SHORT COMMUNICATION

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Contribution of a phytotoxic compound to the allelopathy of *Ginkgo biloba*

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Ginkgo (*Ginkgo biloba* L.) has not changed over 121 million years. There may be unknown special strategy for the survival. Gingko litter inhibited the growth of weed species ryegrass (*Lolium multiflorum* L.). The inhibition was greater with the litter of the close position than that of the far position from the gingko tree. A phytotoxic substance, 2-hydroxy-6-(10-hydroxypentadec-11-enyl)benzoic acid (HHPEBA) was isolated in the litter. HHPEBA concentration was greater in the litter of the close position than that of the far position from the tree. HHPEBA inhibited the ryegrass growth at concentrations greater than 3 μM. HHPEBA was estimated to be able to cause 47–62% of the observed growth inhibition of ryegrass by the gingko litter. Therefore, HHPEBA may contribute to the inhibitory effect caused by ginkgo litter and may provide a competitive advantage for gingko to survive through the growth inhibition of the neighboring plants.

Ginkgo (*Ginkgo biloba* L.; Maidenhair tree) appeared in the Jurassic period and is only one surviving species in the family of *Ginkgoaceae*. It has not changed over 121 million years according to paleontological studies,[1] which indicates that the species may have unknown special survival strategies.

A novel phytotoxic substance with allelopathic activity, 2-hydroxy-6-(10-hydroxypentadec-11-enyl) benzoic acid (HHPEBA) has recently been isolated from Ginkgo leaves.[2] The activity of HHPEBA was 10 – to 52-fold greater than that of nonanoic acid (pelargonic acid), which occurs naturally as esters in the oil of pelargonium, and its ammonium salt, ammonium nonanoate, is used as a herbicide.[3] The allelopathic substances can provide a competitive advantage for host-plants to survive through the growth inhibition of the other plants in the local ecosystems.[4-6]

Ginkgo is a deciduous tree, and the tree drops their leaves in the late autumn. The leaf fall accumulates on the forest floor as litter and becomes one of the main components of the soil of the forest floor.[7,8] In this report, it was investigated whether gingko litter contains HHPEBA, and HHPEBA serves as an allelopathic substance, which is able to provide a competitive advantage.

Allelopathic activity of ginkgo litter

Gingko litter was taken with soil in the depth of 5 cm from the surface at the position of 1, 2, 3, and 4 min from gingko tree (**Fig. 1**) on November 2011. The litter was then extracted with 80% aqueous methanol, and the biological activity of the extract was determined by weed species ryegrass (*Lolium multiflorum* Lam) as described previously.[2] All extracts of ginkgo litter inhibited coleoptile and root growth of ryegrass (**Fig. 1**). However, the inhibitory activity of the litter was the greatest at 1 min from the gingko tree, and the smallest at 4 min. The result suggests that ginkgo litter may have allelopathic activity and contain allelopathic active substances.

Concentration of HHPEBA

A phytotoxic substance, HHPEBA, was found in the bioassay medium of all gingko litter (**Table 1**). The concentration of HHPEBA was the highest (9.2 μM) in the medium of the litter at 1 min from the gingko tree and the lowest (1.3 μM) in that at 4 min (**Table 1**). The result indicates that the litter contained HHPEBA since the medium was prepared only from gingko litter extracts as described in **Figure 1**.

HHPEBA had been isolated from ginkgo leaves[2] and the leaves are the main source of plant litter.[7,8] Therefore, HHPEBA in ginkgo leaves may be delivered onto gingko forest floor by the defoliation and accumulate in the litter.

Inhibitory activity of HHPEBA

HHPEBA inhibited the growth of ryegrass coleoptiles and roots at concentrations greater than 3 μM (**Fig. 2**). When the inhibition of coleoptiles and roots was plotted against the logarithmic concentration of HHPEBA as described by Streibig,[9] there were the significant logistic functions. The equations of HHPEBA were $Y = [(4.024–89.276)/\{1+(X/12.515)^{1.487}\}]+89.27$; ($r^2 = 0.998$) and $Y = [(3.449–86.252)/\{1+(X/10.091)^{1.062}\}]+86.252$; ($r^2 = 0.997$) for coleoptiles and roots, respectively. Y is the growth inhibition (%) and X is the HHPEBA concentration (μM) as shown in **Figure 2**.

Contribution of HHPEBA to allelopathy of gingko litter

The potential ability of HHPEBA in the bioassay medium for the growth inhibition was estimated by the equations

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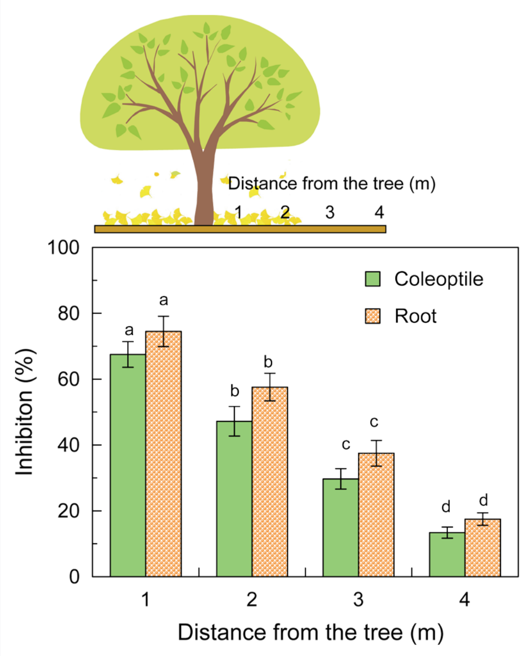

Figure 1. Effect of aqueous methanol extracts of ginkgo litter on the growth of ryegrass. Ginkgo litter (100 g) was extracted with 1 L of 80% (v/v) aqueous methanol. Extract (10 mL) was evaporated and added on the filter paper the petri dishes. After evaporation of the solvent, the filter paper was moistened with 1 mL of a 0.05% (v/v) aqueous solution of Tween 20. The biological activity of the extract was then determined by ryegrass seedlings as described previously.[2] After the incubation of 48 h in darkness at 25 °C, the length of ryegrass coleoptiles and roots was measured and inhibition % was deterined by the formula: [(control plant length – plant length incubated with the extract) / control plant length] x 100. Control ryegrass was incubated on the medium in the absence of the extract. Different letters indicate significant differences ($P < 0.05$) according to the Tukey's test.

