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ORIGINAL ARTICLE

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Comparison of phenolic compounds and the effects of invasive and native species in East Asia: support for the novel weapons hypothesis

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Abstract One prediction of the novel weapons hypothesis (NWH) for the dominance of exotic invasive plant species is that the allelopathic effects of successful invaders will, in general, be more biochemically inhibitory to native species and microbes in invaded regions than the native plants themselves. However, no study has compared biochemical concentrations, compositions, or effects of large numbers of native species to those of large numbers of invasive species. In this context we tested the allelopathic and antimicrobial potentials of nine native plant species and nine invasive species in East Asia by comparing their broad phenolic contents and the effects of extracts made from each of the species on target plants and soil fungi. Three of the invasive species, including *Eupatorium rugosum*, had higher concentrations of total phenolic compounds than any of the native species, and the mean concentration of total phenolics for invasive species was 2.6 times greater than the mean for native species. Only scopoletin was novel to the invasive species, being found in all of nine invasive species, but not in the native species. More importantly, the effects of the total suites of phenolic compounds produced by invasive species differed from the effects of phenolics produced by natives. Extracts of invasive species reduced radicle growth of the three test plant species by 60–80%, but extracts of native species reduced radicle growth by only 30–50%. Extracts of invasive species reduced shoot growth of the three test species by 20–40%, but the overall effect of native species' extract was to stimulate shoot growth. The antimicrobial activity of invasive species was also significantly higher than that of native species. It should be noted that phenolics are just one component of a plant's potential allelopathic arsenal and non-phenolic compounds are likely to play a role in the total extract effect. For example, extracts of *P. americana* contained the lowest levels of phenolic compounds, but exhibited the strongest inhibition effect. We could not determine whether the greater inhibitory effects of the extracts from invasive species were due to novel combinations of chemicals or higher concentrations of chemicals, but our results are consistent with the predictions of the NWH.

Keywords Allelopathy · Antimicrobial · Invasive plant · Native plant · Phenolic compound

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Introduction

Large-scale introduction of invasive plants has occurred in many regions and in some cases, threatens the local biodiversity of natural systems by displacing communities of native plants. Often these introductions change community structure and ecosystem functions (Lovel 1997; Keane and Crawley 2002; Kennedy et al. 2002). Interestingly, many invasive plants are not dominant competitors in their natural ranges, but appear to competitively diminish or eradicate neighbors in non-native systems.

Recent comparative studies of invasive and native plants have explored the mechanisms for competitive interactions in invasive plant invasions, including tests of the "novel weapons hypothesis" (NWH). This is the idea that some invasive species may gain disproportionate competitive advantages through the effects of novel secondary metabolites to which species in invasive regions have not had the opportunity to adapt (Callaway and Ridenour 2004). Novel secondary metabolites may have stronger allelopathic, antimicrobial, or herbivore defense

effects. The NWH suggests the possibility of diffuse yet important co-evolution among organisms in different regions. Derived from the NWH, the "allelopathic advantage against resident plant" hypothesis (AARS) posits that some species with novel secondary metabolites may experience strong selection to produce higher amounts of chemicals due to exceptional effectiveness in non-native ranges. However, it is important to note that novelty may both enhance (Callaway and Ridenour 2004) or resist (Weidenhamer and Romeo 2005; Verhoeven et al. 2008) invasions, and it is reasonable to expect selection on novel secondary compounds to either increase or decrease their concentrations in tissues, leachates, or exudates (Callaway and Ridenour 2004; Ridenour et al. 2008).

We focused on phenolic compounds with the potential to affect plants, herbivores, and soil microbes through the release of plant phytotoxins from plant tissues. We also focused on plant species that were invasive or native to a single habitat in the Hanam region on the outskirts of Seoul, Korea. We chose to analyze phenolic compounds, a group of compounds that are widespread in the plant kingdom; low molecular weight phenolics are precursors of a variety of antimicrobial compounds and play an important role in plant defense responses against competitors, predators and pathogens (Nakatani 1994; Wu et al. 1998; Ejechi et al. 1999; Seneviratne and Jayasinghearachchi 2003).

