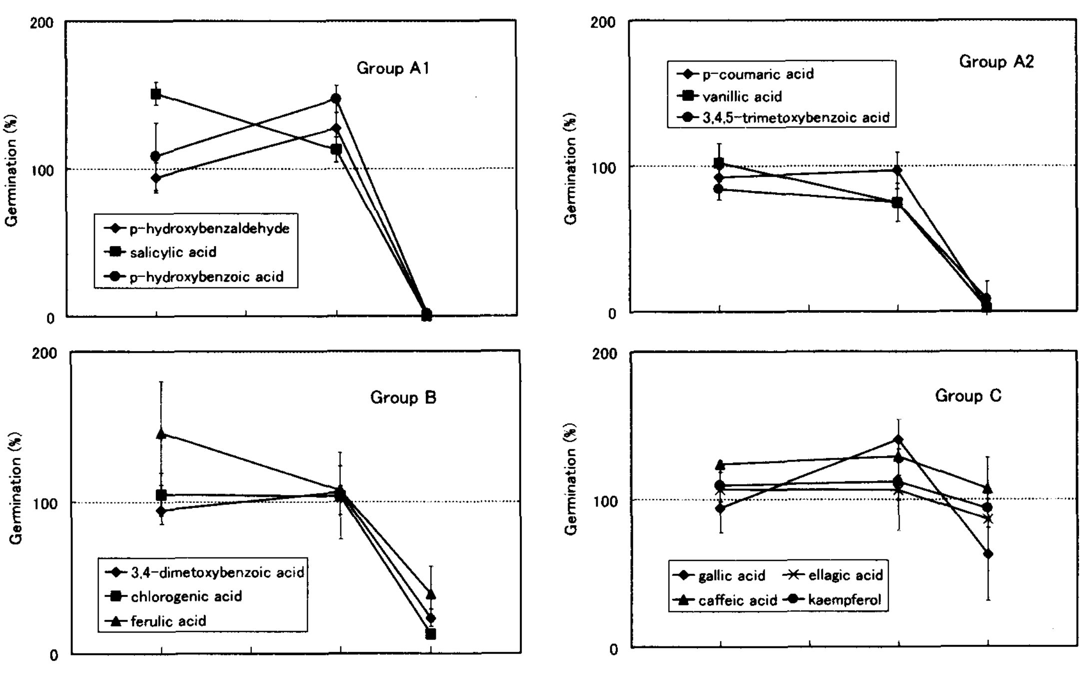


Fig. 2 Phenolic compounds classified by the inhibition behaviors on the seed germination of shirakamba birch, *Betula platyphylla* var. *japonica*. Average seed germination percentage of the control (without phenolic compound) was 31.5%.

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 5×10^{-4} *M* and promoted it at the lower concentration of 10^{-4} *M*.

