BIOLOGIA PLANTARUM 55 (3): 545-548, 2011

BRIEF COMMUNICATION

Involvement of ABA in reduced photosynthesis and stomatal conductance in *Cuscuta campestris* - *Mikania micrantha* association

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Abstract

We investigated whether changes in abscisic acid (ABA) content in leaves of *Mikania micrantha* infected by the holoparasite *Cuscuta campestris* at five growth stages, influenced the host stomatal conductance (g_s), transpiration rate (E) and net photosynthetic rate (P_N). *C. campestris* infection caused a negative effect on g_s, E and P_N of the host plants. ABA content in host leaves infected by *C. campestris* was significantly lower at 6 d after parasitization (DAP) and significantly higher at 13 and 33 DAP, relative to uninfected controls. In the parasite, ABA content was lowest at 13 DAP and then sharply increased to the maximum at 26 DAP. Moreover, the ABA content in the parasite was always lower than in the infected host leaves. The results suggest that an increase in host ABA concentration contributes to reduced host g_s, E and P_N in the holoparasitic *C. campestris* - *M. micrantha* association.

Additional key words: ELISA, growth stages, parasitic plant.

The interaction between parasitic plants and their host plants has been one of the key topics in parasitic plant biology (Shen *et al*. 2006). In addition to resource capture from host plants, many parasitic angiosperms are able to influence host growth *via* effects on host photosynthesis (Watling and Press 2001). Although there are exceptions, most holoparasitic plants enhance host photosynthetic productivity *via* source-sink interactions while hemiparasitic plants usually have a negative impact on host photosynthesis *via* other processes (Press *et al*. 1999, Watling and Press 2001).

The plant hormone, abscisic acid (ABA) plays an important role in mediating response to biotic (Kovač *et al*. 2009) and abiotic stresses (Zhang *et al*. 2008). In some root hemiparasitic associations high ABA content had negative effects on host stomatal conductance which consequently resulted in lower rates of photosynthesis (Drennan and El Hiweris 1979, Taylor *et al*. 1996, Frost *et al*. 1997, Jiang *et al*. 2004). However, the potential role of ABA in stem holoparasitic associations is not known.

Stimulation of host photosynthesis has been found in holoparasitic associations of *Cuscuta reflexa* with leguminous or non-leguminous hosts (Jeschke *et al*. 1997, Jeschke and Hilpert 1997). The enhanced host photosynthesis appeared to compensate for resource loss to the parasites. However, in invasive *Mikania micrantha* plants infected by *C. campestris*, there is a reduction in host photosynthetic rate due to decreased stomatal conductance (Shen *et al*. 2007).

Cuscuta campestris Yuncker (*Convolvulaceae*) is an obligate holoparasite, which 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). The parasite draws all nutrients from its host *via*

Received 19 November 2009*, accepted* 31 March 2010.

Abbreviations: ABA - abscisic acid; DAP - days after parasitization; E - transpiration rate; g_s - stomatal conductance; PFD - photon flux density; P_N - net photosynthetic rate.

Acknowledgements: We thank the anonymous reviewer, Drs. Jane Prider and Jennifer Watling for their constructive comments and revision on the manuscript. This work was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences, the National Natural Science Foundation of China (30470345).

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haustoria and is a very powerful sink for host photosynthates, suppressing host growth, preventing flowering and fruiting, and even resulting in death (Shen *et al.* 2005). *Mikania micrantha* H.B.K. (*Asteraceae*) is native to Central and South America where it is a weed of minor importance (Wirjahar 1976). However, in its exotic range, it has become a horrific invader and been listed as one of the 100 worst invasive alien species in the world (Lowe *et al.* 2001). Previous studies have shown that the generalist stem holoparasite *C. campestris* can effectively restrain the growth of *M. micrantha* and even cause the aerial parts to die (Shen *et al.* 2005, 2007).

In order to better understand the role of ABA in the interaction of the holoparasitic *C. campestris* - *M. micrantha* association, we conducted a glasshouse experiment to test the hypothesis that an increase in ABA concentration in *M. micrantha* hosts infected by the holoparasite *C. campestris* results in a reduction in host stomatal conductance and transpiration rate and a subsequent decline in host photosynthesis.