A total of 33 families containing 194 invasive plants have been recorded in Korea (Park 1995), proving an excellent opportunity to explore mechanisms for invasions across large numbers of taxa. Also, the production of phenolic compounds by many exotic species (e.g., *Pueraria thunbergiana*, *Ambrosia artemisiifolia* var. *elatior* and *Pinus rigida*) has been studied intensively in Korea (Kim and Lee 1996; Kil 1999; Kim et al. 2005a, b).

Our primary objectives were to compare concentrations and composition of large phenolic compounds in invasive and native plant species, and to examine the allelopathic effects of plant extracts on seedling growth and the inhibition of common soil pathogens.

Materials and methods

We collected species in a field near Hanam (37°35′N, 127°12′E) in the outskirts of Seoul, Korea. The 20 ha study area lies on the banks of the Han River. The mean annual precipitation is 1,340 mm, and mean annual temperature is 13.2°C. More than half of the annual precipitation occurs in summer (June–August) and winters are dry and cold, reflecting a typical continental weather pattern. Sixteen invasive and 16 native plant species were selected at random for preliminary seed germination testing to find plants with apparent allelopathic effects. Plant extracts were prepared from leaves of ten individual plants per species, and total phenolic compounds were analyzed in these extracts. Nine native and nine invasive plant species (Table 1) were then selected for more detailed analysis of phenolic compounds and antimicrobial activity. Aqueous extracts from these plants were used for seedling growth inhibition experiments and ethanol extracts were used to assess antimicrobial activity (McMurrough 1992). We used two invasive species (*Rumex acetocella* and *Oenothera odorata*) and one native species (*Plantago asiatica*) for target species against which to test extracts. This does not allow a complete biogeographic test of the NWH because the biogeographic matches are not precise, but does allow a rather conservative comparison of broad biochemical effects of invasive and native species. Seedlings were collected from the field to use for seedling growth bioassays. Six common soil fungi, *Aspergillus* sp. (*A. versicolor* IAM 2080, *A. phoenicis* KCTC 1228, *A. usamii* mut. *shiro-usamii* KCTC 1291, *A. parasiticus* KFU 3074, *A. oryzae* KCCM 1372, and *A. awamori* PC) were obtained from the Rural Development Administration (http://www.rda.go.kr/foreign/eng/) and used for the antimicrobial bioassay.

Analysis of phenolic compounds

Fresh leaves (200 g; collected from ten individual plants) of native and invasive plants were extracted with 1 l distilled water at room temperature then centrifuged at 15,000 rpm for 30 min (Centrikon T-1045, Kontron, Zurich, Switzerland). The supernatant was collected and stored at 4°C. Phenolic compounds were purified from water extracts by adding 10 ml saturated NaCl to 40 ml aqueous extract. The pH was then adjusted to pH 2 with 1 *N* HCl, 20 ml 5% $NaHCO_3$ was added, mixed, and the solution adjusted again to pH 2 with HCl, and 20 ml ether added. The solution was mixed and, after phase separation, the ether layer was collected and evaporated under reduced pressure with a rotary evaporator. The residue was dissolved in 5 ml acetonitrile. Individual phenolics were analyzed by HPLC (Series 1050, Hewlett Packard, Houston, TX) with Diode-Array Detection (250, 254, 284 nm). Extracts (20 µl) were injected into a µBonda-Pak C18 Radial Pak (0.8 × 10 m) column, with a mobile phase of acetonitrile and 0.02 M sodium acetate (pH 4.3), with a flow rate of 1.3 ml/min (Kim and Lee 1996). Total phenolics were measured using the Folin–Ciocalteu phenol reagent (Swain and Hillis 1959). Chemical standards used for analysis of phenolic compounds were purchased from Sigma (St. Louis, MO).

Seedling growth

Extracts of six native and six invasive species (see Fig. 1 for list of plants) were tested for effects on seedling growth of *O. odorata*, *R. acetocella* and *P. asiatica*. Distilled water was used as a control, and extracts were diluted to concentrations of 5, 10, 25, 50, 75 and 100%, and then used in a bioassay to determine the critical concentrations of inhibition. Seeds of uniform size were sterilized for

Table 1 Quantitative analysis by high performance liquid chromatography (HPLC) of concentrations of individual phenolic compounds (mg/l) from nine native and nine invasive species

