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Silver nanoparticles intensify the allelopathic intensity of four invasive plant species in the Asteraceae

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Abstract: This study aimed to estimate the allelopathic intensity of four Asteraceae invasive plant species (IPS), including *Conyza canadensis* (L.) Cronq., *Erigeron annuus* (L.) Pers., *Bidens pilosa* (L.), and *Aster subulatus* Michx., by testing the effect of leaf extracts on the seed germination and seedling growth (SGe and SGr) of lettuce *(Lactuca sativa* L.) in combination with two particle sizes of silver nanoparticles. These four IPS decreased the germination of lettuce seeds but increased the growth of lettuce seedlings. The allelopathic intensity of the four IPS decreased in the following order: *B. pilosa* > *C. canadensis* > *E. annuus* > *A. subulatus*. Silver nanoparticles decreased the SGe and SGr of lettuce. The 20 nm silver nanoparticles affected the competition intensity for water and the absorption of inorganic salts by lettuce more intensively than the 80 nm nanoparticles. Silver nanoparticles intensify the allelopathic intensity of the four invasive plant species on the SGe and SGr of lettuce. The allelopathic intensity of *B. pilosa* was higher than that of the other three IPS when they were polluted with silver nanoparticles. Thus, silver nanoparticles could facilitate the invasion process of the four IPS, particularly *B. pilosa*, via an increase in the intensity of allelopathy.

Key words: Ag ion, allelochemicals, invasion process, lettuce, particle size.

INTRODUCTION

Invasive plant species have a noticeable impact on the composition and structure of the habitats that they encroach upon, and, in particular, those invaders can lead to a loss of biodiversity (Wang et al. 2018a, b, 2019a-c, 2020a, b, 2021, Wu et al. 2019a, Wei et al. 2020a, b). Thus, the mechanisms that lead to the successful invasion by invasive plant species are currently one of the main topics of study on the ecological effects of invasive plant species. In particular, the new weapon hypothesis verifies that numerous invasive plant species can incur evident allelopathic intensity by secreting several types of secondary substances and noxious compounds, i.e., allelochemicals, that can decrease the growth of native plants (Djurdjević et al. 2012, Fabbro et al. 2014, Lyytinen & Lindström 2019, Hsueh et al. 2020, Wei et al. 2020a, b). More importantly, the process of seed germination and seedling growth (SGe and SGr) was most obviously affected by the allelochemicals of invasive plant species at the beginning of their life history (Fabbro et al. 2014, Wang et al. 2017a, b, 2018c, d, 2019d, 2020c, Carvalhoa et al. 2019, Hsueh et al. 2020, Krstin et al. 2021). However, SGe and SGr are critical to individual growth and the development of biomes. Thus, the allelopathic intensity of invasive plant species on the SGe and SGr of natives can decrease the competitive intensity of their growth (Wang et al. 2018c, d, 2019d, 2020c, Carvalhoa et al. 2019, Wei et al. 2020a, b, Krstin et al. 2021). Moreover, the Asteraceae is the plant family with the highest number of invasive plant species in China (Wang et al. 2016). Thus, it is necessary to analyze the allelopathic intensity recruited by invasive plant species in the Asteraceae on the SGe and SGr of natives to obtain a better explanation for the mechanisms that lead to the successful invasion of invasive plant species.

In addition, drivers of the shifts in SGe and SGr beyond the allelopathic intensity created by invasive plant species may presumably mediate a momentous effect on this aspect. Subsequently, the process of plant invasion and its associated ecological effects may be changed or even made more complex under the interaction of one of these drivers. As one of the most widely used nanomaterials, a substantial amount of silver nanoparticles has been released into the environment and totals 500 tons a year across the globe. These nanomaterials are primarily derived from their production and use in a number of industries, including catalysts, optics, electronics, cosmetics, medicine, pharmaceuticals, and food (McGillicuddy et al. 2017, Wu et al. 2019b, Wang et al. 2020d). However, the silver nanoparticles that are released end up in the soil subsystem and then cause ecotoxicological effects over a long period, particularly via the food cycle (McGillicuddy et al. 2017, Wu et al. 2019b, Wang et al. 2020d). Predictably, silver nanoparticles will be released into the environment in the future with the increasing frequency and intensity of anthropogenic activities, particularly as the industry develops further. In particular, the increasing level of pollution mediated by silver nanoparticles can pose an obvious effect on the invasion process and the underlying mechanisms of invasive plant species (Wang et al. 2018c, Wu et al. 2019b). Therefore, it is vital to understand the allelopathic intensity of the Asteraceae invasive plant species on the SGe and SGr of natives that are affected by silver nanoparticles. Previous studies largely focused on the allelopathic intensity of only one invasive plant species on the SGe and SGr of natives. However, research on the allelopathic intensity of a variety of invasive plant species on the SGe and SGr of natives, particularly under silver nanoparticles, is limited.