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

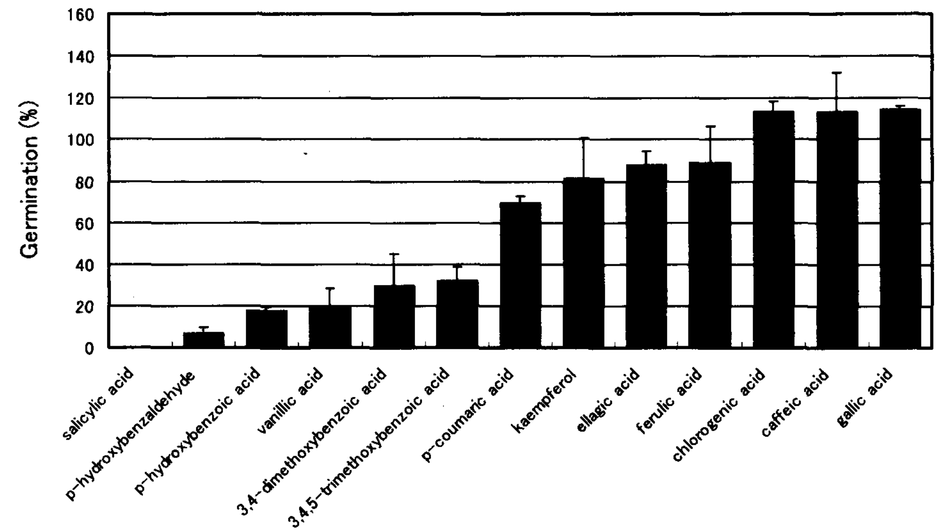


Fig. 3 The results of the seed gernination inhibition of lettuce, *Lactuca sativas* L. by phenolic compounds.
Expressed as percentage of the number of the seeds germinated on that of the control. Average seed germination percentage of the control (without phenolic compounds) was 71%

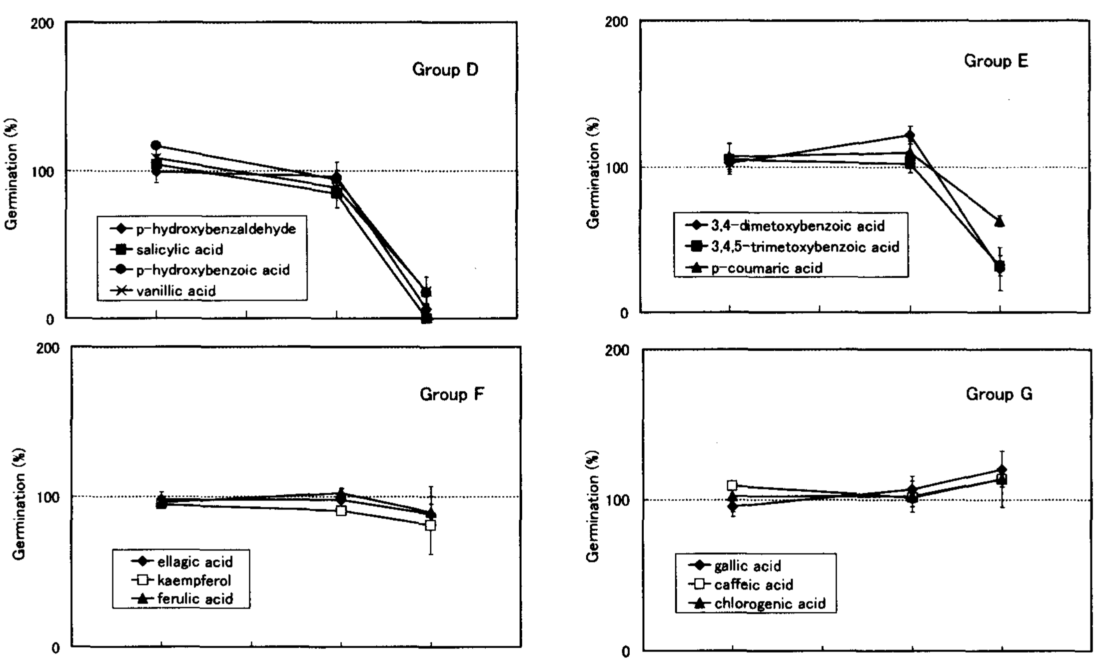


Fig. 4 Phenolic compounds classified by the inhibition behaviors on the seed germination of lettuce, *Lactuca sativas* L.
Average seed germination percentage of the control (without phenolic compound) was 71%.

shows the effect of the thirteen phenolic compounds on lettuce seed germination at a concentration of $5x10^{-4}$ *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*-hydroxyben-zoic 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 $5x10^{-4}$ *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