PC RT	Pro 2.84	*ρ*-hy 4.57	Cat 5.66	Van 6.27	Syr 6.93	Caf 7.31	*ρ*-co 7.82	Ben 8.03	Fer 11.84	Sco 13.67	Cin 18.32	Total
Native plants												
AK	ND	3.0 ± 0.22	ND	ND	ND	**819.4** ± 24.5	ND	30.0 ± 2.42	1.62 ± 0.02	ND	1.8 ± 0.07	**855.8** d ± 23.45
CA	1.1 ± 0.02	2.0 ± 0.04	146.1 ± 18.96	8.6 ± 1.12	ND	ND	16.7 ± 1.32	**368.3** ± 15.6	1.7 ± 0.01	ND	0.1 ± 0.01	**544.6** f ± 13.12
SN	30.1 ± 3.32	ND	33.9 ± 1.45	ND	ND	**213.**1 ± 15.4	ND	30.2 ± 2.45	ND	ND	1.2 ± 0.02	**308.5** i ± 15.34
PT	0.3 ± 0.01	3.6 ± 0.08	**177.4** ± 11.2	ND	ND	19.7 ± 2.32	ND	79.1 ± 4.56	5.4 ± 0.72	ND	5.3 ± 0.82	**290.8** j ± 9.56
CM	9.0 ± 1.12	8.0 ± 0.06	0.2 ± 0.02	4.3 ± 1.02	ND	ND	12.7 ± 0.12	**150.0** ± 10.34	4.1 ± 0.08	ND	2.5 ± 0.032	**190.8** k ± 8.56
FJ	11.8 ± 2.27	7.9 ± 1.32	0.7 ± 0.04	0.1 ± 0.02	2.1 ± 0.12	32.4 ± 5.12	ND	**83.7** ± 7.02	ND	ND	ND	**138.7** m ± 10.02
CK	4.7 ± 1.22	5.9 ± 0.72	1.0 ± 0.02	4.2 ± 0.42	ND	ND	6.5 ± 0.23	70.8 ± 3.44	7.8 ± 0.72	ND	0.5 ± 0.01	**101.5** o ± 7.34
PO	0.2 ± 0.01	ND	1.2 ± 0.07	ND	ND	ND	2.1 ± 0.82	**11.0** ± 1.32	0.1 ± 0.02	ND	1.4 ± 0.52	**16.0** q ± 2.56
TM	0.3 ± 0.02	ND	**6.5** ± 0.86	0.4 ± 0.03	ND	ND	ND	ND	ND	ND	0.7 ± 0.03	**7.9** q ± 0.72
Total	57.5 h ± 8.42	30.4 h ± 2.45	367.1 e ± 21.3	17.6 h ± 0.96	2.1 h ± 0.13	984.6 c ± 33	38.0 h ± 2.69	823.1 d ± 14.7	20.7 h ± 2.54	ND	13.5 h ± 1.55	**2,354.6** ± 32.34
Invasive plants												
ER	7.3 ± 1.22	6.0 ± 0.92	ND	ND	ND	**1,460.9** ± 34	ND	109.7 ± 12.32	ND	0.8 ± 0.05	2.7 ± 0.52	**1,587.4** a ± 21.12
AAR	1.1 ± 0.06	14.1 ± 1.42	ND	1.5 ± 0.32	25.6 ± 2.23	ND	23.8 ± 1.52	**1,237.5** ± 25	ND	134.4 ± 4.52	10.8 ± 1.65	**1,448.8** b ± 17.54
RB	8.3 ± 1.32	9.7 ± 0.56	ND	4.8 ± 0.52	ND	120.9 ± 6.62	22.5 ± 1.82	**753.4** ± 13.45	5.6 ± 0.32	7.2 ± 0.82	1.6 ± 0.01	**934.0** c ± 22.03
AAL	ND	2.6 ± 0.05	ND	0.2 ± 0.01	ND	**418.3** ± 7.22	144.4 ± 5.67	153.5 ± 9.54	ND	0.9 ± 0.02	4.5 ± 0.42	**734.4** e ± 14.23
EC	0.3 ± 0.02	6.9 ± 0.72	ND	13.7 ± 2.12	**227.5** ± 6.82	ND	ND	113.2 ± 3.87	45.7 ± 2.42	112.9 ± 5.45	ND	**520.2** g ± 8.34
OO	ND	1.6 ± 0.08	106.4 ± 5.67	1.3 ± 0.05	ND	**311.0** ± 12.3	ND	78.2 ± 2.34	4.1 ± 0.12	3.7 ± 0.22	0.5 ± 0.02	**506.8** h ± 11.71
RC	ND	ND	55.0 ± 3.42	ND	0.6 ± 0.07	ND	ND	**109.2** ± 9.56	3.7 ± 0.32	1.3 ± 0.05	ND	**169.8** l ± 8.43
PA	ND	1.3 ± 0.01	2.3 ± 0.12	ND	3.5 ± 0.52	12.5 ± 1.4	4.3 ± 0.32	**82.5** ± 5.78	4.3 ± 0.52	0.1 ± 0.02	4.0 ± 0.82	**114.8** n ± 4.45
TO	ND	0.8 ± 0.06	ND	6.5 ± 1.42	13.7 ± 2.22	ND	ND	**60.8** ± 8.34	ND	1.3 ± 0.12	3.1 ± 0.86	**86.2** p ± 5.23
Total	17.0 h ± 0.64	53.0 h ± 2.79	163.7 g ± 5.62	28.0 h ± 1.31	270.9 f ± 118	2,323.6 b ± 56	195.0 g ± 6.68	2,698.0 a ± 58	63.4 h ± 5.2	262.6 f ± 11.2	27.2 h ± 1.5	**6,102.4** ± 62.41

