The response of the invasive weed *Mikania micrantha* to infection density of the obligate parasite *Cuscuta campestris* and its implications for biological control of *M. micrantha*

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(Received February 12, 2008; Accepted May 28, 2010)

**ABSTRACT.** The efficacy of a biological control agent depends upon the impact it has on the target species. We investigate the use of the obligate parasitic plant, *Cuscuta campestris* Yuncker as a biological control for the invasive weed *Mikania micrantha* H.B.K. In this experiment we test whether the impacts of the parasite on host growth, biomass allocation, photosynthesis, chlorophyll content, and soluble protein are affected by the density of the parasite. We examined the response of *M. micrantha* to infection densities of 0, 1, 2, 4 and 8 *C. campestris* seedlings per host plant. By day 30 after parasitization, *C. campestris* infection had significantly reduced *M. micrantha* biomass and the net photosynthetic rate of the 8th fully expanded leaf. These negative effects were greater as the number of parasites increased from 1 to 4 per host, but not from 4 to 8. *Mikania micrantha* stomatal conductance and transpiration rate were significantly reduced by *C. campestris* infection, but among 2, 4, 8 parasites per host there were no significant effects of infection densities on the host plants. Water use efficiency remained stable. The aerial parts of the infected *M. micrantha* plants at densities of 1, 2, 4 and 8 parasites per host died 83, 62, 50 and 46 d after parasitization on average, respectively. All infection densities decreased host chlorophyll content (a and b), and the infection by more than 1 parasite also significantly lowered soluble protein concentration. The results indicated that the effects of *C. campestris* infection on *M. micrantha* are density dependent, which provides a basis for refining the use of the parasite for biological control of *M. micrantha*. The optimal cost-effective number of parasites to control *M. micrantha* is 4 per host plant in the field.

**Keywords:** Biological control; Chlorophyll content; Growth; Invasive weed; Parasitic plant; Photosynthesis; Soluble protein; Weed management.

**INTRODUCTION**

Biological invasion is one of the major threats to native biodiversity and ecosystems (Mack et al., 2000; Mooney and Hobbs, 2000; Pimentel et al., 2005), and it is also an important element of global change (Vitousek et al., 1997; Dukes and Mooney, 1999). The control of invasive species is one of the most urgent challenges in ecology today (Hastings et al., 2006) and many effective control strategies have been developed (Moody and Mack, 1988; Zavaleta et al., 2001; Taylor and Hastings, 2004; Buhle et al., 2005; Culliney, 2005; Hulme, 2006). Biological control has been widely recognized as one of the most promising methods as it is permanent, energy-efficient, non-polluting, and inexpensive (Culliney, 2005).

*Mikania micrantha* H.B.K. is a fast-growing perennial climbing vine belonging to the family Asteraceae and is native to tropical Central and South America, where it is a weed of minor importance (Holm et al., 1977). However, in its palaeotropical exotic range, it has become a horrific invader and a notorious weed, severely damaging forestry and plantation crops (Parker, 1972; Holm et al., 1977; Zhang et al., 2004). It has been listed as one of the 100 worst invasive alien species in the world (Lowe et al., 2001), and is one of the top ten worst weeds in the world (Holm et al., 1977). *Mikania micrantha* entered South
China after 1910. Since the 1980s, it has invaded widely and caused vast economic loss in Hong Kong, Guangdong and Hainan (Feng et al., 2002; Zhang et al., 2004). In China, the obligate parasite Cuscuta campestris infects M. micrantha and has negative impacts on its biomass and physiology (Deng et al., 2003; Zan et al., 2003; Shen et al., 2005, 2007). Studies suggest that Cuscuta spp. effectively restrains the growth of M. micrantha (Zhang et al., 2004) and shows potential as a biological control agent. At the community level, field studies have shown that C. campestris significantly reduced the biomass and coverage of M. micrantha, but had minor effects on other plants (Zan et al., 2003; Lian et al., 2006).

Cuscuta campestris (Convulvulaceae) is the most widespread species in the genus and the only parasitic weed of North America that has spread to the Old World (Dawson et al., 1994). In South China, it is distributed in Guangdong Province (Liao et al., 2005). The parasite draws all nutrients from its host via haustoria and is a very powerful sink for host photosynthates, suppressing host growth, preventing flowering and fruiting, and even resulting in host death (Dawson et al., 1994; Shen et al., 2005). It can infect diverse host species, including some horticultural crops and cause damage in agriculture (Malik and Singh, 1979; Dawson et al., 1994).

