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Influence of light and salt on the growth of alien invasive tropical weed *Ageratum conyzoides*

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Abstract

The study reports the effects of salinity and light on growth of tropic ageratum, an annual invasive weed that is widespread in South China and Southeast Asia. Independent effect of light on leaf gas exchange and chlorophyll fluorescence, and combined effects of light and salinity on plant height, leaf area, chlorophyll content, soluble sugar, malondialdehyde (MDA), soluble protein and proline were measured over the growth period of ageratum. Low light availability decreased the light compensation point (LCP), maximum net photosynthetic rate (Pmax) and electron transport rate (ETR), but increased apparent quantum yield (AQY). High salinity combined with high light intensity reduced plant height, leaf area and chlorophyll content, and increased soluble sugar, MDA, soluble protein and proline contents. Factor analysis revealed that shade could alleviate the damage of salinity stress, but moderate and heavy shade have different effects depending on treatment duration. We conclude that light and salt are important factors controlling the growth of ageratum, and our results partly explain the distribution pattern of this species in the mudflat reclamated for agriculture in the southeast China coast. Further, this weed acclimatizes well to the various light and salinity environments, even high light intensity and severe salinity stresses. Thus, effective management should be taken to prevent further distribution and spread of this weed by environmentalists, ecologists and farmers.

Keywords: Ageratum conyzoides; chlorophyll fluorescence; factor analysis; light; photosynthesis; salinity.
Abbreviations: AQY-apparent quantum yield; Ci-intercellular CO_2 concentration; E-transpiration rate; ETR-electron transport rate; Gs-stomatal conductance; LCP-light compensation point; Pn-net photosynthetic rate; Pmax-maximum net photosynthetic rate; qN-non-photochemical quenching; qP-photochemical quenching; Yield-effective quantum yield of photochemical energy conversion.

Introduction

Tropic ageratum (*Ageratum conyzoides* L., Compositae), is an annual weed native to Central America that has now migrated to South China, Southeast Asia and Sub-Saharan Africa (Ekeleme et al., 2005; Kong et al., 2004; Okunade, 2002). Because of its allelopathic properties, tropic ageratum is an effective invader of native plant communities. It often becomes a dominant understory species in tropical and subtropical agro-ecosystems and invades cultivated fields, reducing crop productivity (Caton et al., 2004). Tropic ageratum constitutes more than 70% of the total above and below ground weed population biomass in some farms in Nigeria, India and Brazil (Akobundu et al., 1999; Devi et al., 1993; Garcia, 1995), and its continued expansion is of great concern for famers. Currently, very little is known about its tolerance to natural environmental stressors, such as salinity or its light requirements for successful establishment. This information will drive innovative solutions for control of this notorious weed. Light is one of the major abiotic factors influencing growth and distribution of plant species (Tang, 1997). Tolerance to low light is an important characteristic of plants developing with insufficient light resources, such as under dense canopies of other plants. Tropic ageratum appears to fall into this category, with strong potential for such invasions, perhaps due to advantage gained by morphological adaptations, high photosynthetic light-use efficiencies, low light compensation points, and lower respiration rates under low light conditions (Brainard et al., 2005). Salinity is another major abiotic stress affecting approximately 20% of the world's total cultivated area (Munns and Tester, 2008). Salt leads to the accumulation of osmolytes in plant tissues dependent upon its level of tolerance to salinity; plants must balance stress associated with direct ion effects found at higher salinity, with stress imposed by osmotic factors found at lower salinity (Munns and Tester, 2008). Particularly, it is important to investigate the role of soluble sugars, malondialdehyde (MDA), soluble protein and proline in salt-stressed weeds such as Parthenium hysterophorus (Hegde and Patil, 1982), Echinochloa crusgalli (Yamamoto et al., 2003) and Echinochloa oryzicola (Nguyen et al., 2005). Compared to crop plants, responses of alien weeds to salt exposure have received little attention. Salinity stress often occurs in conjunction with flooding, drought, and/or high temperature stress. Shade may even improve the physiological response of plants to drought (Duan et al.,

