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ALLELOPATHIC EFFECTS OF AQUEOUS EXTRACTS FROM SIX HYGROPHYTE SPECIES ON ACTIVITIES OF *P. JAPONICA*

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ABSTRACT: Little has been reported on ecological characteristics of *Phragmites japonica* Steud. (Poaceae), perhaps due to its narrow distribution in the Far East. In this study, therefore, allelopathic effects of aqueous extracts (5.0 g L^{-1}) from leaves or stems of six hygrophytes, *Typha latifolia* L., *Phragmites australis* (Cav.) Trin., *Lythrum salicaria* L., *Phalaris arundinacea* L., *Phragmites japonica* Steud. and *Scrirpus tabernaemontani* Gmel., on the activities of *P. japonica* seed and epigeal stolon were assayed under laboratory conditions, and growth after transplanting was also evaluated. All aqueous extracts from six hygrophytes produced no inhibition on seed germination. The radicle length of the seedlings, however, was inhibited by treatment with aqueous extracts from leaves of *P. australis*, *L. salicaria* and *P. arundinacea* (35.5-57.2% of untreated control). Further, these inhibitory effects on the radicle length of the seedlings are likely caused by some kind of allelochemicals, based on the lack of effect with preparations of the aqueous extracts on a polymeric absorbent (Amberlite XAD-4 resin). For epigeal stolon activity, no negative effects were observed for aqueous extracts of any of the hygrophytes. Dry weights of the above- and below-ground organs formed from epigeal stolon also reached 7.3 times of those of seedlings after the transplanting.

Keywords: Allelopathy, Epigeal stolon, Phragmites japonica, Polymeric absorbent, Seed

1. INTRODUCTION

Safety and security, convenience, comfort, and aesthetic beauty in cities have been ensured. Over about the last decade, "biodiversity" in cities has been also achieved through management of natural and manmade features, such as ponds, parks, wetlands, rivers, gardens, groves, and masonry revetments [1, 5, 6, 9, 13, 18, 20, 24, 26, 34, 36]. Further, a new keyword, green infrastructure, frequently appears when considering urban design and planning as the "strategic utilization, maintenance, connection and/or creation of natural and semi-natural areas, such as groves, parks, farmlands, ponds and rivers in urban areas as infrastructure features equivalent to roads, bridges and ports that function to mitigate the heat island effect, deterioration in biodiversity and damage due to weather events, such as severe rainstorms" [2, 16, 17, 25, 37, 39].

Many kinds of plant species have been used in green infrastructure projects. Among them, especially *Phragmites australis* (Cav.) Trin. is more suitable in the projects along waterfronts because it exhibits a wide range of functions, such as filtering nutrients and sediments of water (purification), binding soils (erosion control), and offering habitats for wild animal communities, fish, birds, and mammals [8, 10, 11, 19, 21, 30, 31, 38, 40, 41].

Phragmites (Poaceae) includes several species including the aforementioned *P. australis*. Among them, in Japan, *Phragmites japonica* Steud. along with *P. australis* is a frequently used plant material in green infrastructure projects along waterfronts. Compared to *P. australis*, which is used along relatively stable waterfronts, *P. japonica* has been used in more unstable areas such as along rivers owing to its high tolerance to flooding (disturbance); it can regrow rapidly after disturbance by elongating the epigeal stolons, which are also known as runners (Photo 1) and which attain lengths of 3-4 m or more and form clonal shoots at each node, by which its territory is expanded [22].

Although *P. australis* is a cosmopolitan species with a long and extensive body of research into its ecological, morphological and cytogenetical properties [3, 4, 7, 8, 10, 11, 19, 21, 23, 30, 31, 33, 38, 40, 41], little research has been conducted on *P. japonica*, likely due to its restricted distribution in Japan, Korea, China and Ussuri [12].

In this study, the objective is to collect ecological information on *P. japonica* relevant to its use in green infrastructure. Here, the allelopathic effects of aqueous extracts from leaves or stems of six hygrophytes on the activities of *P. japonica* seed and epigeal stolon in laboratory studies, and their growth after transplanting were examined.