In previous studies we found that C. campestris can affect M. micrantha photosynthesis through both an adverse impact on stomatal conductance (gs) and direct effects on photosynthetic metabolism, such as carboxylation efficiency and CO2-saturated rate of photosynthesis (Shen et al., 2007). Additionally, studies of hemiparasitic associations, such as Striga, Rhinanthus and Cassytha, found lower soluble protein concentrations (Rubisco content) and lower chlorophyll concentrations that might also be responsible for declined host photosynthesis (Watling and Press, 2000; Cameron et al., 2005; Shen et al., 2010), which is characteristic of holoparasitic associations.

In this study we examine whether increasing the C. campestris infection density of M. micrantha increases the parasite’s negative effects on host growth, biomass allocation, photosynthesis, and soluble protein and chlorophyll concentrations. This data will also enable us to determine the likely mechanisms that regulate host photosynthetic dysfunction in the holoparasitic association. The work will provide useful practical information for the deployment of C. campestris for the biological control of the invasive species M. micrantha.

**MATERIALS AND METHODS**

**Experimental materials and design**

The study was conducted during the May-December 2004 growing season at the field station of South China Botanical Garden (23°10’ N, 113°21’ E, elevation 40 m a.s.l.) in Guangzhou, Guangdong Province, China. The region is characterized by a typical south subtropical monsoon climate with annual average temperatures of 20-22°C, RH 77% and annual precipitation of 1982.7 mm. On 31 May 2004, whole M. micrantha plants were collected from a M. micrantha population in Dongguan, Guangdong Province. Two-node segments, similar in size, were selected from the middle of the stems. The segments were planted in 89 L pots filled with a mixture of pool mud and paddy field clay (1:2 v/v). The mixed soil had a pH of 6.5 ± 0.1, organic mater content of 3.3 ± 0.09%, total nitrogen of 0.182 ± 0.08%, ammonia nitrogen of 103.57 ± 5.86 mg kg⁻¹, available P of 23.98 ± 1.36 mg kg⁻¹, and available K of 132.83 ± 6.32 mg kg⁻¹ (mean ± SE). In each pot, three segments were planted with the lower node buried below and the upper one about 5 cm above the soil surface. The upper nodes began to sprout leaves 3 days later. When the plants were about 350 cm in height (23 August), they were thinned to one per pot, and 100 individuals of similar height and stem diameter were selected and placed at random in an open field. Of these, 80 were randomly chosen as host plants (infected group), leaving the remaining 20 uninfected (control group). To prevent M. micrantha from climbing between pots, plants were spaced at least 1 m apart, and a 5-m bamboo cane was placed vertically in each pot for M. micrantha to climb on.

On 12 August, C. campestris seeds were sown at a depth of about 1 cm in pots filled with sand, and they had germinated by 20 August. On 26 August, 5 cm tall C. campestris seedlings with wet sand were placed on the soil surface of each M. micrantha pot in the infected group. Density treatments (1, 2, 4 or 8 C. campestris seedlings per host plant) were randomly assigned to M. micrantha plants in the infected group, with 20 host plants for each treatment density. By 29 August, all the M. micrantha plants in the infected group had become infected with C. campestris stems. The experiment ended on 27 December, 120 d after parasitization (DAP) or 210 d after planting, when the uninfected M. micrantha plants had started to wither. During the experiment, the pots were weeded when necessary and watered twice daily with tap water at 06:00 h and 18:00 h, except on rainy days. No fertilizer was added throughout the experimental period.

**Growth measurements and observations**

On day 30 after parasitization, eight M. micrantha plants at each infection density were selected at random and harvested. All the dead material of both M. micrantha and C. campestris were carefully removed and weighed, while living parts were separated and handled as follows. M. micrantha plants were separated into stems, leaves, and underground roots. Roots were soaked in tap water, washed and separated carefully in running water over a 2-mm-mesh sieve. Stems, tendrils and reproductive organs of C. campestris were carefully dissected from M. micrantha stems and leaves. Plant materials were oven-dried at 70°C until constant weight was achieved. The remaining 12 plants were maintained to enable recording of their initial flowering date and the death date of the infected plants’ aerial parts.
Measurements of photosynthesis

