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## N deposition affects allelopathic potential of *Amaranthus retroflexus* with different distribution regions

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### ABSTRACT

This study aims to determine the allelopathic potential of *Amaranthus retroflexus* (*Ar*) with different climatic zones on seed germination and growth of *A. tricolor* (*At*) treated with a gradient N addition. *Ar* leaf extracts only displayed significantly allelopathic potential on the underground growth of *Ar* but not the aboveground growth of *At*. The allelopathic potential of *Ar* leaf extracts on root length of *At* were enhanced under N addition and there may be a N-concentration-dependent relationship. The effects of the extracts of *Ar* leaves that collected from Zhenjiang on seed germination and growth of *At* may be higher than that collected from Jinan especially on root length of *At* under medium N addition. This reason may be the contained higher concentration of secondary metabolites for the leaves of plants that growths in high latitudes compare with that growth in low latitudes. This phenomenon may also partly be attributed to the fact that *Ar* originated in America and/or south-eastern Asia which have higher similarity climate conditions as Zhenjiang rather than Jinan. The allelopathic potential of *Ar* on seed germination and growth of acceptor species may play an important role in its successful invasion especially in the distribution region with low latitudes.

**Key words:** Allelopathic potential, invasive plant, N deposition, seed germination, *Amaranthus retroflexus*.

### INTRODUCTION

At present, biological invasion has triggered notable changes on the structure and functioning of native ecosystems (Gurevitch et al. 2011, Powell et al. 2013). Numerous studies have suggested that some plants can invade certain environments successfully via the released toxic compounds, a mechanism referred to as allelopathy (Callaway

and Ridenour 2004, Yuan et al. 2013). Allelopathics compounds can pose a remarkably allelopathic effect on seed germination and growth of native species (Mlakar et al. 2012, Yuan et al. 2013, Wang et al. 2016a). Meanwhile, seed germination and growth are highly important for plant recruitment and resource competition (Turk and Tawaha 2003). Thus, the inhibition of seed germination and growth of native species mediated by the allelopathic effects of invasive species may obviously decrease the competitiveness of native species.

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Currently, one of the most significant global environmental problems has been an increase in atmospheric nitrogen (N) deposition (Galloway et al. 2004, 2008). Meanwhile, the rapidly increased deposition of atmospheric N is expected to continue in the coming decades (Galloway et al. 2004, 2008, Liu et al. 2013). East Asia (mainly China) is currently one of the world's three largest areas of N deposition (Galloway et al. 2004, Wang et al. 2007, Liu et al. 2013). However, different regions in China may suffer different levels of atmospheric N deposition (Wang et al. 2007, Zhan et al. 2014). As the world's third largest country, meanwhile, China covers five climatic zones: cold-temperate, temperate, warm-temperate, subtropical, and tropical (Weber et al. 2008, Li et al. 2009). A wide range of habitats and environmental conditions allow China obviously vulnerable to the successful plantation of the invasive species that originating from diverse regions due to the potential invasive species from most areas of the world might searched out the suitable habitat somewhere in China eventually (Weber et al. 2008, Yan et al. 2014). Thus, the invasion of many invasive plants may cross multiple distribution regions (Yan et al. 2014). To illustrate, the notorious invasive plant *Amaranthus retroflexus* L. has been distributed in many regions of China (Weber et al. 2008, Yan et al. 2014). Thus, understanding the allelopathic potential of *A. retroflexus* L. with different distribution regions on seed germination and growth of native species is important to elucidate the mechanism underlying its successful invasion especially under the condition with increasing amounts of anthropogenic N being deposited into ecosystems gradually.

The present study aims to determine the allelopathic potential of the invasive plant *A. retroflexus* with different distribution regions that belong to different climatic zones on seed germination and growth of acceptor species *A. tricolor* in the presence of a gradient of simulated

N deposition (control, 0 g m<sup>-2</sup> yr<sup>-1</sup>; low N, 2.5 g m<sup>-2</sup> yr<sup>-1</sup>; medium N, 5 g m<sup>-2</sup> yr<sup>-1</sup>; and high N, 10 g m<sup>-2</sup> yr<sup>-1</sup>). Both are members of the *Amaranthus* genus. *A. retroflexus* is a summer annual weed (Horak and Loughlin 2000) native to America (Mandák et al. 2011, Yang et al. 2011) and/or south-eastern Asia (Yang et al. 2011). At present, *A. retroflexus* is a common weed that grows vigorously in orchards, roadside verges, fields, woods, and scrubland in China and has been listed as a destructive and widespread invasive species in China (Yang et al. 2011). Allelopathy is invoked to explain the successful invasion of *A. retroflexus* (Mlakar et al. 2012, Konstantinović et al. 2014).