2005) or to excess boron stress (Sotiropoulos et al., 2004) compared to unshaded plants. Indeed, Syvertsen et al. (2003) showed that under 50% shading, high leaf temperature and leaf-to-air vapour pressure differences were reduced at midday such that the net photosynthetic assimilation, stomatal conductance, and photosynthetic leaf water use efficiency increased over that of unshaded leaves. Thus, salt stress as rated through ecophysiological metrics is reduced by shading. In China, tropic ageratum occurs on mudflat reclamated for agriculture that has been historically observed (Gedan et al., 2009) and its total area is still increasing (Wang, 1983), making one speculate that the interactive effects of light and salinity together allow tropical ageratum to flourish on these soils. Tropic ageratum cultivated in Hoagland's solution was tested to determine their physiological responses to salinity in sun versus shade. We hypothesized that high light, by increasing substrate temperature and leaf transpiration, would intensify the negative effects of salinity on tropic ageratum growth, and these findings would help weed managers understand how the two stressors can interact to restrict or exacerbate its invasion to croplands.

Results and discussion

Photosynthetic gas exchange and chlorophyll fluorescence in shade treatments

The slopes of net photosynthetic rate (Pn) versus intercellular CO_2 concentration (Ci) appeared greater for the non-shaded than for shaded leaves, and these slopes were significantly different when tested by analysis of covariance at $P < 0.05$ (Fig. 1A, Table 1). Stomatal conductance (Gs) increased linearly with decreasing Ci, and the slopes (absolute values) were greater in the full sunlight treatment than in the shade treatments (Fig. 1B, Table 1). The slopes of Pn versus Gs appeared greater for plants kept in sunlight (Fig. 1C, Table 1). Compared to the relatively weak correlations between Pn and Ci in shaded plants, the strong correlation between Pn and Gs implied that Gs was the dominant limitation to Pn for the shaded plants (Table 1). Excessively high leaf temperature of shaded leaves at high light intensity accelerates the leaf transpiration causing leaf water stress. The activity of Ribulose bisphosphate carboxylase oxygenase (RuBPCO) may be negatively affected by the water stress (Parry et al., 2002) and light-induced photoinhibition may occur. Meanwhile, a small light-absorbing chlorophyll antenna for photosystem II and higher rates of light-saturated photosynthesis help the sun-exposed plants to avoid or alleviate the physiological stress of high light (Öquist et al., 1992). Highest apparent quantum yield (AQY), lowest light compensation point (LCP) and lowest maximum net photosynthetic rate (Pmax) were found in 75% of shaded plants (Fig. 1D). Weeds that grow in shade typically have low dark respiration rates and lower LCP at which respiratory CO_2 loss equals photosynthetic uptake (Beneragama and Goto, 2010). Under low irradiation, the high AQY of shaded plants was probably due to the increased stomatal activity that had a direct influence on photosynthetic efficiency by regulating RuBPCO activity and Ribulose-1,5-bisphosphate regeneration capacity (Irmak et al., 2008). The electron transport rate (ETR) value of 0% shade exceeded that of 50% shade when photosynthetic photon flux density (PPFD) was above 600 μmol m^{-2} s^{-1} (Fig. 2A). LSP of these treatments exceed 2000 μmol m^{-2} s^{-1}. Similarly, when PPFD was above 600 μmol m^{-2} s^{-1}, yield of 0% shade was higher than that of 50% and 75% shade (Fig. 2B). The photochemical quenching (qP) was relatively higher in 0% shade than in 50% and 75% shade (Fig. 2C), which indicates a higher activity of electron transport in PS II in unshaded plants (Maxwell and Johnson, 2000). In Figure 2D, non-photochemical quenching (qN) exhibited complex changes as PPFD increased gradually, but no significant difference could be found. The relatively low ETR, Yield, qP and Pmax in 75% shade suggested that low light intensity significantly influenced the growth of tropic ageratum. The low LCP and high LSP in shaded plants indicate that tropic ageratum has the ability to acclimatize to different shade conditions, although heavy shade may have a negative impact on its growth. These results are consistent with the characters of weeds that possess plasticity to acclimatize to changing light environments in different regions (Haraguchi et al., 2009).