Gas exchange measurements of leaves were made at around 10:00 h on 30 DAP, using a LI-6400 portable photosynthesis system with a standard 6 cm² leaf chamber (LI-COR Inc., Lincoln, NE, USA), and photosynthetic parameters were calculated based on von Caemmerer and Farquhar (1981). Irradiance was provided by an integrated red-blue light-emitting diode source (model 6400-02B, LI-COR, Inc.). To ensure all sampled leaves were of similar age and developmental stage, only the 8th fully expanded mature leaf from the tip of each stem was used, one leaf per plant and 8 randomly selected M. micrantha plants per infection density. Net photosynthetic rate (Pₚ), stomatal conductance (gₛ), and transpiration rate (E) were recorded when steady-state photosynthesis had been reached under a photosynthetic photon flux density (PPFD) of 1000 µmol photons m⁻² s⁻¹, [CO₂] 360 µmol mol⁻¹, leaf temperature of 30°C, and flow rate of 500 µmol s⁻¹. Water use efficiency (WUE, µmol CO₂ mmol H₂O⁻¹) was calculated as Pₚ/E for each measurement.

Determination of chlorophyll concentration

Leaf chlorophyll concentration was measured on 5-6 randomly selected leaves per infection density on which photosynthesis measurements had been made. Chlorophyll was extracted from ~70 mg of leaf samples in 10.0 mL 80% acetone for 16 h in the darkness, and chlorophyll a and b concentrations were determined spectrophotometrically at 663 nm and 645 nm according to the method of Arnon (1949).

Soluble protein

On 30 DAP after the photosynthesis measurement, the 8th fully expanded sun leaf from four randomly selected M. micrantha plants per infection density was collected for determination of soluble protein content. Approximately 0.5 g of fresh leaf material (midvein excluded) per sample was homogenized in 5 ml of ice-cold 50 mM potassium phosphate buffer (pH 7.8) in an ice-cold mortar. The homogenate was centrifuged at 16,000 g for 15 min at 4°C (CR22G Ultracentrifuge, Hitachi, Japan), and the supernatant was collected and stored at 4°C for soluble protein determination. Total soluble protein content in the samples was determined by the protein dye-binding method of Bradford (1976), using bovine serum albumin as standard.

Statistical analysis

All tests were carried out at α < 0.05 level using SPSS (Version 11.5, SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to determine statistical significance for the effects of infection density on the number of days from 0 DAP to the death time of the aerial parts of the infected plants, and for the variables of growth and photosynthesis, chlorophyll concentration and Ca:Cb ratio, and total soluble protein content. Means for significant ANOVA effects were compared using Tukey post hoc comparisons.

RESULTS

Mikania micrantha growth and biomass accumulation

During the experiment, the uninfected M. micrantha plants grew vigorously, but the infected plants did not. On 16 October, 108 days after planting, the uninfected M. micrantha plants started to develop terminal inflorescences, were in full bloom ten days later, and fruit on 5 November. However, the infected plants did not flower throughout the experiment. The uninfected M. micrantha plants started to wither on 7 December, 190 days after planting. However, all parts of the infected M. micrantha plants above the first node from the base of the stem died, and the rate of death was a function of parasite density. At densities of 1, 2, 4 and 8 parasites per host all died 83, 62, 50 and 46 DAP on average, respectively. There was a significant effect on the number of days to almost complete death of treated plants with 1, 2 and 4 parasites per host plant, and no significant difference between the plants infected with 4 and 8 parasites per host plant.

At 30 DAP, infected and uninfected M. micrantha plants showed significant differences in total plant dry weight (Figure 1a). Among the infected, significant differences occurred among the 1, 2 and 4, but not between the 4 and 8 parasite per host plants (Figure 1a). Moreover, the dry masses of the infected system (host plus parasite) at all infection densities were significantly less than those of the uninfected M. micrantha plants (Figure 1a). There was a non-linear inverse relationship between host dry weight and dry weight of C. campestris. Decreases in M. micrantha growth were greater when C. campestris biomass was less than 30 g (Figure 1b).

Cuscuta campestris biomass

As the number of C. campestris increased from 1 to 2 and 2 to 4 per M. micrantha host, parasite dry weight (including fruit) increased significantly (Figure 1a). However, there was no significant difference between the dry weights of 4 and 8 C. campestris per M. micrantha host plants.

Shoot/root dry weight ratio (S/R)

At 30 DAP, infected M. micrantha plants had higher S/R ratios than the uninfected control at all infection densities, and significantly so when inoculated with 2 parasites per host plant (Figure 2).