The experiment was carried out during the March-July 2007 growing season in Guangzhou (23°10'N, 113°21'E, 40 m a.s.l.), Guangdong Province, China. On 20 March, seeds of *Mikania micrantha* H.B.K. were sown in 0.4 dm^3 pots and placed in a growth chamber with a constant temperature of 25 °C and 12-h photoperiod with irradiance of 60 $\mu mol(photon)\ m^{-2}\ s^{-1}$. After germination, the seedlings were firstly moved into a laboratory, and 55 d later 100 healthy ones were randomly chosen and transplanted to 6.3 dm^3 pots filled with washed moist sand and placed in a naturally lit glasshouse with an average daily temperature of 30.7 °C and relative humidity 90.3 % during May - July 2007. The plants were watered daily, initially with tap water, after five days with half-strength Hoagland nutrient solution, and later with full strength daily until the end of the experiment. On 6 June, 20 *C. campestris* seeds were sown around each of 70 of the 100 *M. micrantha* plants with a height about 120 cm, and the remaining 30 *M. micrantha* plants were kept as the control group. 15 d later, each *M. micrantha* host plant in the infected group was successfully infected by one *C. campestris* seedling, and thus we defined 21 June as the 1st day after parasitization (DAP). On 20 June and the day before each harvest day (see below), the net photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (E) of leaves of *M. micrantha* plants were measured using a *LI-6400* portable photosynthesis system with a standard 6 cm^2 leaf chamber (*LI-COR*, Lincoln, NE, USA). To ensure that leaves measured were of similar age and developmental stage, only fully expanded mature sun leaves at the same positions on the main stems were sampled. At each measurement, one leaf per plant of five randomly selected *M. micrantha* plants per treatment was sampled. The measurements were made at an irradiance of 1000 $\mu mol\ m^{-2}\ s^{-1}$, CO_2 concentration of 400 $\mu mol\ mol^{-1}$ and leaf temperature of 35 °C. The photosynthetic parameters were calculated as described by Von Caemmerer and Farquhar (1981).

Based on our previous observations on anatomy, growth, and development of *C. campestris*, we divided the parasite life cycle into five stages: initial parasitization (6 DAP), rapid-growing (13 DAP), flower bud (20 DAP), blooming (26 DAP), and fruiting (33 DAP). The time of a stage was defined as the day when at least 90 % of *C. campestris* plants entered the corresponding stage. At 6, 13, 20, 26 and 33 DAP, five uninfected *M. micrantha* plants and five *M. micrantha* - *C. campestris* associations were randomly chosen and harvested following the sampling method of Jiang *et al.* (2004). On each harvest day, 0.5 g of fresh tissue of *M. micrantha* leaves and the parasites were collected, frozen in liquid nitrogen and stored at -20 °C for later hormone extraction.

The extraction, purification and determination of endogenous ABA were performed using an indirect ELISA technique (He 1993). ABA contents were calculated following the previous studies (Weiler *et al.* 1981, Mwange *et al.* 2005). The percentage ABA recoveries were above 90 % and all sample extract dilution curves were parallel to the standard curves, indicating the absence of nonspecific inhibitors in the extracts. The accuracy of the ELISA has been verified in earlier studies (Zhao *et al.* 2006).

All statistical tests were carried out at $\alpha = 0.05$ level using *SPSS* software (version *11.5*, *SPSS*, Chicago, IL, USA). The differences in g_s, E, P_N, and ABA content between control and infected *M. micrantha* leaves as well as ABA content between host and parasite leaves were tested by Student's *t*-test for each harvest.

During the experiment, growth of *M. micrantha* infected by *C. campestris* was severely suppressed and showed growth abnormalities after 13 DAP. At the time, when *C. campestris* grew rapidly, the leaves of the infected hosts turned yellow and later withered. At about 33 DAP, *C. campestris* fruited and P_N of infected host plants practically ceased.

