

## Inhibition of invasive specie *Mikania micrantha* H.B.K. by native dominant trees in China

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### ABSTRACT

Laboratory bioassays and pot experiments were done to evaluate the allelopathic effects of 6-native dominant trees [*Pinus massoniana* Lamb., *Schima superba* Gardn. et Champ., *Castanopsis chinensis* Hance, *Castanopsis fissa* (Champ. ex Bench.) Rehd. et Wils., *Cryptocarya chinensis* (Hance) Hemsl. and *Cryptocarya concinna* Hance] of 3-forests on *Mikania micrantha* H.B.K. in lower subtropical China. In laboratory bioassays, the litter leachates of all 6-dominant trees reduced the root length of *M. micrantha* seedlings. Likewise in pot experiments also, the seedling growth of *M. micrantha* was inhibited significantly when planted with *S. superba*, *C. chinensis*, *C. fissa*, or *Cryptocarya chinensis*, but the total biomass was not affected by *Cryptocarya concinna*. Compared to *Cryptocarya concinna* treatment, the stem mass ratio (SMR) of *M. micrantha* decreased significantly with *S. superba*, *C. chinensis*, *C. fissa* and *Cryptocarya chinensis* treatments. This decrease in aboveground biomass results in less space occupied by *M. micrantha* and will consequently reduce the plant's further expansion. Our results support the assumption that the dominant trees exerted different inhibitory effects on the growth of *M. micrantha*, implying that the plant-plant interactions may play an important role in determining the habitat invasion resistance.

**Keywords:** Allelopathy, *Castanopsis chinensis*, *Castanopsis fissa*, *Cryptocarya chinensis*, *Cryptocarya concinna*, dominant trees, invasion resistance, invasive plants, *Mikania micrantha*, *Pinus massoniana*, *Schima superba*, seedling growth

### INTRODUCTION

Biological invasion represents one of the most serious threats to biodiversity, hence the invasion ecology research has become one of the central issues of contemporary environmental science (14,25,39). *Mikania micrantha* H.B.K. (Asteraceae), a perennial vine weed, is one of the top ten most invasive weeds in the world (13). It is native to tropical Central and South America and was introduced in Indonesia as ground cover in 1940s, from there it spread rapidly to the Pacific Islands, Southeast Asia, New Guinea and Australia (33,42). In these regions, *M. micrantha* became most invasive plant, resulting in dramatic reduction in agricultural production and degeneration of forest ecosystems (11,44). *M. micrantha* was introduced in China after 1910 and has invaded and spread widely since the 1980s (19,40). In recent years, it has infested and damaged various ecosystems in South China, which is one of the biodiversity centres in China (6,28,45).

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The *M. micrantha* invasion varies among the different forest types (44). Plant-plant interactions play a key role in regulating the composition and function of a given community (3). Exotic invasive plants could influence the accompanying species through competition etc, altering ecosystem processes (31). However, the relative inhibitory effects of native dominant trees on performance of exotic invasive species have received little attention.

This study aimed to determine the effects of native dominant trees of 3-different forests in lower subtropical China on the growth of *M. micrantha*. Laboratory bioassays were done to indicate the effects of aqueous extracts derived from the litter of dominant trees on the root growth of *M. micrantha*. Meanwhile, we also cultivated the *M. micrantha* with different dominant tree seedlings in the same microcosm respectively, to test the hypothesis that dominant trees exerted different inhibitory effects on the growth of *M. micrantha*, and plant-plant interactions may play an important role in determining the habitat invasion resistance.

## MATERIALS AND METHODS

The UNESCO/MAB Dinghushan Biosphere Reserve (DHSBR) Guangdong Province, South China (112°10'E, 23°10'N) was selected as study site. It has area of approx 1200 ha. The reserve is located in a subtropical moist forest life zone with a monsoon climate (*sensu* 12). Mean annual temperature: 21.0°C, Mean coldest temperature: 12.6°C (January) and hottest: 28.0°C (July) (26). The average annual relative humidity is 80%, and the mean annual precipitation is 1927 mm (15).