This study aimed to estimate the allelopathic intensity of the notorious four invasive plant species of the Asteraceae, i.e., *Conyza canadensis* (L.) Cronq., *Erigeron annuus* (L.) Pers., *Bidens pilosa* (L.), and *Aster subulatus* Michx., on the seed germination and seedling growth of lettuce (*Lactuca sativa* L.) after exposure to 20 nm and 80 nm particle sizes of silver nanoparticles. In particular, the four Asteraceae invasive plant species originated in North America, and therefore, they may share a similar or even the same evolutionary history during their colonization and invasion in China (Wang et al. 2016). Furthermore, the four Asteraceae invasive plant species are currently considered to be the most harmful invasive plant species in China owing to their notable influence on native plant communities. Additionally, farmlands and wastelands are more vulnerable to the process of invasion of the four Asteraceae invasive plant species in China. In addition, the allelopathic intensity of the four Asteraceae invasive plant species on the SGe and SGr of native ones is a vital issue in their successful invasion (Khanh et al. 2009, Djurdjević et al. 2012, Fabbro et al. 2014, Wang et al. 2017a, He et al. 2019, Hsueh et al. 2020, Lu et al. 2020, Wei et al. 2020b). Moreover, as one of the most frequent native species in the environments occupied by the four Asteraceae invasive plant species, lettuce seedlings are highly sensitive to stress in the environment. Therefore, the growth of lettuce seedlings is more sensitive to the allelopathic intensity of invasive plant species on the SGe and SGr of natives (Khanh et al. 2009, Wang et al. 2017a, b, 2018c, d, 2019d, 2020c, Carvalhoa et al. 2019, Wei et al. 2020a, b). In addition, the four Asteraceae invasive plant species and lettuce can co-exist in the same habitat, particularly in the farmlands and wildlands. Lastly, the four Asteraceae invasive plant species and lettuce belong to the Asteraceae, which currently contains the largest number of invasive plant species of any family of plants in China (Wang et al. 2016).

We present the following hypotheses: (I) the four Asteraceae invasive plant species can create intensive allelopathic intensity on lettuce the SGe and SGr of lettuce, and there may be remarkable differences in the allelopathic intensity of these invasive plant species on lettuce; (II) silver nanoparticles can reduce the SGe and SGr of lettuce, and silver nanoparticles that are 20 nm tend to more toxic than that are 80 nm; and (III) silver nanoparticles can intensify the allelopathic intensity of the four Asteraceae invasive plant species on lettuce SGe and SGr.

MATERIALS AND METHODS

Preparation of plant materials and allelopathic solutions

Fully mature leaves samples of the four Asteraceae invasive plant species, including *C. canadensis*, *E. annuus*, *B. pilosa*, and *A. subulatus*, were randomly collected from Zhenjiang, Jiangsu (32.21°N, 119.52°E), China in the middle of September 2019. Zhenjiang has a subtropical monsoon humid climate (annual mean precipitation: ≈ 1,101.4 mm; annual mean temperature: ≈ 15.9°C; annual mean hours of sunshine: ≈ 1,996.8 h) (Jia & Wu 2020). The harvested leaves of the four Asteraceae invasive plant species were moderately washed and then thoroughly air-dried at approximately 25 °C. The air-dried leaves of the four Asteraceae invasive plant species were then soaked using sterile distilled water in the flasks at approximately 25 °C to yield the allelopathic solution (20 g L^{-1}). This concentration was used to simulate the plants that were subjected to the growth of invasive plant species. In contrast, undisturbed wild growth was simulated using distilled water as the control (CK, 0 mg L^{-1}). The allelopathic solution was stored at 4°C for less than a week.

Preparation of the silver nanoparticles solutions

Solutions with silver nanoparticles of two particle sizes, 20 nm and 80 nm, were prepared using AgNP (purity ≥99.9%). Silver nanoparticles of 20 nm and 80 nm are widely used in the study area. A silver nanoparticles solution with 20 nm and one with 80 nm were all established at 100 mg L^{-1}, which is comparable to the concentrations found in soils polluted with silver nanoparticles. Distilled water was used as the control (0 mg L^{-1}), and it simulated soil that had not been polluted with silver nanoparticles pollution. In particular, a AgNP solution was homogenized by stirring with an ultrasonoscope (Q-250DE) at 25 °C at approximately 40 kHz with its strongest power at 100 W for approximately 4 h to sufficiently disperse the particles to avoid the accumulation of silver nanoparticles (Wang et al. 2018c, 2020d, Wu et al. 2019b).