2. MATERIALS AND METHODS

2.1 Allelopathic Effects of Aqueous Extracts

2.1.1 Aqueous extract preparations

Leaves of five species of perennial hygrophytes, *Typha latifolia* L., *P. australis*, *Lythrum salicaria* L., *Phalaris arundinacea* L. and *P. japonica*, and stems of one species of perennial hygrophyte, *Scrirpus tabernaemontani* Gmel., which shows no leaf, were used as test materials. The leaves and stems of these hygrophytes were collected during early summer (May to June) in Hokkaido, Japan (Table 1), rinsed with distilled water and air dried at room temperature (18-20 °C). They were then cut into lengths of 2-4 cm and ground up into a fine powder using a MC210-YE grinder (Sanyei Corporation, Tokyo, Japan). To prepare solutions for testing effects on *P. japonica*, 0.5 g of each hygrophyte powder was weighed and suspended in 100 mL of distilled water. After 24 h at room temperature, the aqueous extracts (5.0 g L^{-1}) were strained through two layers of cheesecloth and then through two layers of Whatman No. 2 filter paper (Whatman International Ltd., Maidstone, England) to remove solid materials. These aqueous extracts were further strained through 0.2 µm membrane filters (Nalge Nunc International Corporation, Rochester, USA) to remove bacteria prior to use in bioassays.

The extracts had a pH in the range of 5.1 to 6.0 and EC of 12.56 to 94.21 mS m^{-1} at room temperature.

2.1.2 Bioassay-I

(i) Seeds

Phragmites japonica seeds were obtained from

[A] epigeal stolons elongated from *P. japonica* community; [B] close-up of clonal shoot and adventitious roots formed from node of an epigeal stolon in [A]; [C] mature clonal shoots formed at each node- *P. australis* shows no epigeal stolon.

Photo 1 *Phragmites japonica* epigeal stolon.

Table 1 Outline of sampling sites of test hygrophytes from Hokkaido.

Test plant	Sampling site †	Field condition	Growth condition ††	
			Plant height (cm)	Number of stems (No. m^{-2})
T. latifolia	Obihiro	Around pond	143.8 ± 10.1	5.3 ± 1.5
P. australis	Taiki	Marsh	183.2 ± 13.3	17.3 ± 2.5
L. salicaria	Obihiro	Around pond	101.2 ± 9.9	12.3 ± 2.1
P. arundinacea	Obihiro	Riverside	151.2 ± 4.0	85.7 ± 7.2
S. tabernaemontani	Taiki	Marsh	145.2 ± 9.8	51.7 ± 6.0
P. japonica	Obihiro	Riverside	175.0 ± 8.7	22.3 ± 4.0

†, City name. ††, Investigated in mid-August. Mean ± S.D. (n=5).

Snow Brand Seed Co., Ltd., Sapporo, Japan. For each hygrophyte, 5 mL of aqueous extract (or distilled water as a control) was added to eight Petri dishes (90 mm diameter) each lined with three layers of Whatman No. 2 filter paper and containing 36 *P. japonica* seeds. The Petri dishes were sealed with film paraffin (American National Can Company, Chicago, USA) to prevent loss of moisture and incubated at 25 °C under daylight at the greenhouse of the Center for Regional Collaboration in Research and Education, Obihiro University, Japan for 7 days. And then, seed germination was recorded. Radicles were fixed in FAA (ethanol:formalin: acetic acid: distilled water= 10:1:1:8), and the radicle length was measured and expressed as a percentage of the control.