Table 1. Concentration of HHPEBA in the bioassay medium

Position (m)	Concentration (μM)
1	9.2 ± 1.7
2	6.2 ± 1.5
3	3.4 ± 1.1
4	1.3 ± 0.5

Concentration of HHPEBA in the bioassay medium of **Figure 1** was determined by HPLC. Means ± SE from 5 independent experiments are shown.

Table 2. Potential ability of HHPEBA in the medium to growth inhibition

	Potential growth inhibition (%)	
Position (m)	Coleoptile	Root
1	37.1	42.8
2	26.2	34.4
3	14.8	23.3
4	6.3	10.6

The potential growth inhibition on ryegrass caused by HHPEBA was calculated using the equations of logistic functions (**Fig. 2**) with substituting X values by the concentrations of the compound found in the bioassay medium (**Table 1**) as described in the text.

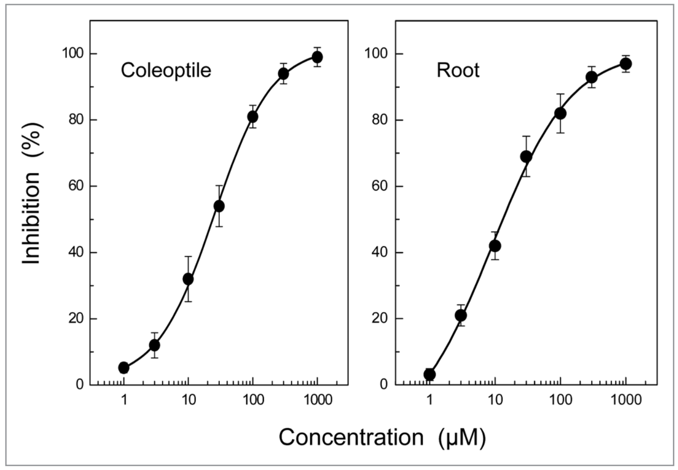

Figure 2. Effect of 2-hydroxy-6-(10-hydroxypentadec-11-enyl)benzoic acid (HHPEBA) on coleoptile and root growth of ryegrass seedlings. Ryegrass seeds were incubated in aqueous solution of HHPEBA for 48 d, and the length of coleoptiles and roots of ryegrass seedlings were measured as described previously.[2] Inhibition % was then determined as described in **Figure 1.**

Table 3. Contribution of HHPEBA to the growth inhibition by gingko litter

	Contribution (%)	
Position (m)	Coleoptile	Root
1	55.0	57.4
2	55.5	50.0
3	49.8	62.1
4	47.0	60.6

Contribution of HHPEBA to the growth inhibition on ryegrass by gingko litter was calculated by the formula: [potential ability of HHPEBA (**Table 2**)] / [growth inhibition observed (**Fig. 1**)] x 100.

obtained by inhibitory activity of HHPEBA (**Fig. 2**) and substituting X values with HHPEBA concentrations in the bioassay medium (**Table 1**). The estimated values in **Table 2** suggest that HHPEBA in the medium of the litter at 1 min has the ability to inhibit ryegrass coleoptile and root by 37.1 and 42.8%, respectively, and that at 4 min has the ability to inhibit the coleoptile and root growth by 6.3 and 10.6%, respectively.

The contribution of HHPEBA to the growth inhibition by ginkgo litter was calculated with the formula: [potential ability of HHPEBA (**Table 2**)] / [growth inhibition observed (**Fig. 1**)] x 100. The calculated values in **Table 3** suggest that HHPEBA explains 47.0–55.5% and 50.0–62.1% of the observed growth inhibition of ryegrass coleoptiles and roots (**Fig. 1**) by the gingko litter, respectively. Thus, HHPEBA may account for 47–62% of the growth inhibitory activity of the gingko litter.

The ginkgo litter showed growth inhibitory activity on weed species ryegrass (**Fig. 1**) and a phytotoxic substance, HHPEBA, existed in the bioassay medium of all gingko litter (**Table 1**). Judging from the activity of HHPEBA for growth inhibition (**Fig. 2**) and the concentration of HHPEBA in the gingko litter (**Table 1**), HHPEBA may be one of important contributors to the growth inhibitory effect of the litter (**Table 3**).

Plant litter is able to affect the microbial community, and the physical and chemical properties of the soil of the plant rhizosphere.[10-12] In addition, plant litter has positive and negative influences on the growth of neighboring plants. These effects are related to several mechanisms such as nutrient cycling, reducing light penetration, physical impediment, and releasing allelopathic substances.[11,13,14]

The plant rhizosphere is a very competitive area where plant root systems fight each other to get sufficient nutrients, minerals, and water as well as space.[10,15,16] Allelopathic substances with inhibitory activity in plant litter are involved in the competitive ability of host-plants to survive because of their effects on the seed germination, seedling establishment, plant growth, and distribution.[7,17-19] Therefore, HHPEBA may serve for ginkgo survival strategy as an allelopathic active substance that is able to give an advantage in such competition for gingko to survive through the growth inhibition of neighboring plants in the local ecosystems.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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