There are three major forest types in DHSBR: (i). Pine forest (PMF), (ii). Pine-broadleaf mixed forest (PBMF) and (iii). Monsoon evergreen broadleaf forest (MEBF) (27). These forests are located at 150-300 m above sea level and belong to different natural successional stages, from the pioneer community PMF to the regional climax vegetation MEBF (46). PMF is dominated by *Pinus massoniana*. PBMF originated from a planted *P. massoniana* forest that was naturally invaded and colonized by broadleaf species. PBMF is a transitional forest type between pine and monsoon evergreen broadleaf forest. The dominant trees in PBMF are *Schima superba*, *Castanopsis chinensis* and *C. fissa*. The selected MEBF has been well protected from the human disturbance for > 400 years. The major dominant trees in MEBF are *Cryptocarya chinensis* and *Cryptocarya concinna* (41).

In January 2007, *M. micrantha* seeds were collected from the Qi-Ao Island, Zhuhai (113°39'E, 22°24'N), where it has heavily infested the area. In March 2007, the newly fallen litter of following 6-dominant trees was collected in the DHSBR, *P. massoniana* from PMF, *S. superba*, *C. chinensis*, and *C. fissa* from PBMF, *Cryptocarya chinensis* and *Cryptocarya concinna* from MEBF, respectively. The seedlings of these dominant trees (about 15 cm height) were also collected from these 3-forests. We used seed-germinated seedlings of *P. massoniana* because of low availability of its seedlings in the field.

### Laboratory bioassays

To determine the allelopathic effects of dominant trees litter on root growth of *M. micrantha*, litter leachates (0.05 g/ml) were prepared in March 2007 by soaking 50 g dry

plant litter of different plants in 1000 mL distilled water for 24 h. The solution was filtered twice through filter paper. The leachates pH was adjusted to 7.0 with 1 M NaOH and HCl. Twenty seeds of *M. micrantha* were placed on filter paper in Petri dishes (9 cm dia) containing 5 ml leachate solution. Distilled water was used as control. Five replicates were set up for each treatment. All Petri dishes were placed in light incubator at 28°C/light (10 h) and 25°C/dark (14 h). Root length of *M. micrantha* was measured on 7th day.

### Pot experiments

Laboratory bioassay is useful to study the plant-plant interactions (17), but it lacks soil medium, in which chemical compounds can function directly or with the help of soil biota (30). To further elucidate the effects of native dominant trees from the 3-forests on *M. micrantha* growth, pot experiments were done in greenhouse in DHSBR with ambient light. The pots (18 cm height, 23 cm dia) were filled with same soil collected from the grassland near the greenhouse and then seedlings of 6-native dominant trees were planted in pots (one plant per pot) in March 2007. Two weeks later, 50 seeds of *M. micrantha* were placed on the soil surface of each pot. There were five replicates for each treatment (6-dominant tree species) and a blank control (without tree seedling). Seedlings of *M. micrantha* were thinned to one plant per pot at 90 day after sowing. All pots were watered twice daily to keep the soil moist. As the *P. massoniana* seedlings couldn't survive in pots, there were no data about its effects on performance of *M. micrantha* in this experiment.

*M. micrantha* were allowed to grow for another 5-months and then harvested. Shoots were immediately clipped at the ground level and the stem lengths of individual *M. micrantha* plants were measured. Leaves were clipped and passed through a leaf-area meter (Li-3000; Li-Cor Corp.) to record the total leaf area. Roots were rinsed gently with water to remove soil particles. Roots, stems and leaves of the *M. micrantha* were separated and dried for 48h at 60°C and then weighed. The measurements were used to calculate: root mass ratio (RMR, root mass/total mass), stem mass ratio (SMR, stem mass/total mass), leaf mass ratio (LMR, leaf mass/total mass), leaf mass fraction (LMF, leaf mass/ shoot mass) and root-shoot ratio (R/S, root mass/ shoot mass).

### Statistical Analysis

One-way ANOVA was used to examine the effects of treatments on the performance of *M. micrantha*. A Student-Newman-Keul multiple comparison test was used to compare the difference among treatments if the one-way ANOVA produced a significant difference ( $P < 0.05$ ). All analyses were performed on SPSS11.5 (SPSS Inc., USA).

The magnitude of inhibition or stimulation was denoted as response index (RI) and calculated as under:

$$RI (\%) = (T/C - 1) \times 100$$

Where, T: Treatment data, C: Control data.