Experimental design of lettuce seed germination and seedling growth

The experiment comprised 15 treatments with all independent or mixed treatments of an allelopathic solution of the four Asteraceae invasive plant species and silver nanoparticles solutions that were 20 or 80 nm. The information of the experimental design is defined in Table I.

Lettuce seeds (the cultivar: *Lactuca sativa* L. cv. Kexing-Jiuzhouhong) were hydroponically cultured in Petri dishes (9 cm in diameter) from October 10 to 18, 2019. The lettuce seeds

Table I. Summary of experimental design.

	Abbreviation	Treatment	Concentration
I	CK	Control (distilled water)	0 g L^{-1}
II	CC	*Conyza canadensis* (L.) Cronq. leaf extracts	20 g L^{-1}
III	EA	*Erigeron annuus* (L.) Pers. leaf extracts	20 g L^{-1}
IV	BP	*Bidens pilosa* L. leaf extracts	20 g L^{-1}
V	AS	*Aster subulatus* Michx. leaf extracts	20 g L^{-1}
VI	AgNP20	Silver nanoparticles with 20 nm	100 mg L^{-1}
VII	CCAgNP20	The combined treatment of CC and AgNP20	The former with 20 g L^{-1} and the latter with 100 mg L^{-1}
VII	EAAgNP20	The combined treatment of EA and AgNP20	The former with 20 g L^{-1} and the latter with 100 mg L^{-1}
IX	BPAgNP20	The combined treatment of BP and AgNP20	The former with 20 g L^{-1} and the latter with 100 mg L^{-1}
X	ASAgNP20	The combined treatment of AS and AgNP20	The former with 20 g L^{-1} and the latter with 100 mg L^{-1}
XI	AgNP80	Silver nanoparticles with 80 nm	100 mg L^{-1}
XII	CCAgNP80	The combined treatment of CC and AgNP80	The former with 20 g L^{-1} and the latter with 100 mg L^{-1}
XIII	EAAgNP80	The combined treatment of EA and AgNP80	The former with 20 g L^{-1} and the latter with 100 mg L^{-1}
XIV	BPAgNP80	The combined treatment of BP and AgNP80	The former with 20 g L^{-1} and the latter with 100 mg L^{-1}
XV	ASAgNP80	The combined treatment of AS and AgNP80	The former with 20 g L^{-1} and the latter with 100 mg L^{-1}

were surface-sterilized using 1% NaClO for approximately 15 min. The surface-sterilized lettuce seeds were then cleaned carefully using sterile deionized water. The washed lettuce seeds were then carefully moved to Petri dishes with 30 seeds per Petri dish. Two layers of filter paper were enclosed under the lettuce seeds in each dish. The cultured lettuce seeds in the Petri dishes were placed in an electronic-controlled incubator (LRH-250-G) with 27.5 μmol m^{-2} s^{-1} (12 h of alternating light and dark per day) at approximately 25°C. On the first day, 5 mL of deionized water was used to soak the lettuce seedlings and the filter paper. After that, 0.5 mL of sterile deionized water, the allelopathic solution of the four Asteraceae, and/or a solution of AgNPs were added to each Petri dish daily using a pipette (Eppendorf Research® Plus). The number of germinated lettuce seeds, measured by exposure of the radicle, was recorded daily (Wang et al. 2017a, 2018c, d, 2019d, Wei et al. 2020b). Three Petri dishes were utilized per treatment.

Determination of lettuce seed germination and seedling growth indices

After 8 d, 10 lettuce seedlings per Petri dish, i.e., three Petri dishes per treatment * 10 seedlings per Petri dish for determination (with 30 seeds per Petri dish for germination) = 30 seedlings per treatment for determination, were randomly selected to assess the values of lettuce SGe and SGr indices. In particular, the germination percentage was calculated as the ratio of the number of the germinated seeds to the total number of the tested seeds and denotes the germination capacity. The germination potential was calculated as the germination percentage after 3 d of cultivation and denotes the germination intensity and homogeneity. The germination index was calculated as the following equation: germination index = $\boldsymbol{\Sigma} G_i/D_t$, where G_i is the number of the germinated seeds in D_t, i.e., the time after cultivation (day), which denotes germination power. The germination rate index was calculated as the arithmetic product of the two values of germination percentage and germination index and denotes germination rate and vitality. The germination vigor index was calculated as the arithmetic product of the two values of germination index and seedling fresh weight and denotes germination rate and vitality. The promptness index was calculated as the following equation: $(1.00) * nd_2 + (0.75) * nd_4 + (0.50) * nd_6 + (0.25) * nd_8$, where nd_2, nd_4, nd_6, nd_8 are the number of the germinated seeds in the second, fourth, sixth, and eighth days after cultivation, respectively, and denotes the response capability of seed germination. The seedling height, which indicates the distance between the base of the stem and the apical shoot, was measured by a ruler that was accurate down to 0.1 cm and denotes the competitive intensity for sunlight capture. The root length was the distance between the base of the root and the root tip and was measured by a ruler that was accurate to 0.1 cm; it denotes the competitive intensity for water and the absorption of inorganic salts. The leaf length was considered to be the maximum value parallel with the midrib, which was measured by a ruler that was accurate to 0.1 cm, and denotes the competitive intensity for sunlight capture. The leaf width, which is the maximum value perpendicular to the midrib, was measured by a ruler with 0.1 cm accuracy and denotes the competitive intensity for the capture of sunlight. The green leaf area was calculated as the following equation: the green leaf area = $0.75 \times$ leaf length $\times$ leaf width, which denotes the photosynthetic area. The seedling biomass, which includes the fresh and dry weight, was estimated by an electronic balance with an accuracy of 0.001 g and denotes the growing competitive intensity. The moisture content was calculated as the ratio of the difference between the seedling fresh weight and the seedling dry weight to the seedling fresh weight and denotes the water content (Steinmaus et al. 2000, Toscano et al. 2017, Ding et al. 2018, Huang et al. 2018, Wang et al. 2018c, 2019d, 2020e, Lu et al. 2020, Wei et al. 2020b).

