BRIEF COMMUNICATION

Effects of clonal integration on photosynthesis of the invasive clonal plant *Alternanthera philoxeroides*

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Abstract

A greenhouse experiment examined whether clonal integration improves photosynthesis of ramets of alligator weed [*Alternanthera philoxeroides* (Mart.) Griseb.], a widespread invasive clonal plant in China, in heterogeneous (He) nutrient habitats. The connected pairs of ramets experienced different nutrient levels [high homogeneous (Ho) nutrient, low Ho nutrient, and two He nutrient treatments]. Clonal integration significantly improved the net photosynthetic rate, stomatal conductance, transpiration rate, and minimal and maximal chlorophyll fluorescence of ramets of alligator weed in low nutrient condition. These characteristics may contribute to the success of the ramets of alligator weed in invading contrasting habitats. The clonal integration of the invasive clonal plants may contribute significantly to their invasiveness.

Additional key words: alligator weed; biological invasions; chlorophyll fluorescence; clonal plants; net photosynthetic rate; photosystem 2; nutrient heterogeneity; stomatal conductance; sub-stomatal CO_2 concentration; transpiration rate.

Plant invasions have received increasing attention worldwide because of their environmental impacts and huge economic costs (Wilcove *et al.* 1998, Mack *et al.* 2000, Pimentel *et al.* 2000, Grosholz 2005, Liu *et al.* 2005). Understanding of the factors influencing invasiveness is a fundamental goal of invasion ecology. Many invasive plant species have the capability of vigorous clonal propagation and their invasiveness is related to clonality (Leakey 1981, Dong 1996, Kolar and Lodge 2001, Liu *et al.* 2006). A comparative study found that clonality of the invasive plant species contributed significantly to their invasiveness in China, but the mechanism is not clear (Liu *et al.* 2006).

Clonal plants may occupy heterogeneous habitats in terms of nutrients (Bell *et al.* 1993, Stuefer 1996, Dong *et al.* 2002). Clonal integration between ramets may include water, nutrients, and photoassimilates (Ashmun *et al.* 1982, Alpert 1996). Integration among ramets may help clonal plants resist abiotic stress and increase the fitness (Alpert 1996, Yu *et al.* 2002, 2004). Therefore, clonal integration may improve the invasion success through enhancement of photosynthesis of ramets in heterogeneous habitats. The study of the effects of clonal integration on photosynthesis of the invasive clonal plant is helpful to understand the mechanism of clonal integration and clonality's contribution to plant invasions.

Alligator weed [*Alternanthera philoxeroides* (Mart.) Griseb.], a C_3 member of the Amaranthaceae (Balagtas-Burow *et al.* 1993), is of South-America origin and has become one of the worst invasive plant species in China and the world (Buckingham 1996, Li and Xie 2002, Ye *et al.* 2003). Although alligator weed possesses very low genetic diversity in China (Xu *et al*. 2003, Ye *et al*. 2003, Wang *et al*. 2005), this alien weed has successfully invaded diverse habitats (from swamps to dry lands) in China (Geng *et al.* 2006). Most likely, rapid expansion of

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the alligator weed is the result of a massive vegetative propagation and re-sprout (Xu *et al.* 2003, Ye *et al.* 2003, Wang *et al.* 2005, Wilson *et al.* 2007). These made alligator weed a model species suitable for the study of the mechanism of clonal integration and clonality's contribution to the plant invasions.

We conducted a greenhouse experiment to examine the effects of clonal integration on the photosynthesis of the invasive clonal plant alligator weed experiencing different nutrient levels. We asked the following questions: (*1*) Will the clonal integration affect the net photosynthetic rate (P_N), stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i), and transpiration rate (E) of the ramets in resource heterogeneity in alligator weed? (*2*) Will the clonal integration affect the F_0, which is the minimal level of chlorophyll (Chl) fluorescence, F_m (maximal level of Chl fluorescence), and F_v/F_m (maximum quantum yield of photosystem 2, PS2) of the ramets in nutrient heterogeneity in alligator weed?