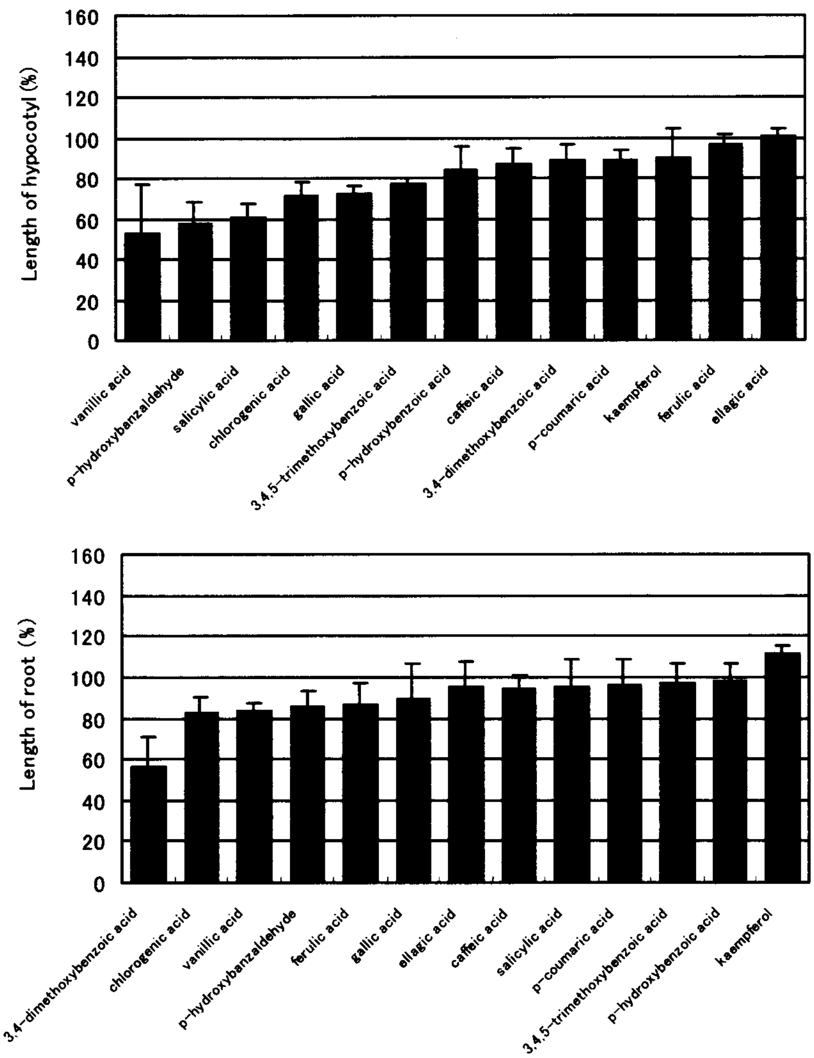


Fig. 5 The results of the seedling growth inhibition of shirakamba birch, *Betula platyphylla* var. *japonica*, by phenolic compounds.
Expressed as percentage of the length of hypocotyls and roots on those of the control. Average length of hypocotyls of the control (without phenolic compound) was 23.5 mm. Average length of roots of the control (without phenolic compound) was 5.5 mm.

strongly in the hypocotyls than in the roots. Salicylic cid (1), *p*-hydroxybenzaldehyde (9), and vanillic acid (4) inhibited the growth of hypocotyls at a concentration of $5 \times 10^{-4} M$.