This study addressed the following hypotheses: (1) *A. retroflexus* exhibits allelopathic potential on seed germination and growth of *A. tricolor*; (2) the allelopathic potential of *A. retroflexus* on seed germination and growth of *A. tricolor* will be changed under exogenous N addition; and (3) there may be significant difference in the allelopathic potential of *A. retroflexus* between different distribution regions that belong to different climatic zones on seed germination and growth of *A. tricolor*.

## MATERIALS AND METHODS

### EXPERIMENTAL DESIGN

In September 2015, leaf samples of *A. retroflexus* were obtained from the following two distribution regions with different climatic zones in China: Jinan (JN, 36.68°N, 116.90°E, featuring a warm temperate climate), Zhenjiang (ZJ, 32.21°N, 119.52°E, featuring a subtropical humid climate). The annual mean temperature of Jinan is approximately 13.8°C, and monthly mean temperature reaches a maximum of 27.2°C in July and decreases to a minimum of -3.2°C in January. The annual precipitation of Jinan is approximately 614 mm, and monthly mean precipitation reaches a maximum of approximately 196 mm in July

and decreases to a minimum of approximately 7 mm in January. The annual mean temperature of Zhenjiang is approximately 15.6 °C, and its monthly mean temperature reaches a maximum of approximately 25 °C in July and decreases to a minimum of approximately -1 °C in January. The annual precipitation of Zhenjiang is approximately 1088 mm, and monthly mean precipitation reaches a maximum of approximately 206 mm in July and decreases to a minimum of approximately 22 mm in December. The collected leaves of *A. retroflexus* were washed, air dried at room temperature for 48 h, and then stored at 4°C for further study. *A. tricolor* seeds were chosen as the acceptor seeds and bought from a local vegetable market.

Approximately 10 g of air-dried leaves of *A. retroflexus* were placed in flasks containing 500 mL of distilled water and soaked for 48 h at room temperature. *A. retroflexus* leaf extracts were strained using a cheese cloth and then through two layers of filter paper to remove solid particles. The stock solution was stored at 4°C for further study. The concentrations of *A. retroflexus* leaf extracts from Jinan and Zhenjiang (abbreviated as JN and ZJ hereafter, respectively) were both set at 20 g L<sup>-1</sup> with distilled water as control (CK, 0 g L<sup>-1</sup>).

A mixed N solution was prepared with a 1:1:1 ratio of KNO<sub>3</sub>-N : NH<sub>4</sub>Cl-N : urea-N. The ratio of different N forms in the mixed N was similar to the global average ratio of natural atmospheric N deposition (Neff et al. 2002, Cornell et al. 2003). The concentrations were made with distilled water prior to use to create a series with gradient contents, namely, control 0 g m<sup>-2</sup> yr<sup>-1</sup> (CK, fertilized with sterile distilled water only), low N, 2.5 g m<sup>-2</sup> yr<sup>-1</sup> (N-2.5); medium N, 5 g m<sup>-2</sup> yr<sup>-1</sup> (N-5); and high N, 10 g m<sup>-2</sup> yr<sup>-1</sup> (N-10). N-5 corresponded to levels of N deposition already recorded in the Yangtze River Delta region of China (Wang et al. 2007). N-10 represented a high deposition level that may be reached in the future.

The experiment included twelve treatments, each consisting of a combination of N form and aqueous extract regime. Five replicates were performed per treatment.

#### SEED GERMINATION EXPERIMENT

Experiments were performed through incubation in Petri dishes (Wang et al. 2016a). The *A. tricolor* seeds were surface sterilized (1% NaClO for approximately 15 min) and then thoroughly washed thrice with deionized water. Thirty healthy seeds of *A. tricolor* were placed in a 9 cm Petri dish and covered with two layers of filter paper. The seeds were treated with deionized water, N solutions, and/or *A. retroflexus* leaf extracts. The Petri dishes were placed in a climate-controlled incubator at 25 °C for 7 d with 12 h light per day (light intensity was set at 27.5 μmol · m<sup>-2</sup> · s<sup>-1</sup>). The number of germinated seeds was counted every day at incubation time, and each seed was considered to have germinated when its radicle protruded (Wang et al. 2016a).