## RESULTS AND DISCUSSION

Laboratory bioassays are used to indicate the role of chemical compounds in plant-plant interactions (17). Root growth is very sensitive to allelochemicals (17,30), hence, used as indicator of allelopathic effects. We found that aqueous leachates from litter of all 6-dominant trees (*P. massoniana*, *S. superba*, *C. chinensis*, *C. fissa* and *Cryptocarya chinensis*) reduced the root growth of *M. micrantha* by 39.6%, 45.1%, 46.5%, 33.7% and 48.4%, respectively, than control (distilled water) (Fig. 1). However the aqueous litter leachates of *Cryptocarya concinna* proved least inhibitory (14.3%) to root growth.

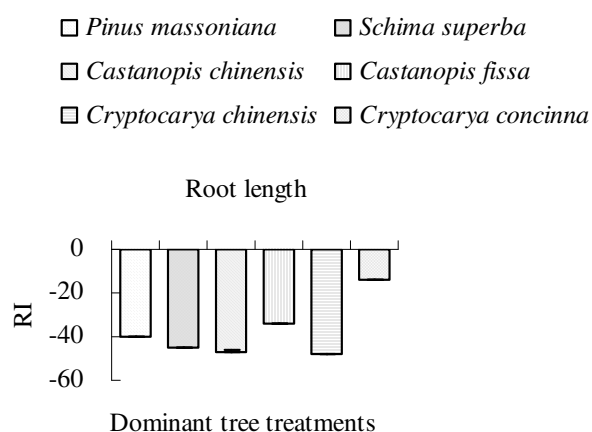


Figure 1. Effects of litter leachate of different dominant trees on root length of *Mikania micrantha*. Data are expressed as RI values.

Allelopathy is most common pathway in plant-plant interactions, affecting numerous ecosystem processes including community composition and function (16). The secondary compounds from invasive species could facilitate their invasions, which turned allelopathy into a “novel weapon” for invasive plant species attacking against the native species (4,5). On the other hand, many native plants, especially dominant trees in forests, contain allelochemicals (22,32,36). Our results indicated that root length of *M. micrantha* was inhibited by the litter of native dominant tree species from 3-forest types in lower subtropical China. Allelochemicals, which affects the development of plant roots, would further affect the absorption of water and nutrients, thereby, affecting the performance of plant. Therefore, allelopathic effects of native dominant trees on exotic species might be one of the pathways by which native communities resist the exotic species invasion.

The pot experiments results showed that *S. superba*, *C. chinensis*, *C. fissa* and *Cryptocarya chinensis* significantly inhibited the growth of *M. micrantha* seedlings. Compared to control (no tree seedling), total leaf area of *M. micrantha* was reduced significantly by 80.8%, 77.1%, 74.1% and 73.9%, when planted with *S. superba*, *C. chinensis*, *C. fissa* and *Cryptocarya chinensis*, respectively (Fig. 2-(A)). Stem length was reduced by 76.7%, 71.3%, 62.9% and 70% than control when planted with *S. superba*,

*C. chinensis*, *C. fissa* and *Cryptocarya chinensis*, respectively (Fig. 2-(B)). Total biomass of *M. micrantha* was also decreased by 87.6%, 82.0%, 76.0% and 74.1% compared to control when planted with *S. superba*, *C. chinensis*, *C. fissa* and *Cryptocarya chinensis*, respectively (Fig. 2-(C)). However, *Cryptocarya concinna* planted with *M. micrantha* slightly influenced its total leaf area, stem length or total biomass.