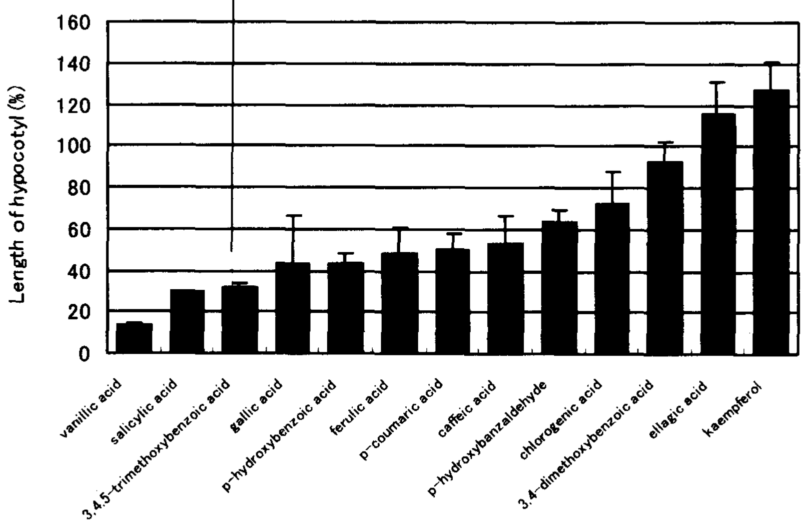
2.2 Lettuce (*Lactuca sativa* L.): All the compounds, except for ellagic acid (18) and kaempferol (17), inhibited both hypocotyl and root elongation at a concentration of 5×10^{-4} *M*. Growth inhibition of the roots was stronger than that of the hypocotyls (Fig. 6).

Discussion

1. Seed germinationaffectedbyphenolic 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 $5 \times 10^{-3} M$. 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 5×10^{-4} *M*, they may inhibit



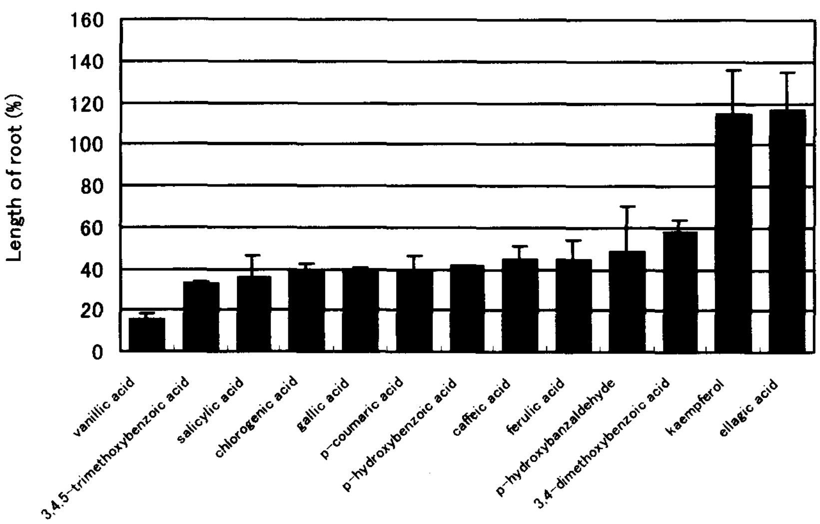


Fig. 6 The results of the seedling growth inhibition of lettuce, *Lactuca sativas* L., by phenolic compounds. Expressed as percentage of the length of hypocotyls and roots on those of the controls. Average length of hypocotyls of the control (without phenolic compound) was 8.9 mm. Average length of roots of the control (without phenolic compound) was 13.6 mm.

seed germination at concentrations higher than the concentration of our study ($5x10^{-4}M$). Phenolic acids seem to more strongly inhibit the germination of *B. platyphylla* var. *japonica* seeds than lettuce, *Lactuca sativa* L. This sensitivity might be one of the reasons why no regeneration of shirakamba birch, *B. pltyphylla* var. *japonica*, was observed beneath the trees of mizunara oak, *Q. mongolica* var. *grosserrata*. (Shibuya *et al.* 1996).

Kuiters (1989) reported that *p*-coumaric acid (11), along with *p*-hydroxybenzoic acid (2), vanillic acid (4), ferulic acid (13), caffeic acid (12) and syringic acid (6), had a stimulatory effect on seed germination at a low concentration (10^{-5} *M*) and had an inhibitory effect at higher concentrations (10^{-3} *M* or higher) in herbaceous woodland plants. In our study, similar tendency was observed. Namely, salicylic acid (1) and feruic acid (13) had a stimulatory effect (about 150% germination promotion) at a low concentration (10^{-5} *M*) and had strong inhibitory effects (about 60-100%inhibition) at a higher concentration ($5x10^{-4}$ *M*) on the seed germination of *B. platyphylla*. var. *japonica* (Fig. 2).