Statistical analyses

Differences in the lettuce SGe and SGr indices among all treatments were estimated using a one-way analysis of variance (ANOVA) with a Tukey's test. $P \leq 0.05$ was defined as the threshold for statistical significance. The statistical analyses were completed using IBM SPSS Statistics 25.0 (IBM, Inc., Armonk, NY, USA).

RESULTS

Influence of the allelopathic intensity of the four asteraceae invasive plant species on lettuce seed germination and seedling growth compared with the CK

The germination percentage, germination index, germination vigor index, and promptness index of lettuce decreased, but the green leaf area, leaf length, moisture content, fresh weight, and seedling height of lettuce increased under CC ($P < 0.05$; Figs. 1 and 2). The germination index, germination vigor index, promptness index, and root length of lettuce decreased, but the green leaf area and leaf length of lettuce increased under EA ($P < 0.05$; Figs. 1 and 2). The germination index, germination potential, germination rate index, germination vigor index, promptness index, root length, and seedling biomass (dry weight) of lettuce decreased under BP ($P < 0.05$; Figs. 1 and 2). The root length of lettuce decreased, but the leaf length of lettuce increased under AS ($P < 0.05$; Figs. 1 and 2).

Differences in the allelopathic intensity of the four asteraceae invasive plant species on lettuce seed germination and seedling growth

The germination potential of lettuce under BP was lower compared with that under AS ($P < 0.05$; Fig. 1). The germination index of lettuce noticeably declined in the following order: AS, EA, CC, and BP ($P < 0.05$; Fig. 1). The germination rate index of lettuce under BP was lower than that under the other three Asteraceae invasive plant species ($P < 0.05$; Fig. 1). The germination vigor index of lettuce under CC was lower than that under AS ($P < 0.05$; Fig. 1). The promptness index of lettuce under CC was lower than that under EA and AS ($P < 0.05$; Fig. 1). The germination vigor index and promptness index of lettuce under BP were also lower than those under EA and AS ($P < 0.05$; Fig. 1). The seedling height of lettuce under BP was lower than that under CC ($P < 0.05$; Fig. 2). The root length of lettuce under BP was lower than that under CC and EA ($P < 0.05$; Fig. 2). The green leaf area, leaf length, and root length of lettuce under CC were higher than those under the other three Asteraceae invasive plant species ($P < 0.05$; Fig. 2). The fresh weight of lettuce under CC was also higher than that under BP and AS ($P < 0.05$; Fig. 2).

Influence of silver nanoparticles with two particle sizes on lettuce seed germination and seedling growth

The germination rate index and seedling biomass (including fresh weight and dry weight) of lettuce decreased under AgNP20 and AgNP80 ($P < 0.05$; Figs. 1 and 2). The root length of lettuce also decreased under AgNP20 ($P < 0.05$; Fig. 2). The root length of lettuce under AgNP20 was lower than that under AgNP80 ($P < 0.05$; Fig. 2). The silver nanoparticles did produce any other significant effects on the lettuce SGe and SGr indices ($P > 0.05$; Figs. 1 and 2).

