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Original Research

Effects of Soil Substrate Contaminated by Knotweed Leaves on Seed Development

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Abstract

The aim of our experiment was to evaluate phytotoxicity of three knotweed species (*Reynoutria japonica*, *R. sachalinensis*, *R. x bohemica*). The tests examined suppression of germination in the seeds of two crop plants (*Leucosinapis alba*, *Brassica napus*) and two weed plants (*Chenopodium album* agg., *Echinochloa crus-galli*) using dried knotweed leaves mixed with soil. Data processing by two-way ANOVA has shown that the type of seed tested was a more important factor affecting germination than the type of knotweed leaves used to contaminate the soil. The tested crop plants were more sensitive than either weed plant. The highest phytotoxicity was found for crop plants cultivated in soil contaminated with *R. japonica* leaves (wherein seed germination for *L. alba* was 35% and for *B. napus* 43%). Reaction of weed plant was stimulatory (seed germination for *E. crus-galli* in soil contaminated with *R. sachalinensis* leaves was 191%). This response is probably plant hormesis.

Keywords: seed germination, early growth, hormesis, allelopathy, phytotoxicity, *Reynoutria,* biotechnology

Introduction

Knotweeds are invasive plants [1] capable of crowding out all other vegetation and competing successfully with native plant species. They change the habitat by shading out native vegetation, reducing species diversity, and suppressing wildlife habitat [2]. Three knotweeds exist in the Czech Republic: Japanese knotweed, originating from Japan, Korea and China; Giant knotweed, which is native to Sakhalin; and Bohemian knotweed, which is a hybrid of the two aforementioned species. Japanese knotweed is listed by the World Conservation Union as one of the world's most invasive species.

One effective way to be competitive as an invasive species is to produce allelopathic substances that inhibit the germination of seeds and thus the sprouting and growing of other plants in the same location. A number of phenolic compounds show allelopathic activity [3]. These substances are able to inhibit germination in many sorts of seeds [4, 5]. Such phenolic compounds as catechins, stilbens, derivatives of quercetin, quinones, chlorogenic acid, and caffeic acids have been identified in the mentioned species of knotweed [6-11].

Knotweed species belong to the *Polygonaceae* family. Some species from this taxonomic group, such as *Polygonum persicaria* L. or *P. plebeium* R. Br., are characterized by the allelopathic effect [12, 13]. Phytotoxic activities of knotweeds have been evaluated in the laboratory using lettuce seeds [6] and mustard seeds [14, 15]. Knotweed extracts also have been found to be toxic for the larvae of *Spodoptera littoralis* [16]. It has been discovered that the most toxic knotweed species is Giant knotweed, and the most phytotoxic part of the plant body is the leaf [14].

Knotweed extracts are used against powdery mildew on vegetables [17] and knotweed extract has been tested against powdery mildew on wheat [18]. The active sub-

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stances can move into the soil after spraying the vegetables and plants. That may affect the subsequent germination and growth of other cultivated plants or weeds. The aim of this study was to test phytotoxic activity in soil that was contaminated with knotweed leaves. The test was performed using two common crop plants and two intractable weed species. The differences in germination and early growth of the tested seeds were compared.

Material and Methods

Plant Materials

The tested seeds of weed plants (*Chenopodium album* agg., *Echinochloa crus-galli* (L.) P.B.) and the leaves of knotweeds (*Reynoutria japonica* Hoult, *R. sachalinensis* (F. Schmidt) Nakai, *R. x bohemica* Chrtek et Chrtková) were collected on their natural locations in the vicinity of České Budějovice, Czech Republic, during autumn 2006. We collected only undamaged vital leaves. The dried leaves of knotweed were stored in darkness under laboratory conditions (at 21ºC). Before use, the dried leaves were shattered into particles no larger than 0.3 cm and mixed with soil substrate (see below).

The mustard seeds (*Leucosinapis alba* (L.) Spach VERONICA) were purchased in a special shop. The rapeseeds (*Brassica napus* L. OPONENT) were obtained from the Oil Crops Research Institute in Opava, Czech Republic. The seeds were manually sorted, cleaned of vegetative residues, and held at 6ºC until the start of the tests.