Values are mean ± SE ($N = 3$); means with the same letter are not significantly different (Duncan's multiple range test, $P < 0.001$)

PC Phenolic compound, *RT* retention time (min), *ND* not detectable. Plants: AK *Aster koraiensis*, PO *Portulaca oleracea*, CM *Cassia mimosoides* var. *nomame*, SN *Solanum nigrum*, TM *Taraxacum mongolicum*, CA *Chenopodium album* var. *centrorubrum*, PT *Pueraria. thunbergiana*, FJ *Fatsia japonica*, CK *Cephalotaxus korea*, EC *Erigeron canadensis*, AAR *Ambrosia artemisiifolia* var. *elatior*, PA *Phytolacca americana*, AAL *Ailanthus altissima*, RB *Rudbeckia bicolor*, RC *Rumex crispus*, ER *Eupatorium rugosum*, TO *Taraxacum officinale*, OO *Oenothera odorata*. Phenolic acids: *Pro* protocatechuic acid, *ρ-hy* ρ-hydroxybenzoic acid, *Cat* catechol, *Van* vanillic acid, *Syr* syringic acid, *Caf* caffeic acid, *ρ-co* ρ-coumaric acid, *Ben* benzoic acid, *Fer* ferulic acid, *Sco* scopoletin, *Cin* cinnamic acid

The highest and total phenolic compound levels for each species are indicated in bold face