Influence of the allelopathic intensity of the four asteraceae invasive plant species on lettuce seed germination and seedling growth under two particle sizes of silver nanoparticles

The germination index, germination vigor index, promptness index, root length, and seedling biomass (dry weight) of lettuce decreased under the combined treatment of the four Asteraceae invasive plant species leaf extracts and silver nanoparticles regardless of particle size ($P < 0.05$; Figs. 1 and 2). The germination rate index of lettuce also decreased under the combined treatment of the four Asteraceae invasive plant species leaf extracts and silver nanoparticles regardless of particle size (except ASAgNP20)

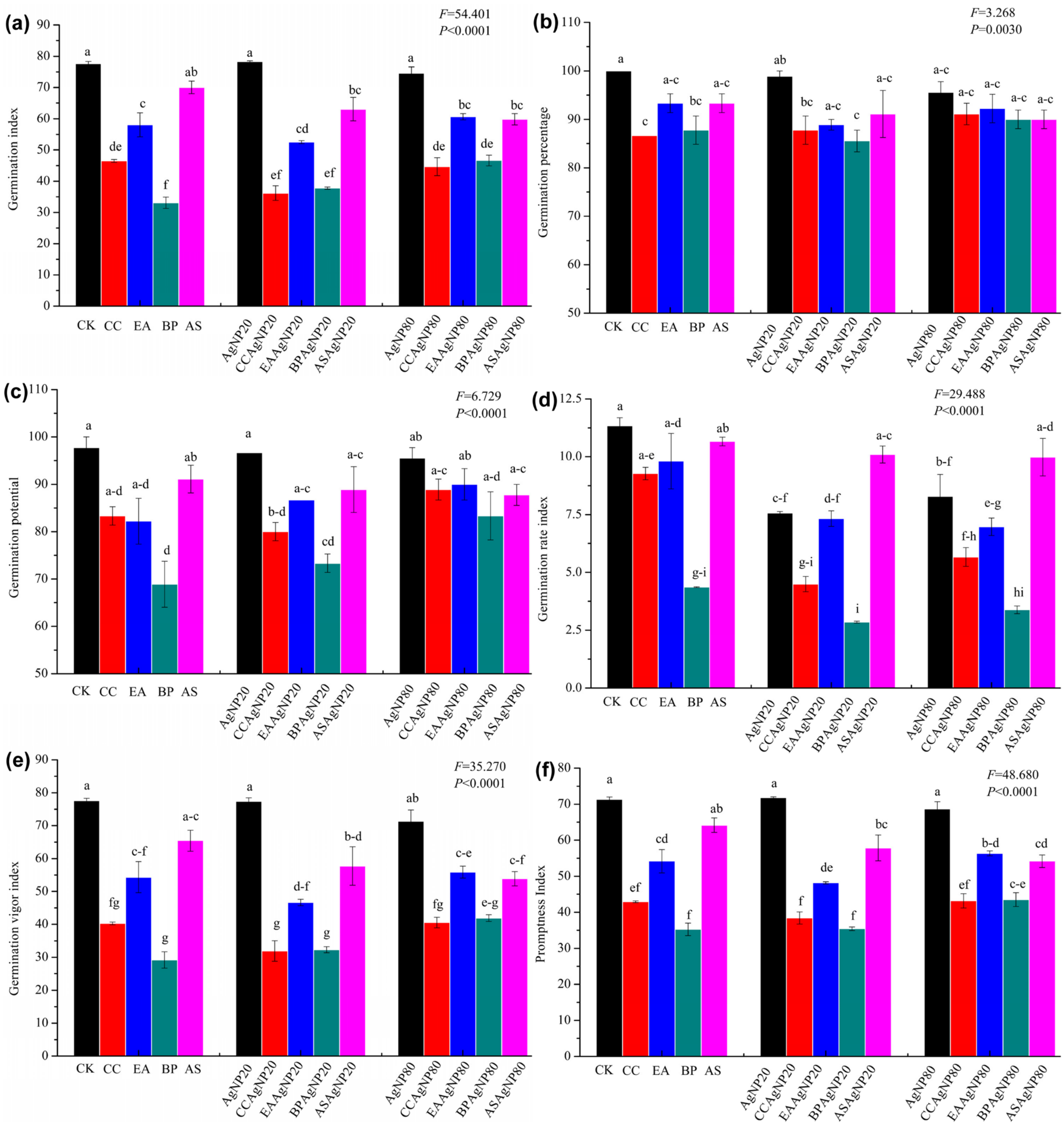

Figure 1. Seed germination indices of lettuce. Bars (means ± SE) with different letters mean statistically significant differences (*P* < 0.05). The lowercase letters on top of the bars are presented as "a-c", "a-d", "b-d", "g-i", "d-f", "f-h", "e-g", and "c-e" means "abc", "abcd", "bcd", "ghi", "def", "fgh", "efg", and "cde", respectively.

(*P* < 0.05; Fig. 1). The germination percentage and germination potential of lettuce decreased under CCAgNP20 and BPAgNP20 (*P* < 0.05; Fig. 1). The moisture content, fresh weight, and seedling height of lettuce decreased under BPAgNP20 (*P* < 0.05; Fig. 2). The leaf length, moisture content, and fresh weight of lettuce decreased under BPAgNP80 (*P* < 0.05; Fig. 2). Conversely, the leaf length and seedling height of lettuce increased under ASAgNP20 and ASAgNP80 (*P* < 0.05; Fig. 2). The moisture content of lettuce also increased under ASAgNP80 (*P* < 0.05; Fig. 2).