#### DATA MEASUREMENTS

Ten seedlings per Petri dish were randomly selected for growth measurement on the same day. The plant height (PH), root length (RL), seedling biomass (BM, fresh weight), germination rate (GR), germination potential (GP), germination index (GI), germination rate index (GRI) and vigor index (VI) of *A. tricolor* were determined. PH and RL were measured using a ruler (Wang et al. 2016a). BM was determined using an electronic balance with an accuracy of 0.001 g (Wang et al. 2016a). GR was calculated using the ratio of the final number of germinated seeds to the total number of seeds when no new germination occurred after 7 d of incubation (Wang et al. 2016a). GP was determined by dividing the number of germinated seeds on the third day by the total number of seeds (Wang et al. 2016a). GI was calculated using the following

equation:  $GI = \sum G_i / I$ , where  $G_i$  represents the number of germinated seeds, and  $I$  represents the time after cultivation (day) (Schmer et al. 2012). GRI was calculated using the following equation:  $GRI = GR \times GI$  (Steinmaus et al. 2000). VI was determined using the following equation:  $VI = BM \times GI$  (Lin et al. 2000).

#### STATISTICAL ANALYSES

Differences among various dependent variables were assessed using analysis of variance between groups followed by a multiple comparisons correction using the Student–Newman–Keuls test. All statistical analyses were performed using SPSS Statistics (version 22.0; IBM, Armonk, NY, USA). Statistical significance was set at  $P$  values equal to or lower than 0.05.

### RESULTS

#### ALLELOPATHIC POTENTIAL OF *A. RETROFLEXUS* LEAF EXTRACTS ON SEED GERMINATION AND GROWTH OF *A. TRICOLOR*

*A. retroflexus* leaf extracts exerted significant effects on seed germination and growth of *A. tricolor* (Table I). In particular, the PH, BM, and VI of *A. tricolor* under JN and ZJ were significantly higher than those of *A. tricolor* under CK (Table I,  $P < 0.05$ ). While, the RL of *A. tricolor* under JN and ZJ were significantly lower than those of *A. tricolor* under CK (Table I,  $P < 0.05$ ). Both JN and ZJ did not significantly affect the GR, GP, GI, and GRI of *A. tricolor* (Table I,  $P > 0.05$ ). There was no significant difference in seed germination and growth of *A. tricolor* between JN and ZJ (Table I,  $P > 0.05$ ).

#### EFFECTS OF N ADDITION ON SEED GERMINATION AND GROWTH OF *A. TRICOLOR*

N addition remarkably affected seed germination and growth of *A. tricolor* (Table I). In particular, the PH, BM, and VI of *A. tricolor* under N addition

were significantly higher than those of *A. tricolor* under CK (Table I,  $P < 0.05$ ). By contrast, the RL of *A. tricolor* decreased markedly with increasing concentrations of N addition (Table I,  $P < 0.05$ ). There was no significant difference in the GR, GP, GI, and GRI of *A. tricolor* between N addition and CK (Table I,  $P > 0.05$ ).

#### ALLELOPATHIC POTENTIAL OF *A. RETROFLEXUS* LEAF EXTRACTS ON SEED GERMINATION AND GROWTH OF *A. TRICOLOR* UNDER N ADDITION

The PH, BM, VI of *A. tricolor* under *A. retroflexus* leaf extracts and N addition, regardless of concentration, were significantly higher than those of *A. tricolor* under CK (Table I,  $P < 0.05$ ). Whereas, the RL of *A. tricolor* under *A. retroflexus* leaf extracts and N addition, regardless of concentration, was significantly lower than that of *A. tricolor* under CK (Table I,  $P < 0.05$ ). The GI of *A. tricolor* under N-10\*JN was also significantly lower than that of *A. tricolor* under CK (Table I,  $P < 0.05$ ) but not the rest of the combined treatment of *A. retroflexus* leaf extracts and N addition (Table I,  $P > 0.05$ ). The combined treatment of *A. retroflexus* leaf extracts and N addition, regardless of concentration, did not pose significant effects on the GR, GP, and GRI of *A. tricolor* compared with CK (Table I,  $P > 0.05$ ).

The allelopathic potential of *A. retroflexus* leaf extracts on *A. tricolor* could be changed in the presence of different levels of N addition (Table I). In particular, RL of *A. tricolor* exposed to both of JN and ZJ with N addition, regardless of concentration, were significantly lower than those of *A. tricolor* exposed only to *A. retroflexus* leaf extracts (Table I,  $P < 0.05$ ). Meanwhile, the RL of *A. tricolor* under both JN and ZJ with N addition decreased significantly with increasing concentrations of N fertilizers (Table I,  $P < 0.05$ ). Moreover, the PH of *A. tricolor* under N-5\*ZJ and N-10\*ZJ was significantly higher than that of *A. tricolor* exposed only to the extracts of *A. retroflexus* leaves that collected from Zhenjiang (Table I,  $P <$

TABLE I  
Differences in the indices of *A. tricolor* with different treatments.