(ii) Epigeal stolons

Epigeal stolons of *P. japonica* were obtained from natural communities growing along the Satsunaigawa River, Obihiro, Japan. The epigeal stolons were cut into several segments, 15 cm in length with one node for bioassay use- a clonal shoot and adventitious root could be formed from the node under appropriate moisture conditions (Appendix). Twenty epigeal stolon segments were placed in a transparent plastic dish (10.0×32.5×3.5 cm height), and 300 mL of aqueous extract (or distilled water as a control) was added to the dish. Five replicates were used for each hygrophyte or control. The total aqueous extract or distilled water volume in the dish was maintained throughout the experimental period by adding distilled water daily. The epigeal stolon segments were incubated at 25 °C under daylight at the greenhouse mentioned above for 21 days. At the end of the incubation period, the number of nodes that formed clonal shoots were counted. The epigeal stolon segments were fixed in FAA, and the length of the formed adventitious root was measured. The number of nodes forming clonal shoots was expressed as a percentage, shoot formation rate (%), using the following Eq. (1), while adventitious root length was expressed as a percentage of the control.

$$\text{Shoot formation rate (\%)} = NNFC / TNN \times 100 \quad (1)$$

Where NNFC is the number of nodes that formed clonal shoots per dish, and TNN is 20 for this study or the total number of nodes (epigeal stolon segments) per dish.

2.1.3 Bioassay-II

In this study, aqueous extracts were passed through Amberlite XAD-4 resin (hereafter, XAD-4 resin; Rohm & Haas, Philadelphia, USA), a polystyrene-based absorbent with a high surface area that removes various allelochemicals [14, 27, 28, 29, 42]; the aqueous extracts were strained twice through an 88 mL volumetric column (15 mm diameter×500 mm height; AGC Inc., Tokyo, Japan) filled with 50 mL of XAD-4 resin at a flow rate of 3 mL min^{-1} using a PST-100N peristaltic pump (Iwaki Glass Co., Ltd., Tokyo, Japan). The resulting solutions (hereafter, XAD-4 preparations) had a pH in the range of 5.1 to 6.6 and EC of 12.14 to 92.11 mS m^{-1} at room temperature.

The same bioassays described in *Bioassay-I* were performed but with XAD-4 preparations with five replicates for only those hygrophytes that showed inhibitory effects on the radicle length of seedlings in *Bioassay-I*.

2.2 Growth after Transplantation

Seeds and epigeal stolons, which were cut into 5, 10 and 15 cm segments with one node each, were incubated at 18 ℃ in the light in distilled water in a MIR-553 incubator (SANYO Electric Co., Ltd., Osaka, Japan). Eight seedlings and epigeal stolon segments that formed clonal shoots from the node were then immediately transplanted to individual 1/5000a Wagner pots (Fujiwara Scientific Company, Tokyo, Japan) filled with soil (volcanic ash soil:alluvial soil:vermiculite=6:3:1) and further incubated at 25 °C under daylight with regular watering at the greenhouse mentioned above. No fertilization was used for cultivation. Dry weights of stems, leaves, radicles (or adventitious roots for clonal shoots) and rhizomes in seedlings and clonal shoots were measured at 120 days after transplanting.

2.3 Data Analysis

Differences among the means were analyzed by one-way analysis of variance (ANOVA). Probabilities less than 0.05 were considered significant. Differences between means were determined using Bonferroni's multiple comparison test.

3. RESULTS

3.1 Allelopathic Effects of Aqueous Extracts

Compared to the control (Table 2), no aqueous extracts from the six tested hygrophytes inhibited seed germination ($p=0.072$). The radicle length of the seedlings, however, was inhibited by treatment with aqueous extracts from *P. australis*, *L. salicaria* and *P. arundinacea* ($p<0.001$) compared to the control. Incidentally, the radicle length of *P. japonica* seedlings was not inhibited by treatment with an aqueous extract from its own leaves compared to the control.

For all aqueous extracts, no inhibitory effects were observed for shoot formation rate from epigeal stolon segments ($p=0.220$). There were significant

Table 2 Allelopathic effects of aqueous extracts on seed germination, radicle length of seedling, shoot formation rate from epigeal stolon, and adventitious root length of clonal shoot in *Phragmites japonica*.

