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Source: American Fern Journal, 102(3):208-215.

Published By: The American Fern Society

DOI: http://dx.doi.org/10.1640/0002-8444-102.3.208
URL: http://www.bioone.org/doi/full/10.1640/0002-8444-102.3.208

American Fern Journal 102(3):208–215 (2012)

Effects of *Ageratina adenophora* on Spore Germination and Gametophyte Development of *Neocheiropteris palmatopedata*

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Abstract.—As one of the worst invasive alien plant species in China, *Ageratina adenophora* has caused serious economic losses and reduced the diversity of native species, possibly due to allelopathic interactions. However, we have little knowledge of its effects on ferns. In Petri dish bioassays, the effects of the aqueous leachates from roots, stems and leaves of *A. adenophora* on spore germination and gametophyte development of *Neocheiropteris palmatopedata* were investigated. All leachates inhibited spore germination and rhizoid growth of *N. palmatopedata*. Furthermore, the inhibitory effects increased with increasing leachate concentrations, and root leachates exhibited the greatest inhibition. Possible inhibitory causes are discussed in the present study. Additionally, the gametophytes of *N. palmatopedata* treated with the leachates of *A. adenophora* did not show morphological differences compared with the control. This result differs from previous studies investigating morphological changes in other fern species when associated with *A. adenophora*. Varying sensitivity of different fern species to the same allelochemicals of *A. adenophora* may partly be responsible for this difference.

Key Words.—*Ageratina adenophora*, *Neocheiropteris palmatopedata*, spore germination, gametophyte development, allelopathic interaction

At present, there are about 170 species of terrestrial invasive plants in China. *Ageratina adenophora* Sprengel (Synonym: *Eupatorium adenophora*) (Asteraceae) is a very successful invasive species (Sang *et al.*, 2010). It is native to Mexico, where it is common (Cronk and Fuller, 1995). It first invaded Yunnan Province in China in the 1940s. Since then it has intensively colonized southwest China, reduced agricultural production and strongly altered plant community structures (Wang *et al.*, 2011). It survives and proliferates under various conditions and may

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adversely affect the surrounding vegetation through allelopathic effects in China (He and Liu, 1990; Song *et al.*, 2000; Yu *et al.*, 2004; Yang *et al.*, 2006; Yang *et al.*, 2008).

Most previous studies of *Ageratina adenophora* (Ageratina) have focused on its deleterious effects on spermatophytes (Wan *et al.*, 2010). It excretes allelochemicals into the soil, which hinder the germination and seedling growth of potential competitors (Sang *et al.*, 2010). However, studies of the effects of *A. adenophora* invasion on ferns (including gametophytes and sporophytes) have received comparatively little attention (Zhang *et al.*, 2007; Zhang *et al.*, 2008a, 2008b).

Yunnan is one of the richest regions in species diversity in China (Wang and Wang, 2006), with 762 documented fern species (Kunming Institute of Botany, Chinese Academy of Sciences, 2006). Our field observations showed that some ferns failed to establish in pure patches of *Ageratina adenophora*. Previous studies by Zhang *et al.* (2007, 2008a, 2008b) provide strong evidence in support of the inhibitory effects of *A. adenophora* on three fern species, *Macrothelypteris torresiana* (Gaud.) Ching, *Pteris finotii* Christ and *Cibotium barometz* (L.) J. Sm. Those studies showed that all leachates of *A. adenophora* inhibited spore germination and growth of the first rhizoid of the three fern species, and inhibitory effects increased with increasing leachate concentrations.

Neocheiropteris palmatopedata (Baker) H. Christ (Polypodiaceae) is endemic to China and is distributed in Yunnan, Sichuan and Guizhou. It is well known for its ornamental and medicinal value (Lin, 2000; Ding, 1982). Furthermore, in China, it is rare in the wild (Yu, 1999). Hence, understanding the biology of *N. palmatopedata* is of great importance for conservation purposes.