Alligator weed was collected on a cropland of Kunming, Yunnan province in April in 2004 and then planted in a greenhouse in Beijing for the propagation. On July 23, newly produced ramet pairs were selected on the base of similarity of plant size. Pairs of ramets were prepared in which the two ramets were connected and the root was rooted in separated plastic pots filled with river sand. The ramets were standardized to two leaves and root 2 cm long every week (three times in all). On August 14, ramets in a pair from 10 plants were given: (*1*) a uniform high level (HN) of nutrient; (*2*) a contrasting level of nutrient [HN in the proximal (older, O) ramet, while low level (LN) in the distal (younger, Y) ramet]; (*3*) a contrasting level of nutrient (HN in Y, while LN in O); (*4*) a uniform LN. There were 10 replicates per treatment, and the stolons were connected during the experiment. Hence there were eight experimental variants: *1* – homogeneous (Ho) pair with HN in O section (HoHNO), *2* – HoHNY; *3* – heterogeneous (He) pair with HN in O (HeHNO); *4* – HeLNY; *5* – HeLNO; *6* – HeHNY; *7* – HoLNO; *8* – HoLNY.

The nutrient treatments were selected on the basis of pilot observation. In LN, every pot was given 0.1 g *Peters Professional* (*The Scotts Company*, Marysville, Ohio, USA) at the beginning of the experiment, which was limiting to growth but nearly sufficient to support survival and growth. The HN was 0.1 g *Peters Professional* in every pot every day during the experiment, which was suitable for the growth of the plants. All the pots were randomly arranged in the same greenhouse. Every pot was given 100 cm^3 of water every afternoon. The photosynthesis-related characters were measured in October 2004, after 6 weeks of growth.

We measured P_N, g_s, C_i, and E by a Portable Photosynthesis System (*LI-COR 6400*, USA) using PAR of 1 200 $\mu mol\ m^{-2}\ s^{-1}$. Leaves were measured under ambient CO_2 concentration [360 $\mu mol(CO_2)\ mol^{-1}$]. We measured alternatively between treatments and 12 fully expanded fresh leaves from 10 replicated plants of each treatment were measured on two sunny days. Instantaneous water use efficiency (WUE) was calculated as P_N/E for each measurement (He and Dong 2003).

We measured F_0 (minimal Chl fluorescence), F_m (maximal Chl fluorescence), and F_v/F_m [maximum quantum yield of PS2, $F_v/F_m = (F_m - F_0)/F_m$] by a Chl fluorescence measuring system (*H. Walz*, Germany). We measured 4–5 leaves per treatment and each leaf experienced 20 min dark adaptation before the measurement.

One-way ANOVA and Duncan test were carried out using the statistical package *SPSS 10.0*.

The ramets in LN can benefit significantly from connecting with the ramets in HN while the ramets in HN were not affected significantly by connecting with the ramets in LN. One-way ANOVA showed that P_N, g_s, and E were significantly different among treatments while C_i and WUE was not (Table 1). Duncan test (Table 2) showed that P_N, g_s, and E of leaves of treatments *7* and *8* were significantly lower than those of other six treatments although the nutrient levels of treatments *4*, *5*, *7*, and *8* were similarly low. The benefit of physiological integration on the studied photosynthesis character was similar for O and Y sections.

In terms of F_m and F_0, the ramets at LN can benefit significantly from connecting with the ramets at HN while the ramets at HN were not affected significantly by connecting with the ramets in LN. One-way ANOVA showed that F_0 and F_m were significantly different among treatments while the F_v/F_m was not. We used Duncan test to compare the F_0 and F_m among treatments**.** The F_0 and F_m of leaves of treatments *1–6* were not significantly different although the nutrient level was not the same. The effects of physiological integration on F_m were similar for O and Y sections while the effects on F_0 showed some differences.

The differences in P_N of plants can result in the variation in biomass accumulation and fitness. Investigation of the effects of clonal integration on P_N of the invasive clonal plant is helpful for understanding the mechanism of clonal integration and the role of clonality in the plant invasions. We found that the ramets in LN could benefit significantly from connecting with the ramets in HN in terms of the determined photosynthesis-related parameters.