2. Relationship between phenolic compounds and plant hormones on seed germination and seedling growth

Tmaszewski and Thimann (1966) reported that monophenols stimulated the decarboxylation of indole-3-acetic acid (IAA), while polyphenols synergized IAA-induced growth by counteracting IAA destruction in assays with oat coleoptile and pea sections incubated with phenolics and IAA. Many investigations have corroborated that the phenolic acids can be divided into two groups: suppressors of IAA destruction such as chlorogenic acid (14), caffeic acid (12), ferulic acid (13), and protocatechuic acid (3) and compounds that stimulate IAA oxidase such as *p*-coumaric acid (11), *p*-hydroxybenzoic acid (2), vanillic acid (4), syringic acid (6), and phloretic acid (15) (Lee *et al.* 1982; Frank, A. E. 1986).

Reports on the effects of phenolic acids on other hormones (Corcoran *et al.* 1972; Jacobson and Corcoran 1977; Ray *et al.* 1980; Li *et al.* 1993-b.) have found that some regulatory polyphenols may reduce growth by binding gibberellic acid (GA), whereas others promote growth by binding abscisic acid (ABA).

Li *et al.* (1993-b) reported interactions of *t*-cinnamic acid (10), ferulic acid (13), chlorogenic acid (14), *p*-coumaric acids (11), coumarin (19) and ABA on seedling growth and seed germination of lettuce. These phenolic compounds along with ABA 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 $5x10^{-4}$ *M*, *p*-coumaric acid (11), ferulic acid (13) and chlorogenic acid (14) inhibited seed germination at the same concentaration of $5x10^{-4}$ *M* (Fig.1, 2).

The contents of *p*-hydroxycinnamic acids in the soil beneath the trees of *Q. mongolica* var. *grosseserrata* in Hokkaido were very high (Li *et al.* 1993-a): the contents of the six main phenolic compounds per 100g soil were *p*-coumaric acid (11) (13,382 μg), ferulic acid (13) (3,542 μg), vanillic acid (4) (2,952 μg), *p*-hydroxybenzoic acid (2) (2,164 μg), *p*-hydroxybenz aldehyde (9) (1,378 μg) and kaempferol (17) (990 μg). Although the contents of 3,4-dimethoxybenzoic acid (7) and 3,4,5-trimethoxybenzoic acid (8) were not reported, their contents seems to be high enough being same order as those of *p*-hydroxybenboic acid and vanillic acid from the HPLC chart reported (Li *et al.* 1993-a). In our study, 3,4-dimethoxybenzoic acid (7) in the group B and 3,4,5-trimethoxybenzoic 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 kulilensis*, *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*-hydroxybenz-aldehyde (9), *p*-hydroxy benzoic 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-dimethoxybenzoic 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 copounds including glycosides 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 sachalinese* Fr. Schm. and found that they inhibited the seedling growth of lettuce, green amaranth and timothy grass. He also isolated glucosides from *P. sachalinese*, emodin-O- β -D-glucoside (22) and physcion-O- β -D-gluco 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 *Q. mongolica* var. *grosseserrata*, similar events might have occurred. Phenolics, including their glycosides, in the green leaves of *Q. mongolica* var. *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-dimethoxyben-zo-ic 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 *p*-hydroxylphenyl derivatives during the decay of the fallen leaves by soil bacteria, because the existence of *p*-methoxy-phenyl derivatives in the green leaves of *Q. mongolica* var. *grosseserrata* has not been reported so far.