C. campestris had negative effects on the P_N of its host *M. micrantha* (Fig. 1*A*), which was apparent already after 5 DAP. There was further significant decrease in P_N of infected plants until 12 DAP, coinciding with the rapid growth phase of *C. campestris*. The decline in P_N of infected *M. micrantha* slowed between 12 and 25 DAP when *C. campestris* formed flowers but P_N decreased again as fruits developed to 32 DAP. The P_N of uninfected *M. micrantha* declined over the course of the experiment, but P_N in control plants was always higher than in infected plants. The lower P_N in infected plants was accompanied by lower g_s from 5 to 32 DAP (Fig. 1*B*). E followed the same pattern as g_s regardless of the infection treatments (Fig. 1*C*). The negative effects of infection by *C. campestris* on P_N, g_s and E of *M. micrantha* host plants were consistent with previous observations in the field (Shen *et al.* 2007). Additionally,

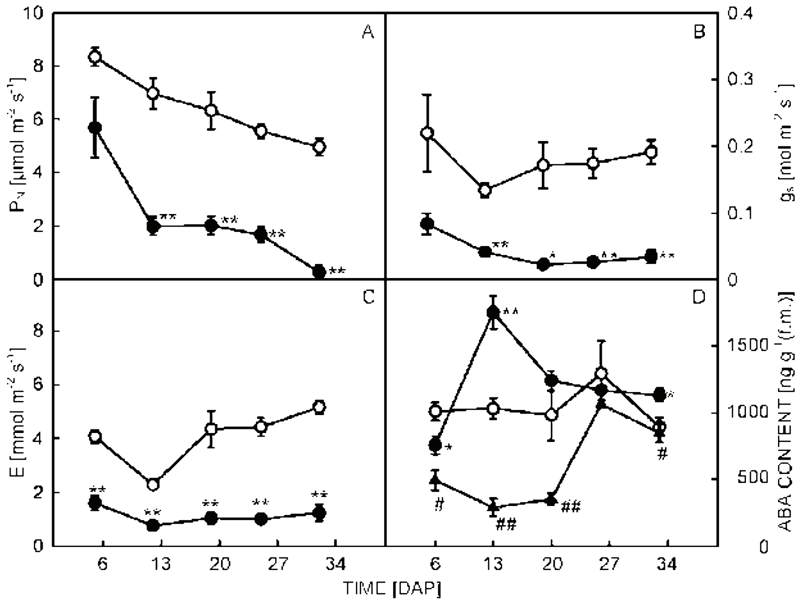

Fig. 1. Net photosynthetic rate, P_N (*A*), stomatal conductance, g_s (*B*), transpiration rate, E (*C*) and ABA content (*D*) in leaves of uninfected (*open circles*) and infected (*filled circles*) *Mikania micrantha* plants by *Cuscuta campestris* and ABA content in the parasite (*filled triangles*) on different days after parasitization (DAP). Means ± SE, $n = 5$. Significant differences between host and control plants at each DAP are indicated as * ($P < 0.05$), ** ($P < 0.01$); and between host plants and parasites at each DAP indicated as $^{\#}$ ($P < 0.05$), $^{\#\#}$ ($P < 0.01$).

this is similar to reports of Taylor *et al.* (1996) and Frost *et al.* (1997) on the root hemiparasite *Striga hermonthica*

We found a rapid increase in ABA content in infected host leaves from 6 to 13 DAP (Fig. 1*D*) along with a steep decrease in P_N, g_s and E (Fig. 1*A*,*B*,*C*). These results indicate that the infection of *C. campestris* can induce elevated content of ABA in host tissues, resulting in a decline in g_s and a subsequently reduction of host E and P_N (Seel *et al.* 1992). The reduced E in the infected host leaves may generate a water potential gradient benefiting the parasite by diverting more water and nutrients from the host (Taylor *et al.* 1996, Jiang *et al.* 2008). Thus, it is probable that both limitations to carbon assimilation and competition by the parasite for resources resulted in the decline of photosynthetic capacity of the infected host plant. However, which factor is more important is worthy of further investigation.