Photosynthesis

Cuscuta campestris infection significantly reduced net photosynthetic rates (Pₚ) of mature M. micrantha leaves at all infection densities, compared with Pₚ of the uninfected leaves (Figure 3a). Among the infection densities, host Pₚ decreased significantly as the number of C. campestris parasites in the host plant moved from 1 to 2 and from 2 to 4, but not from 4 to 8 (Figure 3a).
Stomatal conductance ($g_s$) and transpiration rate ($E$) of host plants among all infection densities (0-8) followed the same variation pattern as $P_n$ although the decline from 2 to 4 $C. campestris$ per host plant produced no statistically significant changes (Figure 3b, c). Water use efficiency (WUE) was not affected by parasite infection at any level (Figure 3d). There was a linear relationship between $P_n$ and $g_s$ in leaves of both uninfected and infected plants (Figure 4). Both the slopes and the y intercepts of the two lines were very different, and uninfected plants always had higher assimilation rates for a given stomatal conductance than infected plants.

Chlorophyll content

Cuscuta campestris infection significantly reduced the amounts of total chlorophyll, chlorophyll $a$, and chlorophyll $b$ of the 8th fully expanded mature $M. micrantha$ leaves at all infection densities, compared with uninfected leaves (Figure 5a). Among the infection densities, total chlorophyll, chlorophyll $a$, and chlorophyll $b$ of host leaves decreased significantly from 1 to 2 $C. campestris$ seedlings per host plant, but not from 2 to 4 and 4 to 8 (Figure 5b). However, infection with $C. campestris$ had no significant effects on the chlorophyll $a:b$ ratio of $M. micrantha$ plants (Figure 5b).

Soluble protein concentration

At 30 DAP, inoculation with 1 parasite had no significant effect on the soluble protein concentration of $M. micrantha$ plants, compared with control plants; however, infection with more than 1 parasite significantly reduced soluble protein concentration (Figure 6). Soluble protein concentrations at 2 and 4 parasites per host plant were not significantly different, but were significantly higher than at 8 parasites per host.

DISCUSSION

Response of $M. micrantha$ to $C. campestris$ infection density

In this study, even a single $C. campestris$ parasite per $M. micrantha$, significantly reduced host growth and $P_n$, as was observed in our previous studies (Shen et al., 2005, 2007). The negative effect of the parasite on host growth and $P_n$ increased up to a density of 4 parasites per host, but density increases beyond this did not result in further decreases in host growth and $P_n$. We also observed that as...
C. campestris biomass increased up to 30 g per host M. micrantha biomass plummeted. However, further increases in parasite biomass did not result in further substantial decreases in host dry weight. In our study, C. campestris infection intensity on M. micrantha growth and Pn, formed a threshold at 4 parasites or 30 g of parasite biomass per host. Therefore, effective control might require only four parasitic plants per M. micrantha plant in the field.

There are relatively few studies on host responses to parasite infection levels (Puustinen and Salonen, 1999), but thresholds in parasite effects on host growth have been reported between tomato and Orobanche aegyptiaca (Barker et al., 1996), faba bean and O. crenata (Kharrat et al., 1994), and sorghum and Striga hermonthica (Cechin and Press, 1993; Gurney et al., 1999). At high infection densities, the growth of the host is severely reduced, and this in turn would reduce its ability to generate resources and support the growth of the parasite. Thus, at high infection densities, the growth of the parasite is limited by the source capacity of the host (Watling and Press, 2001).

Figure 3. Means (± SE, n=8) of net photosynthetic rate (Pn, a), stomatal conductance (gs, b), rate of transpiration (E, c) and water use efficiency (WUE, d) of the 8th fully expanded mature leaf of Mikania micrantha at 30 days after parasitization by different densities of Cuscuta campestris. Bars not sharing a common superscript letter are significantly different (P<0.05); Results of ANOVA: a, $F_{(4, 37)} = 103.322, P<0.001$; b: $F_{(4, 37)} = 34.954, P<0.001$; c: $F_{(4, 37)} = 67.903, P<0.001$; d, $F_{(4, 37)} = 0.467, P=0.760$. Refer to Figure 1 for definitions of C0, C1, C2, C4 and C8.

Figure 4. Relationship between photosynthesis (Pn) and stomatal conductance (gs) of the 8th fully expanded mature leaf of Mikania micrantha either uninfected (open circles; solid line) or infected by Cuscuta campestris (filled circles; broken line). Correlation coefficients are 0.89 and 0.95 for uninfected and infected plants, respectively (P<0.001).
In the present study, the total biomass of the \textit{C. campes\-tris} - \textit{M. micrantha} association was significantly less than the control at all infection densities. Similar results have been reported in our previous studies (Shen et al., 2005, 2007) and among some other parasitic associations (Parker et al., 1984; Press, 1995; Hibberd et al., 1996; Watling and Press, 1997). This indicates that the response of \textit{M. micrantha} to \textit{C. campestris} infection is not solely due to a simple source-sink relationship (Press et al., 1999), of resource competition for s between parasite and host. Non-source-sink interactions (Watling and Press, 2001) may also be important as infection results in a decline in host photosynthesis and growth.