	Seed		Epigeal stolon	
	n =8		*n* =5	
Test plant	Germination (%)	Radicle length (% of control) ††	Shoot formation rate (%)	Adventitious root length (% of control) ††
Control †	89.9 ± 6.8 A	100.0 ± 17.8 B	76.0 ± 4.3 A	100.0 ± 12.2 AB
T. latifolia	89.6 ± 3.9 A	110.1 ± 13.9 B	80.2 ± 4.1 A	99.1 ± 8.7 AB
P. australis	86.5 ± 6.7 A	57.2 ± 18.5 A	76.9 ± 4.8 A	98.8 ± 8.2 AB
L. salicaria	87.8 ± 4.9 A	35.5 ± 6.5 A	80.1 ± 7.2 A	106.5 ± 11.9 AB
P. arundinacea	87.2 ± 4.4 A	38.1 ± 17.1 A	81.8 ± 2.5 A	91.2 ± 18.0 AB
S. tabernaemontani	88.9 ± 4.5 A	101.7 ± 10.0 B	75.9 ± 6.0 A	86.2 ± 9.7 A
P. japonica	94.4 ± 4.2 A	84.7 ± 7.7 B	81.7 ± 3.1 A	110.3 ± 11.2 B
	p=0.072	p<0.001	p=0.220	p=0.025

†, Distilled water. ††, Values are expressed as a percentage of the control.
Mean ± S.D. The levels of significance are also shown.
Within any column, values followed by the different letters are significantly different using Bonferroni's multiple comparison test.

Table 3 Allelopathic effects of XAD-4 preparations from the three hygrophytes on seed germination and radicle length of seedling in *Phragmites japonica*.

Test plant	Solution	Germination (%)	Radicle length (% of control) ††
P. australis	Control †	91.1 ± 6.6 A	100.0 ± 21.5 B
	Aqueous extract	86.1 ± 8.8 A	53.7 ± 14.1 A
	XAD-4 preparation	93.9 ± 3.0 A	47.2 ± 16.4 A
		p=0.209	p<0.001
L. salicaria	Control †	91.1 ± 6.6 A	100.0 ± 21.5 B
	Aqueous extract	89.4 ± 5.0 A	41.2 ± 4.3 A
	XAD-4 preparation	86.1 ± 7.3 A	69.0 ± 8.7 AB
		p=0.475	p<0.001
P. arundinacea	Control †	91.1 ± 6.6 A	100.0 ± 21.5 B
	Aqueous extract	87.8 ± 2.5 A	35.2 ± 16.6 A
	XAD-4 preparation	92.8 ± 4.6 A	96.2 ± 12.2 B
		p=0.295	p<0.001

†, Distilled water. ††, Values are expressed as a percentage of the control.
Values are the means of five replicates with S.D.
The levels of significance are also shown. Values followed by the different letters are significantly different using Bonferroni's multiple comparison test.

differences in the length of adventitious roots formed from clonal shoots between when treated with aqueous extracts of *S. tabernaemontani* and when treated with those of *P. japonica* (p=0.025), but none of the aqueous extracts from the six hygrophytes inhibited adventitious root length of the clonal shoot compared to the control.

3.2 Allelopathic Effects of XAD-4 Preparations

The bioassay with *P. japonica* seeds was repeated using XAD-4 preparations from the three hygrophytes, *P. australis*, *L. salicaria* and *P. arundinacea* that produced inhibition of the radicle length in seedlings (Tables 2 and 3). The aqueous extract from *P. australis* had no effect on seed

Table 4 Growth of *Phragmites japonica* seedlings and clonal shoots from epigeal stolon segments of different lengths at 120 days after transplanting.

Item	Seed	Length of epigeal stolon segment (cm)			
		5	10	15	
Weeks for seed germination or clonal shoot formation	0.59 ± 0.10 A	1.73 ± 0.54 B	1.74 ± 0.51 B	1.86 ± 0.57 B	p<0.001
Stems (g-DW / individual)	0.16 ± 0.05 A	1.78 ± 0.24 B	1.63 ± 0.31 B	1.75 ± 0.28 B	p<0.001
Leaves (g-DW / individual)	0.18 ± 0.05 A	1.71 ± 0.31 B	1.71 ± 0.21 B	1.66 ± 0.24 B	p<0.001
Radicles (g-DW / individual)	0.35 ± 0.08 A	1.95 ± 0.24 B	2.11 ± 0.29 B	2.01 ± 0.19 B	p<0.001
Rhizomes (g-DW / individual)	0.20 ± 0.03 A	1.08 ± 0.16 B	0.99 ± 0.14 B	1.17 ± 0.27 B	p<0.001