The resource sharing between ramets may include water, nutrients, and photosynthates (Ashmun *et al.* 1982, Alpert 1996). The nutrients in soil (N, P, and K) significantly affect the photosynthesis of plants (Gulias *et al.* 2003). Clonal integration could significantly increase P_N of rametes in LN in our experiment, which may be caused by nutrient sharing between ramets. The sharing of nutrients could improve the total photosynthesis of the ramet-pairs for it could enable the ramets in LN to utilize the space and radiant energy. Clonal integration significantly increased g_s of ramets in LN in our experiment which certainly affected P_N by controlling the exchange

Table 1. One-way ANOVA of the effects of treatments on the characteristics of gas exchange: C_i – sub-stomatal CO_2 concentration [μmol mol^{-1}]; E – transpiration rate [mol m^{-2} s^{-1}]; g_s – stomatal conductance [mol m^{-2} s^{-1}]; P_N – net photosynthetic rate [μmol m^{-2} s^{-1}]; WUE – water use efficiency.

	Source of variance	Sum of squares	DF	F	*p*
P_N	Between groups	801.87	7	10.462	<0.001
	Within groups	963.54	88		
	Total	1765.41	95		
g_s	Between groups	0.62	7	6.186	<0.001
	Within groups	1.27	88		
	Total	1.89	95		
C_i	Between groups	22375.07	7	1.642	0.134
	Within groups	171315.92	88		
	Total	193690.99	95		
E	Between groups	51.03	7	8.775	<0.001
	Within groups	73.11	88		
	Total	124.15	95		
WUE	Between groups	12.11	7	1.202	0.310
	Within groups	126.66	88		
	Total	138.77	95		

Table 2. The Duncan test of traits of the photosynthesis between the treatments (see the text). The values sharing the same letter are not different at p=0.05. For abbreviations see Table 1.

Treatments	P_N Mean	SD	g_s Mean	SD	E Mean	SD
1 – HoHNO	10.598^b	3.332	0.292^b	0.137	2.933^b	0.840
2 – HoHNY	10.243^b	3.238	0.275^b	0.114	2.968^b	1.040
3 – HeHNO	8.078^b	2.864	0.208^b	0.093	2.322^b	0.871
4 – HeLNY	7.964^b	2.431	0.238^b	0.105	2.537^b	0.835
5 – HeLNO	9.308^b	3.242	0.244^b	0.146	2.597^b	1.177
6 – HeHNY	10.883^b	5.928	0.293^b	0.199	2.928^b	1.244
7 – HoLNO	3.009^a	1.475	0.079^a	0.019	1.072^a	0.257
8 – HoHNY	3.514^a	2.019	0.087^a	0.055	1.135^a	0.638

of CO_2 and water (Leuning *et al.* 1995, Alpert 1996, Yu *et al.* 2004).

The ramets of alligator weed in HoHN and HeHN did not differ in F_0 and F_m. Thus the clonal integration could help maintain the ability of photosynthesis in alligator weed. The F_v/F_m is a good estimation of the maximum photochemical efficiency of PS2 (Adams *et al.* 1990, He and Dong 2003). No significant effects were found on the F_v/F_m between the treatments, which suggested that alligator weed could maintain the potential ability of Chl in photosynthesis even in very LN treatments. The maintenance of F_v/F_m of ramets in LN could enable the alligator weed to regain the ability of photosynthesis when the nutrient level is suitable. These characters might be an adaptation of alligator weed in response of temporal variation of nutrients.

Many invasive plants are clonal plants (Leakey 1981, Pyšek 1997, Liu *et al.* 2006), but the mechanism of the relationship between plant invasions and clonality is not clear (Maurer and Zedler 2002, Liu *et al.* 2006). Our study showed that clonal integration could significantly improve the rate and ability of photosynthesis and F_0 and F_m of Chl fluorescence of the invasive alligator weed. Clonal integration could enable the clone to grow through unfavourable sites, thus increasing the probability of invading into more favourable conditions, which is also found in other clonal plants (Yu *et al.* 2002, 2004). Clonal integration is adaptation of alligator weed in response of He nutrients. These characteristics may contribute to the success of alligator weed in invading a variety of contrasting habitats. Our study supported the idea that the clonal integration of the invasive clonal plants may contribute significantly to their invasiveness (Liu *et al.* 2006).

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