In this study, \textit{M. micrantha} plants infected by two parasites had a higher S/R ratio than uninfected plants. Similar observations arose in our previous study with a single parasite (Shen et al., 2005). The decrease in root biomass relative to shoot biomass in infected plants may be attributable to strong competition by the parasite, diverting resources from the host root system (Jeschke et al., 1997). Besides, when the upper parts of \textit{M. micrantha} were infected by \textit{C. campestris}, the host would respond by allocating more resources to shoot growth (Shen et al., 2005). However, when the infection density was 1 or more than 2 parasites per host, the difference in S/R ratio between uninfected and infected plants was not significant. For the former, the reason might be that the infection effects were not strong enough to cause a significant shift in resource allocation of the host. For the latter, the reason might be the severe decrease in the shoot growth caused by the parasites exerting an overriding effect on the host shoot relative to the root.

What mechanism contributes to a decline in host photosynthesis?

Previous studies on the \textit{C. campestris} / \textit{M. micrantha} association have indicated that reduced \( g_s \) results in the suppression of photosynthesis in the host (Shen et al., 2007), which is also supported by our present results. The parasite had a significantly negative effect on the stomatal conductance \( (g_s) \) of host plants, and there was a linear relationship between the decline in \( P_{n} \) and \( g_s \). The same pattern between \( g_s \) and \( E \) suggests there were stomatal limitations, which reduced the diffusion of \( CO_2 \) into photosynthetic tissues and subsequently reduced photosynthesis (Taylor et al., 1996; Frost et al., 1997; Shen et al., 2007).
A decrease in chlorophyll concentration can result in a decrease in antenna size, reducing light capture, lowering photosynthesis rate (Cameron et al., 2005). Lower photosynthesis rate may also result in light stress and photodamage for the infected host (Shen et al., 2010). In the current study, the parasite significantly reduced the host chlorophyll content at all infection densities. Thus, the decreased chlorophyll content also explains the decline in photosynthesis. 

Additionally, soluble protein concentrations were lower in M. micrantha plants infected with more than 2 C. camppestris. In C3 plants, Rubisco, the key enzyme to fix CO2 in C3 photosynthesis, is the most abundant soluble protein in leaves (Evans, 1989; Parry et al., 2003). Rubisco generally accounts for 30-60% of the soluble proteins (Ellis, 1979; Sage et al., 1987; Parry et al., 1999). Reducing Rubisco content has been shown to reduce photosynthesis (Hudson et al., 1992; Lauerer et al., 1993; Stitt and Schulze, 1994; Furbank et al., 1996). Therefore, in this study, reduced Rubisco concentration in infected M. micrantha, could also contribute to the reduced photosynthesis of the host.

In conclusion, the results indicate that once a C. camppestris-M. micrantha association has been established, the parasite severely restrains the growth of M. micrantha, inhibits its flowering, and results in almost complete death of the aerial parts during a growing season. Therefore, C. camppestris has great potential as a biological control agent for M. micrantha. The effects of C. camppestris on the performance of M. micrantha depend on the intensity of the parasitic infection. Host growth and photosynthesis decreased significantly from one to four parasite plants per host, but not from 4 to 8. Thus, the minimum number of C. camppestris for optimum control of M. micrantha in the field is four per host plant. However, future experiments are needed to investigate the effect of different infection levels of C. camppestris on M. micrantha community structure and function.

Moreover, C. camppestris not only obtains its resources directly from M. micrantha for its growth and development, but also adversely affects host growth physiologically. C. camppestris infection decreases host stomatal conductance, transpiration, chlorophyll content, and soluble protein concentration, which may directly and indirectly reduce the rate of photosynthesis and thus suppress the growth of the host.

Acknowledgements. We thank Mr. Chris Parker for his research guidance. We also thank two anonymous reviewers, Mr. Chris Parker, and Dr. Jane Prider for their constructive comments and revision on the manuscript. This work was supported by the National Basic Research Program of China (973 Program, 2009CB119200), the Knowledge Innovation Program of the Chinese Academy of Sciences, the National Natural Science Foundation of China (30470345), and the Guangdong Natural Science Foundation (021536).

LITERATURE CITED


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