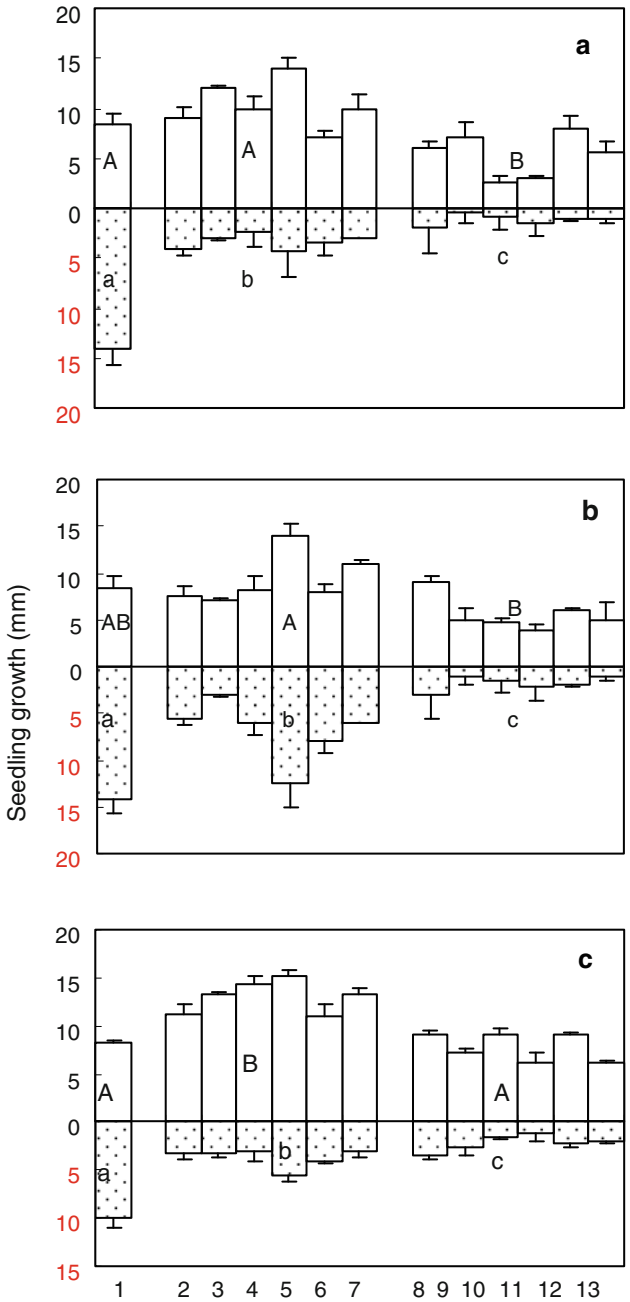

Fig. 1a–c Radicle and shoot growth ($n = 10$) of three plants treated with water (control *1*), aqueous extracts of six native (*2–7*) and six invasive species (*8–13*). **a–c** Length of radicles (*shaded bars*) and shoots (*open bars*) of *O. odorata* (**a**) and *R. acetocella* (**b**) invasive plants, and *P. asiatica* (**c**) native plant. Extracts: *1* Water control, *2 Aster koraiensis*, *3 Chenopodium album* var. *centrorubrum*, *4 Cassia mimosoides* var. *nomame*, *5 Portulaca oleracea*, *6 Taraxacum mongolicum*, *7 Pueraria. thunbergiana*, *8 Erigeron canadensis*, *9 Ambrosia artemisiifolia* var. *elatior*, *10 Ailanthus altissima*, *11 Phytolacca americana*, *12 Eupatorium rugosum*, *13 Taraxacum officinale*. Group (control, native and invasive extracts) means with different letters are significantly different at $P < 0.05$ according to Tukey's test

3 min in a solution of 5% sodium hypochlorite and then rinsed ten times with distilled water. Fifty seeds were sown in Petri dishes (90 mm) and 10 ml extract was added to each dish. The root and shoot length of each seedling was measured after exposure of germinating seeds to a critical concentration of 25% extracts for 7 days. This bioassay was modified from Lodhi (1976) and Rice (1984). The bioassay was repeated four times for each extract collection and against each target species in a growth chamber set at a constant temperature of 28°C, 70% relative humidity, with an alternating photoperiod of 12 h light/12 h darkness using fluorescent lights producing 1,200 μmol m^{-2} s^{-1}.

Antimicrobial bioassay

Potato dextrose agar (PDA) medium was prepared for culturing of soil fungi. Dried leaves (50 g; collected from ten individual plants) were ground in 250 ml 75% ethanol and heated in an 85°C water bath for 3 h and the solution filtered through filter paper (Whatman No. 1), and evaporated at 40°C to a final volume of 50 ml (×5-fold concentration) using a rotary evaporator (Buchii type) before being passed through a 0.2 μm disposable syringe filter. Iron rings (8 mm in diameter), which were used instead of the normal filter disc, were placed in the center of a Petri dish and PDA medium was poured around the rings. After the medium solidified, the iron rings were removed. Fungal spores were then spread evenly over the agar surface and 200 μl of a 75% ethanol solution (control) or 200 μl of a 75% ethanol leaf extract were placed into each ring. Petri dishes were incubated in the growth chamber at 26°C for 6 days. The antimicrobial activity of each assay was calculated as the net zone of inhibition obtained by subtracting the ring diameter (8 mm) from the measured zone of growth inhibition. Ethanol leaf extraction, purification of extraction using a rotary evaporator, preparation of fungal spores and using iron rings on an antimicrobial assay, were modifications of the methods of Hawkey and Lewis (1994), and Mahasneh and El-Oqlah (1999).