Either when ABA content in host leaves was lower (at 6 DAP) or when there was no significant difference in ABA content between uninfected and infected leaves (at 20 and 26 DAP), the P_N, g_s and E in the infected host leaves were significantly reduced. This suggested that the host gas exchange was affected also by other mechanisms and probably different mechanisms operated at different infection stages. For example, the inhibition in P_N might be attributable to a decrease in Rubisco content and/or activity in the infected plants (Press *et al.* 1999, Watling and Press 2001). Additionally, the increased ABA content in infected host leaves at 13 DAP could result in leaf senescence and hence there was no recovery in photosynthetic capacity even after decline in ABA content.

The ABA content of the parasite was significantly lower than that in infected host leaves at each DAP, except 26 DAP (Fig. 1*D*). Conflicting results were found in the obligate root hemiparasitic *Striga* - maize association (Taylor *et al.* 1996), where ABA contents in the leaf tissues of *S. hermonthica* were higher than in the host leaves. Higher ABA content in *C. reflexa* than the host have also been reported (Ihl *et al.* 1984, De Bock and Fer 1992). It appears that ABA content differences between a parasite and its host are highly specific and/or depend on whether ABA is synthesized by the host, the parasite or both. It was suggested that ABA could be synthesized and accumulate in the hemiparasites *S. hermonthica* (Taylor *et al.* 1996) and *Rhinanthus minor* (Jiang *et al.* 2004), but there was little evidence of transport from parasite to host. Further studies on the origin and transfer of ABA in parasitic species are still needed.

References

Dawson, J., Musselman, L., Wolswinkel, P., Dörr, I.: Biology and control of *Cuscuta*. - Rev. Weed Sci. **6**: 265-271, 1994.

De Bock, F., Fer, A.: Effects of abscisic-acid on the transfer of sucrose from host, *Pelargonium zonale* (L.) Aiton, to a phanerogamic parasite, *Cuscuta reflexa* Roxb. - Aust. J. Plant Physiol. **19**: 679-691, 1992.

Drennan, D.S.H., El Hiweris, S.O.: Changes in growth regulating substances in *Sorghum vulgare* infected by *Striga hermonthica*. - In: Musselman, L.J., Worsham, A.D., Eplee, R.E. (ed.): Proceedings of the Second Symposium of Parasitic Weeds. Pp. 144-155. North Carolina State University, Raleigh 1979.

Frost, D.L., Gurney, A.L., Press, M.C., Scholes, J.D.: *Striga hermonthica* reduces photosynthesis in sorghum: the importance of stomatal limitations and a potential role for ABA? - Plant Cell Environ. **20**: 483-492, 1997.

He, Z.: [A Laboratory Guide to Chemical Control Technology on Field Crops.]. - Beijing Agricultural University Press, Beijing 1993. [In Chin.]

Ihl, B., Jabob, F., Sembdner, G.: Studies on *Cuscuta reflexa* Roxb. V. The level of endogenous hormones in the parasite, *Cuscuta reflexa* and its host, *Vicia faba* L. and a suggested role in the transfer of nutrients from host to parasite. - J. Plant Growth Regul. **2**: 77-90, 1984.

Jeschke, W.D., Baig, A., Hilpert, A.: Sink-stimulated photosynthesis, increased transpiration and increased demand-dependent stimulation of nitrate uptake: nitrogen and carbon relations in the parasitic association *Cuscuta reflexa-Coleus blumei*. - J. exp. Bot. **48**: 915-925, 1997.

Jeschke, W.D., Hilpert, A.: Sink-stimulated photosynthesis and sink-dependent increase in nitrate uptake: nitrogen and carbon relations of the parasitic association *Cuscuta reflexa-Ricinus communis*. - Plant Cell Environ. **20**: 47-56, 1997.

Jiang, F., Jeschke, W.D., Hartung, W.: Abscisic acid (ABA) flows from *Hordeum vulgare* to the hemiparasite *Rhinanthus minor* and the influence of infection on host and parasite abscisic acid relations. - J. exp. Bot. **55**: 2323-2329, 2004.