Soil Substrate

The soil substrate consisted of two parts: a horticultural-flower potting soil (purchased from Rašelina Soběslav, Soběslav, Czech Republic) mixed with sand at a 2:1 ratio. The parts of the substrate used were sieved through a screen (mesh 3 × 3 mm). The soil mixture was enriched with pulverized limestone (3 g per 5 l) and 14 g of dried knotweed leaves were added per 1 l of soil substrate. The amount of dry leaves was determined on the basis of previous laboratory experiments with extracts from knotweed [15]. Each knotweed species was tested separately. The soil substrates were neither fertilized nor sterilized.

Seed Germination Test

Plastic 4 × 4 × 6 cm flowerpots were filled with 30 ml of the substrate containing the leaves of one species of knotweed and then placed onto a watering tray. The tray was filled with 750 ml of deionized water to moisten the soil in the flowerpot before setting the tested seeds. Twenty seeds were planted in each flowerpot. For each assay (one species of knotweed leaves x one species of tested seeds), 36 flowerpots were used. The seeds were incubated in a greenhouse at 18ºC during the day and 10ºC at night. The soil was regularly irrigated on the trays with the same amount of water. The weed seeds were incubated for three months. The number of germinating seeds was registered daily and sprouting plants were removed from the flowerpots. We recorded only vital sprouting plants with well-developed cotyledons. The flowerpots with crop plants were incubated for 10 days (8-18 June 2007) and the test with weed plants was prolonged for three months (5 March-8 June 2007).

Data Processing

We used two-way ANOVA (with repetition, balanced design, fixed factors: “leaf” and “seed”) to evaluate the influence of each knotweed-treated soil on germination of the various seeds. The “leaf” factor comprised the soil substrate with dried leaves of *R. japonica*, *R. sachalinensis*, and *R. x bohemica*. In the first assay, the factor “seed” represented the data obtained from the germination of *L. alba* and *B. napus* (crop plants). In the second assay, the factor “seed” comprised the seed germination of *C. album* and *E. crus-galli* (weed plants). The dependent variable was the number of germinated seeds. One-way ANOVA was used for detailed comparison of the experimental variants, followed by Tukey’s HSD test for multiple comparisons. All the statistical tests were performed at the 0.05 level of statistical significance.

Results

Significant differences for the factor “seed” and interaction between the factors “leaf x seed” were found in the germination of weed plants (two-way ANOVA). Thus, type of seeds used (cultural versus weed plants) and both tested factors together were more important than the type of knotweed leaves used in preparing the soil substrate.

All three knotweed species significantly influenced germination of the tested crop plant *L. alba*, but only *R. japonica* and *R. sachalinensis* inhibited the germination of *B. napus* seeds (one-way ANOVA, Table 1). The highest inhibition was recorded in the substrate containing *R. japonica* leaves. *R. japonica* intensely inhibited the seeds of both tested crop plants, as *L. alba* had germination of 35% and *B. napus* 43%. On the other hand, the substrate containing leaves of *R. x bohemica* inhibited germination only of the *L. alba* seeds.

The data obtained using weed plants were quite different (Table 1). We found no significant difference in germination among the tested seeds. The leaves of knotweeds did not influence germination of *C. album*. Seeds of *E. crus-galli* germinated better in the soil substrate contaminated by knotweed leaves. Indeed, the highest germination rate for *E. crus-galli* (in soil substrate with leaves of *R. sachalinensis*) was 191% of that for the control sample. We should note that the numbers of germinated seeds for the weed plants were, on the whole, small (Table 1).

Table 1. Numbers of germinated seeds. Those with same letters did not differ significantly in multiple range analysis (Tukey test, P<0.05, see column HSD).