Statistical analysis

Data were distributed normally, and significant differences in total phenolic production and antimicrobial activity among native and invasive plants were compared using Duncan's multiple-range tests. Significant differences among control, native and invasive extracts in percent germination and seedling growth parameters of each target plant was determined by ANOVA and post-hoc Tukey tests.

Results

Comparisons of the totals of individually measured phenolic compounds showed that invasive and native plant species contained on average, 678 ± 18.67 and

Table 2 Antimicrobial activity of phenolic compound extracts from nine native and nine invasive species from Korea

			Relative zones of microbial inhibition (mm) by native and invasive plant phenolic compound extracts					
Kind	Species	Origin	AV	AP	AU	AR	AO	AA
Native plants	AK	Native	ND	ND	2.2 q ± 0.6	ND	6.2 m ± 0.8	5.2 n ± 0.8
	CA	Native	ND	ND	2.1 q ± 0.7	3.0 p ± 1.2	2.7 q ± 0.3	ND
	SN	Native	ND	ND	3.1 p ± 0.6	ND	5.1 n ± 0.6	2.3 q ± 0.1
	PT	Native	ND	ND	2.2 q ± 0.2	2.2 q ± 0.4	2.4 q ± 0.1	ND
	CM	Native	ND	ND	ND	ND	2.5 q ± 0.2	ND
	FJ	Native	ND	ND	ND	2.3 q ± 0.3	ND	ND
	CK	Native	1.1 r ± 0.3	ND	ND	1.5 r ± 0.6	2.2 q ± 0.5	ND
	PO	Native	ND	ND	ND	2.4 q ± 0.2	ND	ND
	TM	Native	2.1 q ± 0.2	ND	ND	ND	ND	3.0 p ± 0.3
Invasive plants	ER	North America	8.3 k ± 1.2	9.2 j ± 0.3	12.3 h ± 1.6	16.2 d ± 1.3	2.4 q ± 0.5	2.4 q ± 0.6
	AAR	North America	3.3 p ± 0.4	2.1 q ± 0.7	15.4 e ± 1.2	9.3 j ± 0.2	2.0 r ± 0.6	3.4 p ± 0.7
	RB	North America	2.1 q ± 0.1	2.4 q ± 0.6	ND	2.1 q ± 0.4	ND	8.3 k ± 0.5
	AAL	Asia	ND	9.3 j ± 1.4	ND	12.3 h ± 0.6	3.4 p ± 0.3	4.1 o ± 0.2
	EC	North America	ND	3.4 p ± 0.6	7.2 k ± 1.3l	2.2 q ± 0.3	5.2 n ± 0.7	ND
	OO	South America	ND	2.3 q ± 0.4	ND	ND	2.7 q ± 0.8	2.3 q ± 0.8
	RC	Europe	ND	ND	2.4 q ± 0.2	ND	ND	ND
	PA	North America	17.2 d ± 0.4	15.3 e ± 0.2	14.3 f ± 0.7	20.5 a ± 1.5	14.2 f ± 1.6	17.3 c ± 1.5
	TO	Europe	ND	2.6 q ± 0.3	2.1 q ± 0.6	3.3 p ± 0.4	ND	ND

Values are mean ± SE ($N = 4$); means with the same letter are not significantly different (Duncan's multiple range test, $P < 0.001$). See Table 1 for identity of native and invasive plants

Target pathogens: AV *Aspergillus versicolor* IAM 2080, AP *Aspergillus phoenicis* KCTC 1228, AU *Aspergillus usamii* mut. *shiro-usamii* KCTC 1291, AR *Aspergillus parasticus* KFU 3074, AO *Aspergillus oryzae* KCCM 1372, AA *Aspergillus awamolil*

ND not detectable

261 ± 21.23 mg l^{-1} phenolic compounds, respectively. Among native plants, *Aster koraiensis* had the highest total concentration of phenolic compounds (855.8 ± 23.45 mg l^{-1}) and *T. mongolicum* the lowest (7.9 ± 0.72 mg l^{-1}). Among invasive plants, *E. rugosum* had the highest total (1,587 ± 21.12 mg l^{-1}) of phenolic compounds, and *Taraxacum officinale* the lowest (86.2 ± 5.23 mg l^{-1}). Benzoic acid was the most abundant phenolic compound, with syringic acid being the least common. Scopoletin was detected in nine invasive species but not detected in native species (Table 1, $P < 0.001$).