Treatment	PH	RL	BM	GR	GP	GI	GRI	VI
CK	1.03±0.01e	3.09±0.05a	0.03±0.01c	99.33±0.67a	98.67±0.82a	46.29±0.45a	45.98±0.56ab	1.17±0.25c
JN	1.73±0.05cd	2.42±0.06b	0.05±0.00b	99.33±0.67a	98.67±0.82a	45.92±0.35ab	45.62±0.61ab	2.44±0.16b
ZJ	1.91±0.07bc	2.65±0.11b	0.07±0.01ab	99.33±0.67a	98.00±0.82a	46.53±0.42a	46.23±0.69ab	3.34±0.31ab
N-2.5	1.62±0.04d	2.52±0.08b	0.06±0.01b	98.67±0.82a	98.00±0.82a	45.70±0.51ab	45.11±0.85ab	2.58±0.28b
N-5	1.76±0.03cd	2.14±0.09c	0.05±0.01b	100.00±0.00a	99.33±0.67a	47.03±0.28a	47.03±0.28a	2.39±0.25b
N-10	1.65±0.04d	1.64±0.03d	0.08±0.00a	100.00±0.00a	98.67±0.82a	46.18±0.37a	46.18±0.37ab	3.64±0.21a
N-2.5*JN	1.77±0.03cd	1.71±0.06d	0.06±0.00ab	100.00±0.00a	99.33±0.67a	45.40±0.85ab	45.40±0.85ab	2.80±0.22ab
N-2.5*ZJ	2.05±0.04b	1.78±0.11d	0.07±0.01ab	100.00±0.00a	100.00±0.00a	46.72±0.17a	46.72±0.17a	3.41±0.25ab
N-5*JN	1.74±0.07cd	1.19±0.03f	0.06±0.00ab	98.00±0.82a	97.34±0.67a	44.77±0.37ab	43.88±0.61ab	2.72±0.20ab
N-5*ZJ	2.36±0.07a	1.41±0.04e	0.07±0.00ab	99.33±0.67a	97.33±1.25a	44.98±0.61ab	44.69±0.74ab	3.09±0.14ab
N-10*JN	1.82±0.02cd	0.67±0.05g	0.05±0.00b	99.33±0.67a	98.67±0.82a	43.72±0.48b	43.42±0.38b	2.35±0.18b
N-10*ZJ	2.06±0.05b	0.79±0.04g	0.05±0.01b	98.67±0.82a	98.00±0.82a	45.00±0.83ab	44.42±1.16ab	2.43±0.28b

Data with different letters in a vertical row indicate a significant difference ( $P < 0.05$ ). Abbreviations: PH, plant height (cm); RL, root length (cm); BM, seedling biomass (fresh weight) (g); GR, germination rate (%); GP, germination potential (%); GI, germination index; GRI, germination rate index; VI, vigor index; CK, sterile distilled water; JN, the extracts of *A. retroflexus* leaves that collected from Jinan; ZJ, the extracts of *A. retroflexus* leaves that collected from Zhenjiang; N-2.5, 2.5 g m<sup>-2</sup> yr<sup>-1</sup>; N-5, 5 g m<sup>-2</sup> yr<sup>-1</sup>; N-10, 10 g m<sup>-2</sup> yr<sup>-1</sup>; N-2.5\*JN, the combined treatment of N-2.5 and JN; N-2.5\*ZJ, the combined treatment of N-2.5 and ZJ; N-5\*JN, the combined treatment of N-5 and JN; N-5\*ZJ, the combined treatment of N-5 and ZJ; N-10\*JN, the combined treatment of N-10 and JN; N-10\*ZJ, the combined treatment of N-10 and ZJ.

0.05). In addition, under the addition of equal N concentration, the PH of *A. tricolor* under ZJ with was significantly higher than that under JN (Table I,  $P < 0.05$ ). Furthermore, under the addition of medium N, the RL of *A. tricolor* under ZJ with was also significantly higher than that under JN (Table I,  $P < 0.05$ ).