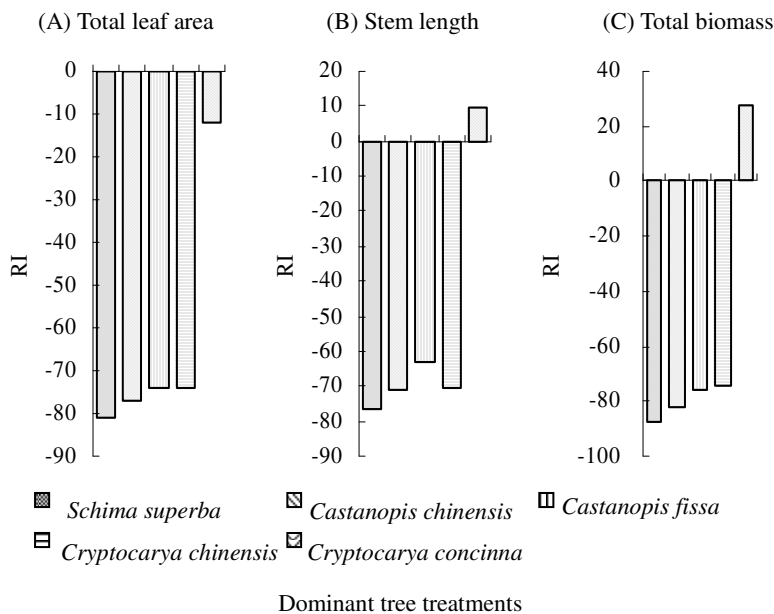


Figure 2. Effects of different dominant trees on (A) Total leaf area, (B) Stem length and (C) Total biomass of *Mikania micrantha*. Data are expressed as RI values.

Meanwhile, compared to *Cryptocarya concinna* treatment, the stem mass ratio (SMR) of *M. micrantha* decreased significantly with *S. superba*, *C. chinensis*, *C. fissa* and *Cryptocarya chinensis* treatments. Contrarily the root mass ratio (RMR) and root-shoot ratio (R/S) of *M. micrantha* seedlings were increased (Table 1). The reduction in aboveground biomass of *M. micrantha* would result in less space occupied by *M. micrantha* and might consequently decrease its further expansion.

Resistance is a well-accepted phenomenon, but the processes contributing to it are unclear (21). Species composition varies with the processes of forest succession and plays an important role in forest ecosystems. Once propagules of invasive plants arrive, the habitat invasion resistance is greatly determined by species interactions (7). To date, most studies exploring the effects of species composition on invasion resistance have focussed on many species (8,18,20,38). However, the species identification in the community is important to interpret the observed effects (1,10). Resistance to the invasion of exotic plants can result from strong effects of a relatively small number of resident species (37). Collectively, we verified that some native plants, especially the dominant species, exerted significant inhibitory impacts on the growth of *M. micrantha*.

Table 1. Biomass allocation of *Mikania micrantha* seedlings when planted with different dominant tree species

Dominant trees	RMR	SMR	LMR	LMF	R/S
<i>Schima superba</i>	0.22a	0.35b	0.55a	0.43a	0.28a
<i>Castanopsis chinensis</i>	0.17a	0.34b	0.59a	0.49a	0.21ab
<i>Castanopsis fissa</i>	0.14ab	0.37b	0.57a	0.50a	0.17ab
<i>Cryptocarya chinensis</i>	0.22a	0.34b	0.57a	0.44a	0.28a
<i>Cryptocarya concinna</i>	0.07b	0.53a	0.43a	0.40a	0.07b
Blank control	0.16ab	0.37b	0.56a	0.48a	0.19ab

RMR: Root mass ratio (root mass/total mass); SMR: Stem mass ratio (stem mass/total mass); LMR: Leaf mass ratio (leaf mass/total mass); LMF: Leaf mass fraction (leaf mass/ shoot mass), and R/S: Root-shoot ratio (root mass/ shoot mass). Numbers within each column followed by the different letters were significantly different according to one-way ANOVAs and Student-Newman -Keul multiple range tests,  $P < 0.05$ .

Apart from allelopathy, resource competition also plays important role in plant-plant interactions (34). The seedlings performance of *M. micrantha* might be influenced not only by allelopathy, but also by competition for resources in the pot experiment. However, the pot experiments results were consistent with our previous bioassay studies, which showed that different species exerted different effects on the growth of *M. micrantha*. Our study revealed that some native dominant trees, as important structural and functional units of terrestrial ecosystems, have inhibitory effects on *M. micrantha* growth and might contribute to the variability in different forest resistance to exotic plant *M. micrantha* invasion.

Litter is most important structural units of forest ecosystems, whose role in forest ecosystems has been assessed in different ways (35). Litter can alter the microenvironment and also act as mechanical barrier to germinated seeds, hence, reducing the plant spread, leaching the inhibitory compounds and altering the microclimates (2,9,29). Our results indicated that litter of native dominant trees had negative effects on exotic invasive plants. In meta-analysis of 35 independent studies, plant responses to litter were found much stronger in fields than in glasshouses (43). Besides litter, the allelochemicals could also be released from plant stems, leaves, and roots (23,24). Consequently, native dominant species have more intense and persistent effects on the invasive plants in the fields.

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