Leaf extract at a concentration of 25% had dramatic inhibitory effects and thus this concentration was chosen as the optimal concentration for all subsequent studies. Overall growth of invasive *Oenothera odorata* and *Rumex actocella* seedlings was inhibited by phenolic plant extracts of virtually all invasive and native plant species, and radicle growth was suppressed more strongly than shoot growth (Fig. 1a–c, $P < 0.05$). Most importantly, seedling growth was suppressed more strongly (inhibition of up to 70%) by extracts of invasive species than by extracts of native species (Fig. 1a–c). The extracts of native plants even had stimulatory effects in some case (8–45%, Fig. 1a–c, $P < 0.05$).

The extracts of invasive species exhibited considerably more antimicrobial activity than the extracts of native species (Table 2, $P < 0.001$) and the cumulative means of microbial inhibitory zones were 1.0 mm for native species versus 4.1 mm for invasive species. Among invasive species, extracts from the invasive *Phytolacca americana* caused the largest inhibitory zone against all microbes (zones of inhibition ranging from 12.2 to 20.5 mm). The following combinations of invasive extracts and microbes resulted in greater than 10 mm inhibition: *Ambrosia artemisiifolia* and *Aspergillus usami* (15.4 mm); *Erigeron canadensis* and *Phytophthora coctorium* (17.4 mm); *Ailanthus altissima* and *Aspergillus parasiticus* (12.3 mm); and *Eupatorium rugosum* and *A. parasticus* (16.2 mm). The invasive *P. americana* was the best inhibitor (20.5 mm) of the *Aspergillus parasiticus* (Table 2, $P < 0.001$). Extracts from native plant species showed little antimicrobial activity against *Aspergillus versicolor* and *A. phoenicis*, and caused less than 6 mm inhibition against other microbes.

Discussion

Our studies indicated that the constitutive production of large amounts of phenolic compounds may provide a competitive edge to invasive plants via suppression of neighboring plants; thus, producing novel weapons may ultimately lead to the proliferation of invasive plants in East Asia. Plant species that we categorized as invasive produced more total phenolics than plant species native to Korea. Extracts from these invasive species were much more allelopathic and anti-pathogenic than phenolic extracts from native species. We do not know whether exotic species or populations with high concentrations of phenolics were favored in invasion over exotic species or populations with low concentrations of phenolics, but these biogeographic differences are consistent with the expectations of the AARS hypothesis (Callaway and Ridenour 2004). Also, we do not know if

extracts from these invasive species would be less inhibitory to species from the native ranges of the invasives, but the difference reported here are consistent with some expectations of the NWH.

Our choice of species to categorize as "invasive", rather than merely "exotic" (Hierro et al. 2005) was subjective, but based on observations of strong patterns and field measurements from other studies. For example, we measured the abundance of *Ambrosia artemisiifloria* var. *elatior*, native to North America, at our study sites and found a 56% increase in cover from 2001 to 2005, and interestingly this species contained the highest levels of phenolic compounds of all species we measured. *Ambrosia artemisiiforia* is also highly invasive in other parts of the world (http://www.issg.org/database). Similarly, *Eupatorium rugosum*, which was first found in Seoul in 1978, has increased in numbers each year and expanded its range in the region (http://cafe.daum.net/greenkeeper). *Eupatorium rugosum* had the second highest level of total phenolic concentrations. Park et al. (2002) surveyed exotic plants across the country from 1997 to 2001 and found that *Ambrosia artemisiifolia* var. *elatior*, *Erigeron canadensis*, *Oenothera odorata*, *Rumex crispus*, *Ailanthus altissima* and *Taraxacum officinale* were found at the almost all survey plots and ranked these species as highly naturalized, or as we define them, "invasive".