Assay	Seed	Leaf	Mean	SD	(%)	HSD
Cultural plant	*L. alba*	*R. japonica*	3.86	2.21	35	a
		R. x bohemica	7.97	2.70	72	b
		R. sachalinensis	7.03	2.60	64	b
		Control	11.00	2.81	100	c
	B. napus	*R. japonica*	5.83	2.86	43	a
		R. x bohemica	12.69	3.13	93	b
		R. sachalinensis	8.61	3.31	63	c
		Control	13.64	2.67	100	b
Weed plant	*C. album*	*R. japonica*	3.03	2.24	76	a
		R. x bohemica	2.83	2.25	71	a
		R. sachalinensis	3.39	1.96	85	a
		Control	3.97	2.25	100	a
	E. crus-galli	*R. japonica*	1.72	1.78	138	ab
		R. x bohemica	1.97	1.59	158	ab
		R. sachalinensis	2.39	1.40	191	ab
		Control	1.23	1.23	100	b

Discussion

Invasive species release substances that alter the soil biota. This change disadvantages native plants [19]. The presence of invasive plant species has in some habitats eliminated many native species [20, 21]. Knotweed species are perennial plants whose stems and leaves grow again each season. They have the ability for rapid vegetative regeneration [22], production of enormous amounts of seeds, and production of vast amounts of biomass per growing season [23]. In autumn, the fallen leaves decompose and assimilate into the soil at the location where knotweed is growing. The leaves of all three knotweed species contain phenolic compounds, namely catechin, epicatechin, chlorogenic acid, caftaric acid, and quercetin derivatives [7, 11]. The content of the compounds differ among all knotweed species. The largest amount of catechin (2,700 mg/kg) has been found in the leaves of Giant knotweed [11]. Catechin exuded from *Centaurea maculosa* roots has been shown to inhibit germination and growth of *Arabidopsis thaliana* species [24]. Catechin may be a compound playing a crucial role in knotweed phytotoxicity.

In addition, persistent rhizomes of knotweed also contain a number of phenolic compounds [1, 8]. These phenolic compounds influence the phytotoxic and allelopathic properties of knotweed plants [3, 5, 6, 25, 26]. One laboratory test has demonstrated that root tips can selectively take up flavonoids and that these are capable of moving in the roots [27]. Thus, the plant body may take up some extracts (e.g. phenolic compounds) from the soil. This may be the reason why knotweed is so successful in competing with other plants. The soil from a location where knotweeds grow inhibits germination of other plant species. This effect of contaminated soil may be as important for highly competitive plants as are dense growth, regeneration from rhizomes, or vegetative propagation.

Findings from laboratory tests using leaf extracts differed from results when examining natural conditions in soil. In the laboratory, free compounds are concentrated into the various extracts [28]. In nature, the components from leaves are assimilated into soil and compounds from rhizomes are subsequently released. The situation under natural conditions may be more complicated. It should be remembered there can also be influences of soil age and type, action of soil microorganisms, and the soil adsorption of active components [29].

Very important is the fact that the seeds of various plant species reveal different sensitivities to the same biochemicals [5, 30, 31]. It is interesting that no sensitivity to knotweed-treated soil was found for the weed seeds and that seeds of *E. crus-galli* were even stimulated (Table 1). Some substances, although toxic at higher doses, can be stimulatory or even beneficial at low doses [32, 33]. This biphasic dose/response effect is termed hormesis. Compared with the common monotonic relationship between the dose of a toxic substance and the resulting response, the hormesis is characterized by an increase in response at low doses that change to inhibition at higher

doses [32]. For example, shoot biomass of *C. album* changed in relation to doses of metamitron (herbicide) after spray application [33]. No sensitivity of both weeds to knotweed-treated soil (Table 1) can probably be explained by a low concentration of knotweed leaves in the used soil.

Both tested annual weed species are widespread in agricultural fields and ruderal habitats. They are characterized by a huge production of seeds in a given growing season [34] and persistent seed banks [35]. The plants exhibit high phenotypic plasticity and genetic variability. They easily adapt to a multitude of agronomic and ruderal habitats [35]. Many weeds develop multiple resistance to various chemicals, and such reproductive traits play an important role in resistance to many pesticides.

Only a limited number of plant species grow close to a knotweed population. Knotweeds probably affect the selective toxicity of the soil, as indicated by the results presented here. Subsequent research will be focused on testing *in situ* the germination of seeds of those plants that do grow on locations together with knotweed plants and on hormesis, probably found in the relationship between tested weeds and soil contaminated by knotweed leaves.

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