The distribution of *Neocheiropteris palmatopedata* overlaps with that of *Ageratina adenophora* in Yunnan. Allelopathic activity is more readily observed in the sensitive stage of plant growth (Petersen and Fairbrothers, 1980). In ferns, the activity is easily detected in stages such as spore germination and gametophyte development (Petersen and Fairbrothers, 1980). Hence, this study had two main objectives: (i) to determine the potential effects of *A. adenophora* on spore germination and gametophyte development of *N. palmatopedata*; and (ii) to examine the allelopathic potential of roots, stems and leaves of *A. adenophora*.

Materials and Methods

Plant Leachates

The effects of *Ageratina adenophora* leachates on *Neocheiropteris palmatopedata* were evaluated in three ways in this study: 1) rate of rhizoid elongation, 2) rate and percentage of spore germination, and 3) gametophyte morphology. Whole fresh plants of *A. adenophora* were collected from Nanjing Forestry University. The root, stem and leaf leachates were made according to Zhang *et al.* (2008a) and were sterilized by passing them through Duropore ® PVDF membrane (0.45 μm, Carrigtwohill, Co. Cork. Ireland). For the determination of rhizoid elongation, the sterile leachates were diluted with sterile double-distilled water to give six concentrations (0, 10, 20, 30, 40 and 50% leachate). For trials of spore germination and gametophyte growth, the sterile leachates were diluted with B_5 basal medium (Gamborg *et al.*, 1968) containing 0.7% agar and 2% sugar to give these six concentrations. In all the trials, B_5 basal medium containing 0.7% agar and 2% sugar was used as a control. All media were added at 6 mL per Petri dish (3.4 cm diameter).

Spore Collection, Incubation, Germination and Gametophyte Growth

Spores of *Neocheiropteris palmatopedata* were collected from 15–20 fertile fronds with mature but closed sporangia from 15 individuals on December 2010 in Yunnan University. The collected fronds were immediately sent to Nanjing. The fronds were unfolded, placed in clean paper bags and air dried at room temperature. Spores were collected and cleared by a mesh with pores 0.088 mm in diameter (Zhejiang Shangyu Yarn and Sieve Factory, Shangyu, China) one week later. About 2 mg of spores was packed into a 1×1 cm^2 bag made of clean filter paper. Thereafter, the spores were immersed into distilled water and washed five times in sterile water after surface-sterilizing in 5% sodium hypochlorite for 4 min. The spore suspension was prepared by adding sterile double-distilled water to the sterilized spores. Spores were inoculated evenly in each Petri dish at a density of 30 spores/cm^2 for the determination of rhizoid elongation and 400 spores/cm^2 for the trials of spore germination and gametophyte development. After inoculation, all Petri dishes were put into larger Petri dishes (12 cm diameter) to avoid desiccation and pollution. The larger Petri dishes were placed in the dark at 25 °C for 24 h and then transferred to fluorescent light (photon flux density 1×10^4 μ mol m^{-2} s^{-1}) at 25 °C at 12 h light photoperiod. Five visual fields were observed in each of the Petri dishes, through a gridding ocular at a magnification of 20 × 1.5 in a microscope (No. XTS 20130, Beijing Tech Instrument Co., Ltd., Beijing, China).

Germination time was calculated as the time elapsed from sowing to the observance of the first germinated spore (i.e., when signs of a first rhizoid were evident). For each dish, fourteen days after the first observed germination, about 400 spores were selected randomly for the calculation of the germination percentage and also for gametophyte development observation. Thereafter, the germination percentage was calculated every 6 days until no further increase was detected. Data for spore germination and gametophyte assays is presented as the mean value of the three Petri dishes of each treatment.

For the determination of rhizoid elongation, after growing for 2 weeks in medium without leachate, six healthy gametophytes with uniform rhizoids in each Petri dish were selected for further observation. Afterwards, all gametophytes were treated with various leachates. One week later, length of rhizoid was recorded using a crossed ocular micrometer in a dissecting microscope (No. XTS 20130, Beijing Tech Instrument Co., Ltd., Beijing, China).