Values are the means of eight replicates with S.D. The levels of significance are also shown.
Within any row, values followed by the different letters are significantly different using Bonferroni's multiple comparison test.

germination and there was also no effect of XAD-4 preparations on seed germination (p=0.209) compared to the control. The aqueous extract from *P. australis* inhibited the radicle length of the seedlings, and the XAD-4 preparation also inhibited the radicle length of seedlings (p<0.001) compared to the control.

Similarly, no allelopathic effect was observed for the aqueous extract from *L. salicaria* or for its XAD-4 preparation on seed germination (p=0.475). The aqueous extract from *L. salicaria* inhibited the radicle length of seedlings (p<0.001), but no inhibition was observed for the XAD-4 preparation compared to the control. Similar results were also observed for *P. arundinacea*. That is, the aqueous extract from *P. arundinacea* and the XAD-4 preparation showed no effect on seed germination (p=0.295), while the aqueous extract from *P. arundinacea* inhibited the radicle length of the seedlings (p<0.001) compared to the control, but the XAD-4 preparation did not.

3.3 Growth of Seedlings and Clonal Shoots

The segment length of epigeal stolon had no effect on the timing of the initiation of clonal shoot formation and dry weight after transplanting (Table 4).

Although the timing of the beginning of seed germination was significantly earlier (p<0.001), the 120 day dry weights of seedlings were remarkably lower in all organs (p<0.001) compared to those of clonal shoots from the epigeal stolon; dry weights of above- and below-ground organs of clonal shoots formed from epigeal stolon segments were 7.3 times of those of the seedlings.

4. DISCUSSION

Phragmites japonica is one of the crucial plant materials in green infrastructure, especially along water edges. However, perhaps due to their restricted distributions in the Far East [12], there have been few ecological studies on *P. japonica*. As allelopathy is one of the mechanisms to explain the propagation strategies of plant species, the allelopathic effects of aqueous extracts from different hygrophytes on the activities of *P. japonica* seeds and epigeal stolons were assayed here, along with growth after transplanting.

Our results show that the epigeal stolon is more tolerant to allelopathic inhibitory effects of aqueous extracts from various hygrophytes than the seed (Table 2). Further, the dry weight of the above- and below-ground organs of clonal shoots from the epigeal stolon reached 7.3 times of those of the seedlings after transplanting (Table 4). These data suggest that the invasion success of *P. japonica* is mainly attributable to epigeal stolons, which can grow vigorously regardless of segment length (Table 4), a finding that is supported by the observation that *P. japonica* seedlings are seldom observed in natural settings.

On the other hand, some allelopathic inhibitory effects on the radicle length of seedlings were not observed in tests with XAD-4 preparations (Table 3), unlike in the case of aqueous extracts (Table 2). That is, some compounds that are absorbed by Amberlite XAD-4 resin, e.g. isovitexin, 3-hydroxy hydrocinnamic acid, vitexin and C-glucosyl flavonoid [14, 15, 27, 28, 29, 42], apparently contribute to the inhibitory effects on the radicle length of seedlings as allelochemicals. Interestingly, self-inhibition among *P. japonica* is unlikely because adverse impacts were not observed in the treatment with an aqueous extract from its own leaves both in seeds and in epigeal stolons (Table 2).

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6. APPENDIX

Clonal shoots and adventitious roots formed from each node of epigeal stolon segments of *Phragmites japonica.*

Pictures were taken separately from the results of this study in September 2018.
[A] epigeal stolons of *P. japonica* in the Mikasagawa River, Fukuoka, Japan; [B] collected epigeal stolons from the river; [C] epigeal stolon segments obtained from [B]; [D] segments soaked into distilled water; [E] clonal shoots and adventitious roots formed from [D] (fourteen days later).

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