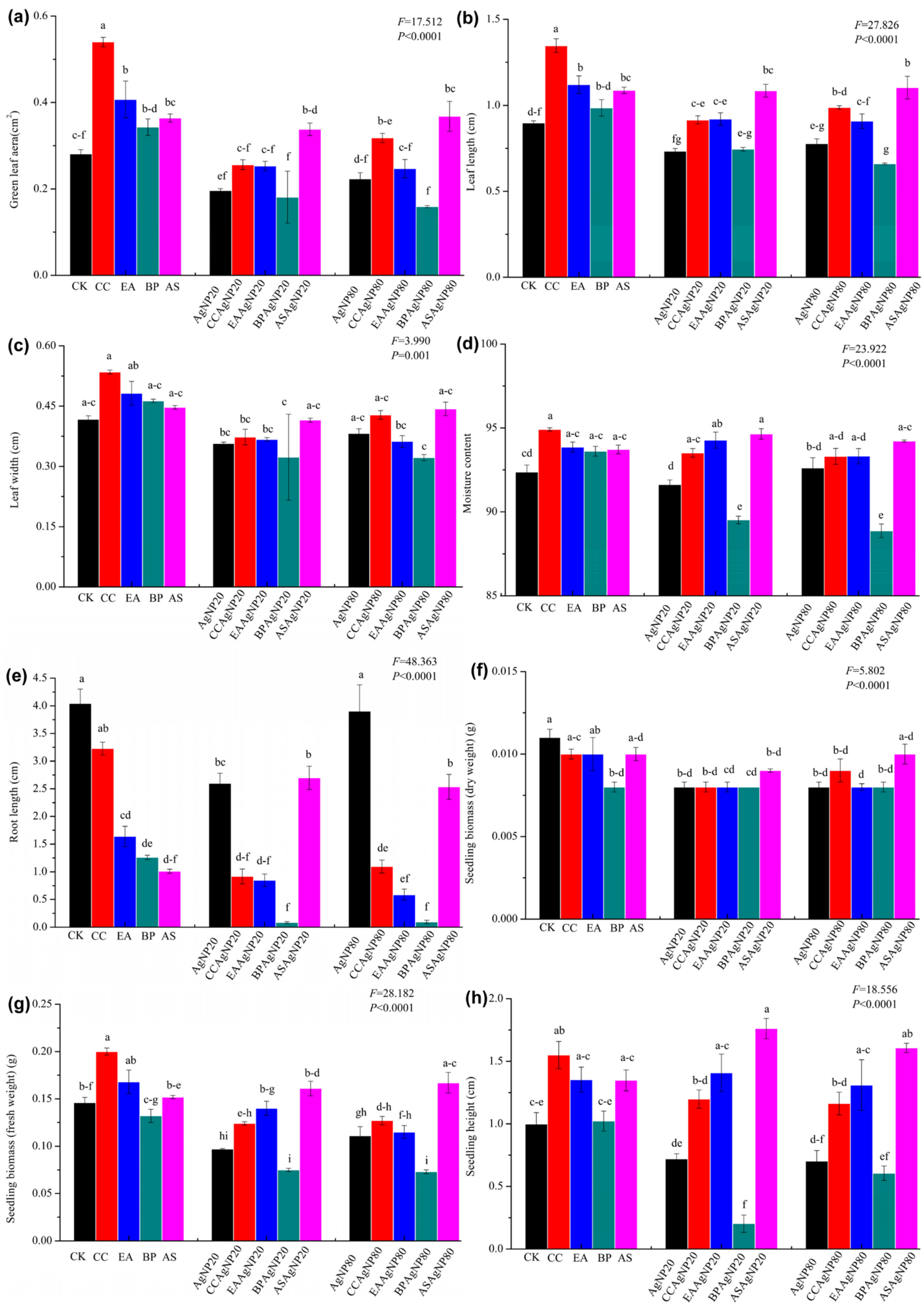

Figure 2. Seedling growth indices of lettuce. Bars (means ± SE) with different letters mean statistically significant differences ($P < 0.05$). The lowercase letters on top of the bars are presented as “c-f”, “b-d”, “d-f”, “b-e”, “e-g”, “a-c”, “a-d”, “d-h”, “f-h”, and “c-e” means “cdef”, “bcd”, “def”, “bcde”, “efg”, “abc”, “abcd”, “defgh”, “fgh”, and “cde”, respectively.