Statistics

The effects of *Ageratina adenophora* leachates on spore germination and gametophyte development of *Neocheiropteris palmatopedata* were analyzed by ANOVA using the SPSS 10.0 package (SPSS, Chicago, IL, USA).

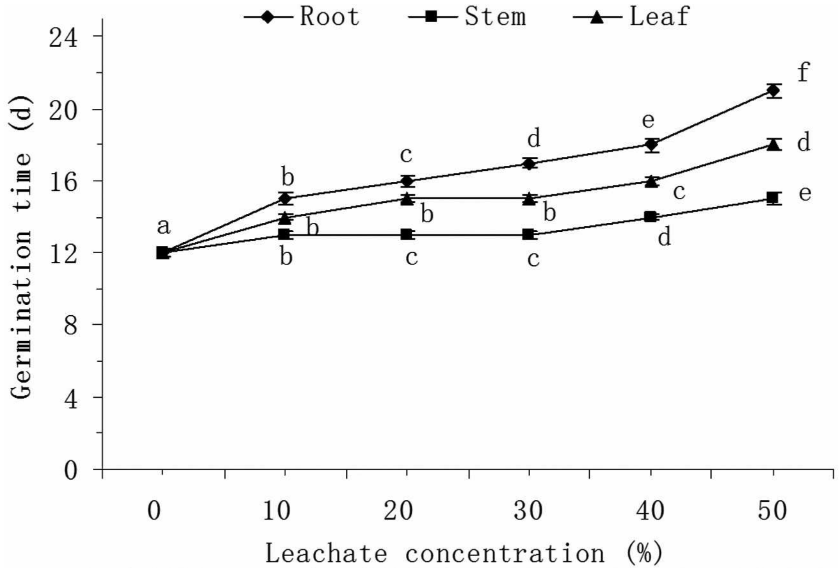

FIG. 1. Germination time of the spores of *Neocheiropteris palmatopedata* in the control and treated with root, stem and leaf leachates of *Ageratina adenophora* after inoculation. Different letters indicate significantly different ($P < 0.05$).

RESULTS

Spore Germination

Spores of *Neocheiropteris palmatopedata* germinated 12 days after inoculation in the control and continued until 35 days. Root, stem and leaf leachates of *Ageratina adenophora* delayed spore germination. The time needed for spore germination differed among treatments. Spores treated with stem leachates germinated faster than those treated with root and leaf leachates (Fig. 1). The inhibition of root, stem and leaf leachates of *A. adenophora* to spore germination percentage of *N. palmatopedata* increased with increasing concentrations ($P < 0.05$) (Fig. 2). As time went by, the germination percentages of *N. palmatopedata* treated with the root, stem and leaf leachates of *A. adenophora* went up and then leveled off ($P < 0.05$) (Fig. 2).

Rhizoid Growth

The root, stem and leaf leachates of *Ageratina adenophora* inhibited the rhizoid growth of *Neocheiropteris palmatopedata* and the inhibitory effect generally increased with increasing leachate concentrations. Root leachates were the most potent inhibitor, followed by the leaf leachates ($P < 0.05$) (Fig. 3).

Gametophyte Development

Gametophyte morphology of *Neocheiropteris palmatopedata* in the control was in agreement with the previous research carried out by Deng *et al.* (2009).