## DISCUSSION

Some invaders can display pronounced allelopathic effects on the growth of native species in the invaded ecosystems to facilitate further invasion (Callaway and Ridenour 2004, Yuan et al. 2013, Wang et al. 2016a). As one of the most notorious invasive species in numerous agricultural areas, the allelopathic potential of *A. retroflexus* on the germination and growth of a wide-variety of

field crops and vegetables are well documented (Mlakar et al. 2012, Konstantinović et al. 2014). This characteristic may play an important role during successful invasion of *A. retroflexus*. It is interesting that the extracts of *A. retroflexus* leaves that collected from both Jinan and Zhenjiang all exerted significantly negative effects on the root length of *A. tricolor* but triggered significantly positive effects on plant height, seedling biomass, and vigor index of *A. tricolor*. This implied that *A. retroflexus* leaf extracts only displayed significantly allelopathic potential on the underground growth of *A. tricolor* but not the aboveground growth of *A. tricolor*. The result was only partly consistent with the study's first hypothesis. The result may be attributed to the difference in the response of the underground and aboveground growth of *A. tricolor* to external effects mediated by *A. retroflexus* leaf

extracts. Meanwhile, *A. retroflexus* leaf extracts increased the aboveground growth of *A. tricolor* may be attributed to the low concentration of allelochemicals released by *A. retroflexus* used in this study. Previous studies founded that allelopathic potential of *A. retroflexus* leaf extracts on the reduced germination and growth of crop seedlings is obviously dependent on its concentration (Mlakar et al. 2012). This finding may also be ascribed to the low concentration of allelopathic compounds released by invasive plants, which stimulated the reactive oxygen molecules in plant cell extension and then promoted growth of native species (Prithiviraj et al. 2007, Duke et al. 2006). Briefly, the low concentration of allelopathic compounds released by invasive species may exert weak stress on plant species; hence, native species adapted to this change and thereby there are stimulatory effects on the growth of native species triggered by the low concentration of allelopathic compounds of invasive plants (Hossain et al. 2016), namely hormesis effects (Viator et al. 2006, Takao et al. 2011). Thus, it is not surprising that *A. retroflexus* leaf extracts promoted the aboveground growth of *A. tricolor* in this study.

N plays an important role in the growth of plants, and enhanced nutrient levels in plant communities lead to increased plant productivity (Jin et al. 2016, Wang et al. 2016b). Meanwhile, exogenous N addition could stimulate seed germination and growth of plant species (Qiu and Zhang 2008). The results of this study showed that the increased levels of exogenous N addition obviously stimulated seed germination and growth of *A. tricolor*, especially the plant height, seedling biomass, and vigor index of *A. tricolor* significantly increased under N addition. The result was consistent with previous studies (Qiu and Zhang 2008). The enhanced seedling growth under exogenous N addition may be due to the elevated levels of soil available N (Bai et al. 2010, Zhang et al. 2013). Meanwhile, the root length of *A. tricolor* decreased significantly

with increasing concentrations of exogenous N addition. This implied that exogenous N addition could inhibit the underground growth of *A. tricolor*. Thus, exogenous N addition promoted aboveground growth of *A. tricolor* but decreased its underground growth and thereby changed its biomass allocation pattern. The result was consistent with previous studies (Janssens et al. 2010, Wang et al. 2015).

The allelopathic potential of invasive species on seed germination and growth of native species may shift and change under the conditions with increased levels of N deposition. The results of this study showed that there may be a synergistic effect between exogenous N addition and *A. retroflexus* leaf extracts on the root length of *A. tricolor*, especially the root length of *A. tricolor*. Thus, the allelopathic potential of *A. retroflexus* leaf extracts on seed germination and growth of *A. tricolor* were enhanced under exogenous N addition and there may be a N-concentration-dependent relationship. However, the combined treatment of the extracts of *A. retroflexus* leaves that collected from Zhenjiang and low as well as medium N treatment showed significant positive effects on the plant height of *A. tricolor*. The reason may be due the fact that exogenous N addition resulted in a shift in biomass allocation from underground to aboveground (Janssens et al. 2010, Wang et al. 2015). Consequently, the result was consistent with the study's second hypothesis. Meanwhile, the effects of the extracts of *A. retroflexus* leaves that collected from Zhenjiang on seed germination and growth of *A. tricolor* may be higher than that collected from Jinan especially on root length of *A. tricolor* under medium N addition. The reason could be a result of the contained higher concentration of secondary metabolites for the leaves of plants that growths in high latitudes compare with that growth in low latitudes (Wright et al. 2004). Gatti et al. (2014) also found that climate conditions can affect plant secondary metabolism (such as precipitation and temperature) and then alter the allelopathic

activity of plant species via alter the production of allelochemicals. The result may also be attributed in part to the fact that *A. retroflexus* originated in America (Mandák et al. 2011, Yang et al. 2011) and/or south-eastern Asia (Yang et al. 2011) which have higher similarity climate conditions as Zhenjiang rather than Jinan. The result was consistent with the study's third hypothesis. Thus, the allelopathic potential of *A. retroflexus* on seed germination and growth of native species may play an important role in its successful invasion especially in the distribution region with low latitudes.

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