Influence of the allelopathic intensity of the four asteraceae invasive plant species on lettuce seed germination and seedling growth under two particle sizes of silver nanoparticles

The germination rate index, green leaf area, leaf length, root length, and fresh weight of lettuce under CCAgNP20 and CCAgNP80 were lower than those under CC ($P < 0.05$; Figs. 1 and 2). The green leaf area, leaf length, and seedling biomass (dry weight) of lettuce under EAAgNP20 were lower than those under EA ($P < 0.05$; Fig. 2). The germination rate index, green leaf area, leaf length, root length, and seedling biomass (includes fresh weight and dry weight) of lettuce under EAAgNP80 were lower than those under EA ($P < 0.05$; Figs. 1 and 2). The green leaf area, leaf length, moisture content, root length, and fresh weight of lettuce under BPAgNP20 and BPAgNP80 were lower than those under BP ($P < 0.05$; Fig. 2). Conversely, the germination index and promptness index of lettuce under BPAgNP80 were higher than those under BP ($P < 0.05$; Fig. 1). The root length of lettuce under ASAgNP20 and ASAgNP80 was also higher than that under AS ($P < 0.05$; Fig. 2).

DISCUSSION

Previous studies (Wang et al. 2018c, d, 2019d, 2020c, Carvalhoa et al. 2019, Lyytinen & Lindström 2019, Wei et al. 2020a, b) indicated that the four Asteraceae invasive plant species, particularly *C. canadensis*, *E. annuus*, and *B. pilosa*, show signficant allelopathic interactions during the the germination of lettuce seeds, particularly on the germination power, germination rate and vitality, and response capability of seed germination of lettuce in this study. Thus, the seed germination of lettuce will decline noticeably under the allelopathic intensity produced by the four Asteraceae invasive plant species. This phenomenon could be caused by the allelochemicals generated, such as polyphenols, by the invasive plant species, which can have deleterious effects on nutrient absorption, carbon assimilation, and cell division (Djurdjević et al. 2012, Fabbro et al. 2014, Lyytinen & Lindström 2019, Hsueh et al. 2020). These factors would have a strong impact on seed germination (Wang et al. 2018c, d, 2019d, 2020c, Carvalhoa et al. 2019, Hsueh et al. 2020, Wei et al. 2020a, b, Krstin et al. 2021). Strangely, the four Asteraceae invasive plant species, particularly *C. canadensis*, *E. annuus*, and *A. subulatus*, can enhance the growth of lettuce seedlings, particularly on competitive sunlight capture and photosynthetic area. The cause could be the generation of reactive oxygen species (ROS) primarily generated within plant cells induced by the low concentration of allelochemicals released by the four Asteraceae invasive plant species, which may stimulate plant growth (Takao et al. 2011, Zhang et al. 2012, Wang et al. 2018c, d, 2019d, 2020c, Wei et al. 2020a, b). This phenomenon has been identified as caused by hormones, as a response mechanism to environmental stress at low levels (Zhang et al. 2012, Agathokleous et al. 2019). In addition, the nutrients in the allelochemicals of the four Asteraceae invasive plant species can promote seedling growth (Wei et al. 2020b). Thus, the four Asteraceae invasive plant species may decrease the seed germination but enhance the seedling growth of natives. The results suggest that the four Asteraceae invasive plant species can colonize new habitat via their allelopathic intensity on seed germination of natives first, but those competitors can obtain a higher fitness rate owing to their ability to more aggressively compete for resources compared with the natives. Based on this, it is necessary to eliminate invasive plant species as early as possible in the sowing of natives or in the early germination of natives to minimize the allelopathic effects of invasive plant species on the growth of natives.

Moreover, the allelopathic intensity of *B. pilosa* leaf extracts on lettuce seed germination was noticeably greater than that of the other three Asteraceae invasive plant species leaf extracts used in this study. However, it was unexpected that the *A. subulatus* leaf extracts had no apparent allelopathic intensity on lettuce seed germination in this study. Typically, the allelopathic intensity of the four Asteraceae invasive plant species on lettuce seed germination was markedly reduced in the following order: *B. pilosa*, *C. canadensis*, *E. annuus*, and *A. subulatus*. The main reason could be owing to the differences in the type and quantity of allelochemicals of the four Asteraceae invasive plant species. Thus, the allelopathic intensity on lettuce seed germination may perform a key role in the effective colonization of *B. pilosa*, *C. canadensis*, and *E. annuus* compared with *A. subulatus*. These results confirm the first hypothesis.