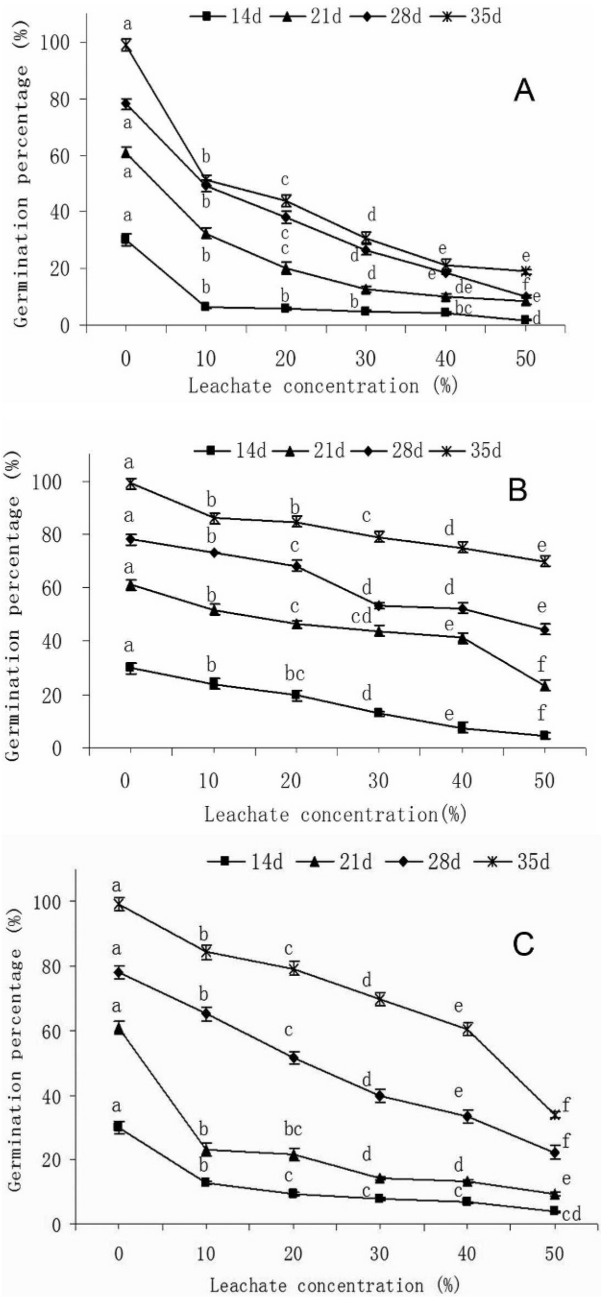

FIG. 2. Germination percentage of the spores of *Neocheiropteris palmatopedata* in the control and treated with root (**A**), stem (**B**) and leaf (**C**) leachates of *Ageratina adenophora*. Statistically significant differences among treatments are indicated by letters above or below lines based on least significant difference (LSD) multiple comparison tests. Two similar letters indicate no significant difference between treatments, and dissimilar letters indicate a significant difference between treatments ($P < 0.05$).

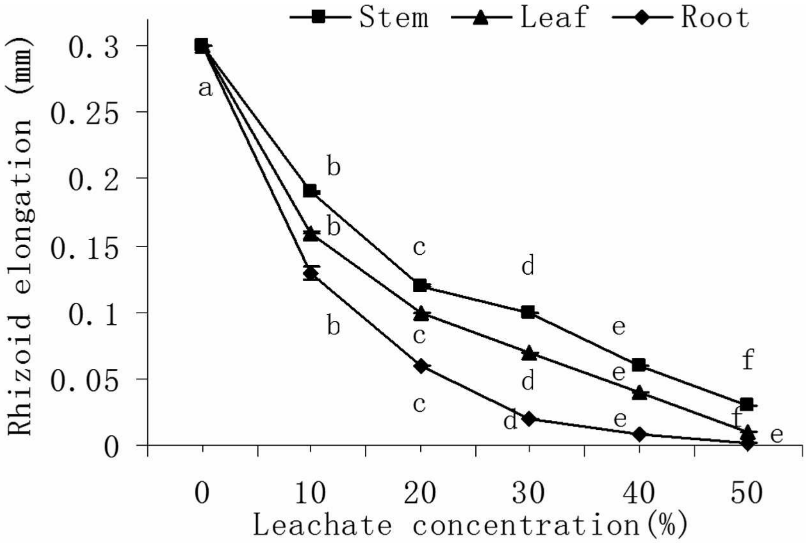

FIG. 3. Mean (± SD) of the rhizoid elongation of *Neocheiropteris palmatopedata* in the control and treated with root, stem and leaf leachates of *Ageratina adenophora*. Different letters indicate significantly different ($P < 0.05$).