Three of the highest concentrations of phenolic acids belonged to invasive plants in the family Asteraceae (*Eupatorium rugosum*, *Ambrosia artemisiifolia* var. *elatior*, and *Rudbeckia bicolor*); whereas the native Asteraceae species (*Aster koraiensis*) had the highest total concentration of phenolics among native species, higher than the mean concentration of all invasive species (Table 1). This raises the possibility that plants in the Asteraceae are unusually well suited for invasion because of their inherent chemical diversity and concentrations. The invasive grass *Buchloe dactyloides*, although not in the Asteraceae, was found to possess very high concentrations of total phenolic compounds (804 mg l^{-1}), with salicylic acid and cinnamic acid the most abundant phenolics (Wu et al. 1998). Other phenolics that appear to contribute to invasion success are the (±)-catechin and 8-hydroxyquinoline reported in invasive *Centaurea* species (Callaway and Aschehoug 2000; Tharayil et al. 2008; He et al. 2009).

We studied native and invasive *Taraxacum* congeners. The invasive *Taraxacum officinale* is widespread, whereas the native *Taraxacum mongolicum* has a narrow distribution, and is a rare plant in Korea. The concentration of phenolic compounds in *T. mongolicum* (7.9 mg l^{-1}) was far lower than *T. officinale* (86.2 mg l^{-1}), raising the question of whether these high concentrations in the exotic contribute to its invasive success.

The only chemical that was unique to the invasive species was scopoletin. This biochemical was not detected in the six native species, but invasive species averaged 262.6 ± 11.2 mg l^{-1} and scopoletin was found in all six invasive species (Table 1). *Avena sativa* produce scopoletin and exude it from their roots. Fay and Duke (1977) reported that the allelopathic effects of *Avena* were correlated with the amounts exuded.

Previous studies of three congeners in the genus *Phytolacca* also support the idea that high or unique concentrations of phenolics or other biochemical could possible contribute to invasion. Kim et al. (2005a) found that the concentration of total phenolic compounds was 3.9 mg l^{-1} for the exotic but not invasive *Phytolacca esculenta*, 4.4 mg l^{-1} for the native *Phytolacca insularis*, but 10.2 mg l^{-1} for the exotic and invasive *Phytolacca americana*, with the latter concentration significantly higher than the other two. Analysis of aqueous extracts found seven dominant phenolic compounds (gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, *m*-hydroxybenzoic acid, *p*-coumaric acid, and cinnamic acid), and in these aqueous extracts the total phenolics in *P. americana* were 8–16 times higher than either *P. esculenta* or *P. insularis*. Also, extracts from *P. americana* inhibited seed germination and dry weight of two assay species more than the other two species. We want to emphasize that, for *P. americana*, the particular compounds produced may be more important than their concentration. Even at low levels, the phenolic compounds produced by *P. americana* had higher biochemical inhibition effects on seedling growth and antimicrobial activity than any other species.

The antimicrobial activity of allelochemicals was first studied by Blum (1995), and since that time antibacterial activity of allelochemicals has been investigated in detail (Silva et al. 1996; Mahasneh and El-Oqlah 1999). However, little is known about the antifungal activity of allelochemicals (Hernandez et al. 1999). In our study, extracts of *A. artemisiifolia*, *E. canadensis*, *P. americana* and *E. rugosum* (all invasive), all of which contain high concentrations of phenolic compounds, had very strong inhibitory effects against all microbes (Table 2). Shukla et al. (1999) reported a similar link between strong antimicrobial activity and high levels of phenolic compounds in *Argyreia speciosa*. *Phytolacca americana* extract, however, had the highest antimicrobial activity despite low concentrations of phenolic compounds and soluble solids. This discrepancy may be explained by mixtures of allelopathic compounds being more effective at lower concentrations than a single compound at high concentrations (Blum 1996; Inderjit 2003).

A number of studies have provided evidence for the NWH (Callaway and Aschehoug 2000; Mallik and Pellissier 2000; Cappuccino and Arnason 2006; McKenny et al. 2007; Callaway et al. 2008; Ens et al. 2009; He et al. 2009; Jarchow and Cook 2009; Inderjit et al. 2010; Ni et al. 2010), but others have not (Blair et al. 2005, 2006). Our study provides further evidence to demonstrate the reason why invasive plants in Korea are successful in new environments, with novel mechanisms of interaction with the recipient community. Therefore, in sum, our results indicate that allelopathic effects and

anti-fungal defense functions of leachates may play a powerful role in the NWH. Our results are consistent in general with predictions of the NWH, adding to the possibility of important co-evolutionary processes in communities that contribute to the success or failure of exotic plant invasions.

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