Silver nanoparticles normally decrease plant growth (Yin et al. 2012, Wang et al. 2018c, 2020d, Wu et al. 2019b). Similarly, silver nanoparticles were found to dramatically decrease the germination rate and vitality, competitive intensity for water and inorganic salts absorption, and growing competitive intensity of lettuce in this study. Thus, silver nanoparticles can inhibit plant growth. The main reason could be owing to the generated Ag ions that enter the environment from the silver nanoparticles (Guo et al. 2017, Wang et al. 2018c, 2020d, Wu et al. 2019b). In particular, Ag ions can be found in high concentrations in the root site of plant species (Vannini et al. 2014). It is believed these ions may decrease the chlorophyll contents and root elongation; inhibit cell division, the electron transport chain, and the synthesis of adenosine triphosphate synthesis; induce lipid membrane peroxidation; and disturb gene expression and the activity of metabolic enzymes (Holt & Bard 2005, Gubbins et al. 2011, Benoit et al. 2013, Qian et al. 2013, Wu et al. 2019b). In addition, silver nanoparticles that are 20 nm had a greater effect on the competitive intensity for water and inorganic salts absorption of lettuce than silver nanoparticles that are 80 nm. This difference is owing to the greater release of Ag ions from silver nanoparticles that have small particles (McGillicuddy et al. 2017, Wang et al. 2018c, 2020d, Wu et al. 2019b). In addition, smaller silver nanoparticles can generate a higher level of reactive oxygen species, which can lead to toxicity toward plant growth (McGillicuddy et al. 2017, Wang et al. 2018c, 2020d, Wu et al. 2019b). The results of this study apparently corroborate the second hypothesis.

In addition, based on previous results (Wang et al. 2018c), silver nanoparticles intensify the allelopathic effect of the four Asteraceae invasive plant species, particularly *C. canadensis*, *E. annuus*, and *B. pilosa*, on lettuce SGe and SGr, particularly on the competitive intensity for water and inorganic salts absorption, competitive intensity for sunlight capture, photosynthetic area, and growing competitive intensity in this study. Thus, silver nanoparticles noticeably increased the allelopathic intensity of invasive plant species on the SGe and SGr of natives, particularly on seedling growth. The finding may be explained by the following reasons: (I) invasive plant species and silver nanoparticles, particularly those with small particle sizes, pose an adverse effect on lettuce SGe and SGr in most cases. Thus, the combined treatment of these two factors generates a (negative) synergistic effect on lettuce SGe and SGr. (II) The combined treatment of these two factors would significantly attenuate the competitive intensity for water and inorganic salts absorption, competitive intensity for sunlight capture, and photosynthetic area of lettuce, and thus, noticeably decrease the growing competitive intensity of lettuce growth. (III) Phenolics, primarily polyphenols, are a group of the maximum abundant secondary compounds in plant species (i.e., allelochemicals for invasive plant species) (Zhang et al. 2011, Djurdjević et al. 2012, Wang et al. 2018d, 2020c, Marksa et al. 2020), but the ecotoxicity of Ag ions released by silver nanoparticles (Guo et al. 2017, Wang et al. 2018c, 2020d, Wu et al. 2019b) on lettuce SGe and SGr may be increased by the weakly acidic phenolics, particularly polyphenols. Previous studies indicated that heavy metals are more toxic in acidic environments (Walker et al. 2004, Wang et al. 2018d, 2020c, Wei et al. 2020a). Thus, these results may lend support to the third hypothesis. Consequently, silver nanoparticles, particularly those with small particle sizes, may facilitate the invasion process of the four Asteraceae invasive plant species. More importantly, most of the lettuce SGe and SGr indices under the combined treatment of *B. pilosa* and silver nanoparticles with two particle sizes were significantly lower than those under the combined treatment of the other three Asteraceae invasive plant species and two particle sizes of silver nanoparticles. Thus, the allelopathic effect of B. pilosa is significantly higher than that of the other three Asteraceae invasive plant species when subjected to pollution by silver nanoparticles. The main reason could be that the reactivity of the allelochemicals of *B. pilosa* is comparatively higher.

In summary, the four Asteraceae invasive plant species, particularly *C. canadensis*, *E. annuus*, and *B. pilosa*, generate apparent allelopathic intensity on the germination of lettuce seeds. Typically, the allelopathic intensity of the four Asteraceae invasive plant species on lettuce seed germination noticeably decreased in the following order: *B. pilosa* > *C. canadensis* > *E. annuus* > *A. subulatus*. In addition, silver nanoparticles increase the allelopathic intensity of the four Asteraceae invasive plant species, particularly *C. canadensis*, *E. annuus*, and *B. pilosa,* on lettuce SGe and SGr. Additionally, the allelopathic intensity of B. pilosa is significantly higher than that of the other three Asteraceae invasive plant species when subjected to pollution with silver nanoparticles. Therefore, increasing levels of silver nanoparticle pollution may stimulate the invasive behavior of the four Asteraceae invasive plant species, particularly *B. pilosa*, via their enhancement in the allelopathic intensity.

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