The gametophytes produced from the spores treated with the leachates of *Ageratina adenophora* did not show morphological differences from the control.

DISCUSSION

Our results indicated that spore germination of *Neocheiropteris palmatopedata* was lower when treated with the root, stem and leaf leachates of *Ageratina adenophora* as compared to the control. Spore germination of *N. palmatopedata* is essential for its sexual reproduction. If this germination inhibition also occurs in nature, where spores of *N. palmatopedata* overlap with plants of *A. adenophora*, these leachates could potentially drastically decrease the abundance of this plant in the community. The delay in germination of *N. palmatopedata* spores could severely affect the competition ability of this fern species for resources.

All leachates of *Ageratina adenophora* inhibited rhizoid growth of *Neocheiropteris palmatopedata*. Furthermore, the inhibitory effects increased with the increasing leachate concentrations. It is generally believed that the rhizoids of fern gametophytes function as organs of uptake and absorption. Inhibition of *N. palmatopedata* rhizoid growth by the root, stem and leaf leachates could reduce the ability of gametophytes to absorb water and nutrients, which could affect the production and potential growth of the future sporophyte and its function in the community. Racusen (2002) demonstrated that rhizoids were the primary site of uptake for monovalent cations in the gametophytes of *Onoclea sensibilis* L. Kamachi *et al.* (2005) showed that *Athyrium* gametophytes had the ability to accumulate lead in the rhizoids. Therefore, we hypothesize that the active component(s) in the root of *A. adenophora* entered and accumulated in the rhizoids of *N. palmatopedata*, which inhibited the rhizoid elongation of *N. palmatopedata*.

In this study, the gametophytes produced from the spores of *N. palmatopedata* treated with the leachates of *A. adenophora* did not show morphological differences when compared with the control. However, in a study of *Macrothelypteris torresiana*, the rhizoids of the young gametophytes treated with various root leachates of *A. adenophora* were erect, curved, or swollen and the curving and swollen rhizoids increased with the increasing concentrations of *A. adenophora* (Zhang *et al.*, 2008a). The gametophytes from the spores of *Pteris finotii* treated with higher concentrations of root leachates of *A. adenophora* suffered morphological changes (Zhang *et al.*, 2007). This difference might be attributed to differences in sensitivity to the same allelochemicals.

In our experiments, greater inhibitory effects on gametophyte development of *N. palmatopedata* occurred with the root leachates of *A. adenophora,* as compared to stem and leaf leachates. This result was consistent with previous studies, which showed that the root leachates resulted in greater inhibition than the stem and leaf leachates (Zhang *et al.*, 2007; Zhang *et al.*, 2008a; Tripathi *et al.*, 1981). However, He and Liu (1990) observed that the aqueous leaf extracts of *A. adenophora* were the most allelopathic to the seed germination of four plants. Tripathi *et al.* (1981) also reported that the aqueous extracts of different parts of *A. adenophora* caused different effects on other plants. This difference might due to the differences in the sensitivity to same allelochemicals, concentration of the allelochemicals, or variations in allelochemicals among the tissues. The leachates of *A. adenophora* were identified as phenolic compounds (Yang *et al.*, 2006), which might act as the allelopathic substances (Kim *et al.*, 2005). Further investigation is needed to better understand the chemistry of the allelopathic activity.

Sustainable management of *A. adenophora* in China includes ecological restoration by competitive replacement of *A. adenophora* and biological control agents against *A. adenophora* (Wan *et al.*, 2010). Future efforts will concern the effective control of *A. adenophora* as well as its utilization.

ACKNOWLEDGMENTS

This work was supported by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), Jiangsu Planned Projects for Postdoctoral Research Funds (1001078C) and China Postdoctoral Science Foundation funded project (20100481152).

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