Jiang, F., Jeschke, W.D., Hartung, W., Cameron, D.D.: Does legume nitrogen fixation underpin host quality for the hemiparasitic plant *Rhinanthus minor*? - J. exp. Bot. **59**: 917-925, 2008.

Kovač, M., Müller, A., Milovanovič, J.D., Milavec, M., Düchting, P.: Multiple hormone analysis indicates involvement of jasmonate signaling in the early defence of potato to potato virus Y^{NTN} - Biol. Plant. **53**: 195-199, 2009.

Lowe, S., Browne, M., Boudjelas, S., Depoorter, M.: 100 of the World's Worst Invasive Alien Species. A Selection from the Global Invasive Species Database. - IUCN-SSC, Auckland 2000.

Mwange, K.N., Hou, H.W., Wang, Y.Q., He, X.Q., Cui, K.M.: Opposite patterns in the annual distribution and time-course of endogenous abscisic acid and indole-3-acetic acid in relation to the periodicity of cambial activity in *Eucommia ulmoides* Oliv. - J. exp. Bot. **56**: 1017-1028, 2005.

Press, M.C., Scholes, J.D., Watling, J.R.: Parasitic plants: physiological and ecological interactions with their hosts. - In: Press, M.C., Scholes, J.D., Barker,M.G. (ed.): Physiological Plant Ecology. Pp. 175-197. University of York, York, 1999.

Seel, W.E., Cechin, I., Vincent, C.A., Press, M.C.: Carbon partitioning and transport in parasitic angiosperms and their host. - In: Pollock, C.J., Farrar, J.F., Gordon, A.J. (ed.): Carbon Partitioning within and between Organisms. Pp: 199-223. BIOS, Oxford 1992.

Shen, H., Hong, L., Ye, W.H., Cao, H.L., Wang, Z.M.: The influence of the holoparasitic plant *Cuscuta campestris* on the growth and photosynthesis of its host *Mikania micrantha*. - J. exp. Bot. **58**: 2929-2937, 2007.

Shen, H., Ye, W.H., Hong, L., Cao, H.L., Wang, Z.M.: Influence of the obligate parasite *Cuscuta campestris* on growth and biomass allocation of its host *Mikania micrantha*. - J. exp. Bot. **56**: 1277-1284, 2005.

Shen, H., Ye, W.H., Hong, L., Huang, H.J., Wang, Z.M., Deng, X., Yang, Q.H., Xu, Z.F.: Progress in parasitic plant biology: host selection and nutrient transfer. - Plant Biol. **8**: 175-185, 2006.

Taylor, A., Martin, J., Seel, W.E.: Physiology of the parasitic association between maize and witchweed (*Striga hermonthica*): is ABA involved? - J. exp. Bot. **47**: 1057-1065, 1996.

Von Caemmerer, S., Farquhar, G.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. - Planta **153**: 376-387, 1981.

Watling, J.R., Press, M.C.: Impacts of infection by parasitic angiosperms on host photosynthesis. - Plant Biol. **3**: 244-250, 2001.

Weiler, E.W., Jourdan, P.S., Conrad, W.: Levels of indole-3-acetic acid in intact and decapitated coleoptiles as determined by a specific and highly sensitive solid-phase enzyme immunoassay. - Planta **153**: 561-571, 1981.

Wirjahar, S.: Autecological study of *Mikania spp*. - In: Proceedings of fifth Asian-Pacific Weed Science Society Conference. Pp: 70-73. Asian Weed Science Society, Tokyo 1976.

Zhang, Y., Xu, W.Y., Li, Z.H., Deng, X.W., Wu, W.H., Xue, Y.B.: F-Box protein DOR functions as a novel inhibitory factor for abscisic acid-induced stomatal closure under drought stress in *Arabidopsis*. - Plant Physiol. **148**: 2121-2133, 2008.

Zhao, J., Li, G., Yi, G.X., Wang, B.M., Deng, A.X., Nan, T.G., Li, Z.H., Li, Q.X.: Comparison between conventional indirect competitive enzyme-linked immunosorbent assay (icELISA) and simplified icELISA for small molecules. - Anal. Chim. Acta. **571**: 79-85, 2006.