4. No regeneration of *B. platyphylla* var. *japonica* beneath the trees of *Q. mongolica* var. *grosseserrata*

Though *B. platyphylla* var. *japonica* seeds do manage to germinate beneath the trees of *Q. mongolica* var. *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 *B. platyphylla* var. *japonica* leads us to suppose that the failure of *B. platyphylla* var. *japonica* to regenerate beneath the trees of *Q. mongolica* var. *grosseserrata* observed by Shibuya *et al.* (1996), is due to allelopathy, which is caused by allelochemicals produced by *Q. mongolica* var. *grosseserrata* and partly bio-converted by soil micro organisms.

The actual contents of the phenolic acids in the soil beneath the trees of *Q. mongolica* var. *grosseserrata,* where no regeneration of *B. platyphylla* var. *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 *B. platyphylla* var. *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 *B. platyphylla* var. *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 *Q. mongolica* var. *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 *B. platyphylla* var. *japonica* on the soil beneath the trees of *Q. mongolica* var. *grosseserrata* at the actual site

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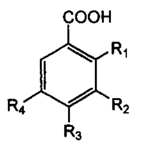
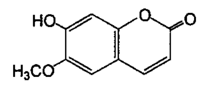
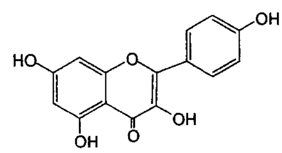


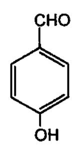
(1) salicylic acid R_1=OH R_2=H R_3=H R_4=H
(2) *p*-hydroxybenzoic acid R_1=H R_2=H R_3=OH R_4=OH
(3) protocatechuic acid R_1=H R_2=OH R_3=OH R_4=H
(4) vanillic acid R_1=H R_2=OCH_3 R_3=OH R_4=OH
(5) gallic acid R_1=H R_2=OH R_3=OH R_4=H
(6) syringic acid R_1=H R_2=OCH_3 R_3=OH R_4=OCH_3
(7) 3,4-dimethoxybenzoic acid R_1=H R_2=OCH_3 R_3=OCH_3 R_4=H
(8) 3,4,5-trimethoxybenzoic acid R_1=H R_2=OCH_3 R_3=OCH_3 R_4=OCH_3



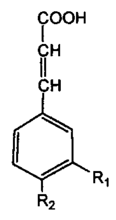
(16) scopoletin



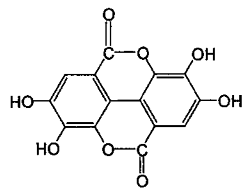
(17) kaemperol



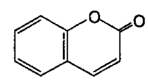
(9) *p*-hydroxybenzaldehyde



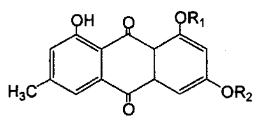
(10) *t*-cinnamic acid R_1=H R_2=H
(11) *p*-coumaric acid R_1=H R_2=OH
(12) caffeic acid R_1=OH R_2=OH
(13) feruic acid R_1=OCH_3 R_2=OH



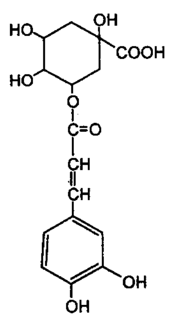
(18) ellagic acid



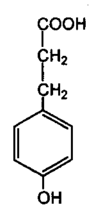
(19) coumarin



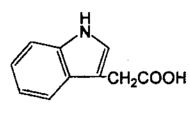
(20) emodin R_1=H R_2=H
(21) physcion R_1=H R_2=CH_3
(22) emodin-1-*B*-D-glucoside R_1=Glu R_2=H
(23) physcion-1-O-*B*-D-glucoside R_1=Glu R_2=CH_3



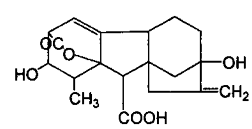
(14) chlorogenic acid



(15) phloretic acid



(24) IAA



(25) GA