Effects of shade and salinity on morphological and biochemical parameters

Effects on all morphological and biochemical parameters before salinity treatment are shown in Table 2. MGLM analysis of treatment main effects (treatment period, light and salinity) and their interactions on tropic ageratum growth are shown in Table 3. There was a negative relationship between morphological parameters [plant height (PH) and leaf area (LA)] and light intensity at the beginning of salinity treatments. LA at 50% and 75% shade were significantly greater than that of full sunlight ($P < 0.05$). After 10 days, the negative relationships did not change, and PH and LA increased as light intensity decreased (Fig. 3A and 3B, $P < 0.05$). PH and LA decreased when salinity levels increased. However, after 20 days the negative relationships changed, especially for PH (Fig. 3C). The plants were significantly taller in 50% shade than in 0% and 75% shade ($P < 0.05$). There was no significant difference in LA under 50% and 75% shade (Fig. 3D). Meanwhile, LA decreased with increasing salinity. There was only a significant main effect of light on PH ($P < 0.05$, Table 3). Compared to PH, treatment period (TP), light and salinity combined with TP-salinity and TP-light interactions have significant effect on the leaf area ($P < 0.001$, Table 3). The morphology changes (increased PH and LA) of tropic ageratum at low irradiance indicate that shade-avoidance response occurred. Such shade-avoidance responses may improve plant fitness by increasing the capture of the most limiting resource under diverse environmental conditions (Sultan, 2000). After 20 days, the small new-born leaves in 75% shade compared to 50% shade implied that lack of photosynthate limited the expansion of leaf area under heavy shade. The lowest Chl a, Chl b and total Chl (Chl a+b) were in un-shaded plants at the beginning of salinity treatments. The lowest and highest chlorophyll a : b ratio (Chl a/b) were in the 50% and 75% shaded plants, respectively. After 10 day exposure to salinity under three shade conditions, Chl a, Chl b and Chl a+b decreased gradually with the increasing NaCl concentrations (Fig. 4A, 4B and 4C). The Chl a, Chl b and Chl a+b were significantly higher in 50% shade at all salinity levels ($P < 0.05$). The Chl a/b increased as shade increased, but decreased when salinity increased (Fig. 4D). After 20 d of salinity treatments under three shade regimes, the Chl a and Chl a+b (Fig. 4E and 4G) also decreased with increased salinity levels (Fig. 4E, 4F, and 4G). However, the highest Chl a and Chl a+b were in plants that received 75% shade with 50 mM NaCl. The Chl a/b (Fig. 4H) increased as shade increased, but not for the cases under 100 and 150 mM NaCl treatments. MGLM analysis

Table 1. Relationship between net photosynthetic rate (Pn) and intercellular CO_2 concentration (Ci), stomatal conductance (Gs) and Ci, and Pn and Gs of ageratum grown under 100%, 50% and 25% light. The linear regression (y = a x + b) for each treatment is indicated.

	100% Light			50% Light			25% Light		
	a	b	r^2	a	b	r^2	a	b	r^2
Pn/Gs	0.75	1.82	0.749**	0.31	-3.11	0.764*	0.16	-3.26	0.348
Gs/Ci	-0.051	42.64	0.596*	-0.037	45.69	0.104	-0.024	88.41	0.321
Pn/Ci	-0.053	41.92	0.848**	-0.022	15.74	0.165	-0.0075	12.31	0.208

[a] slopes of linear regression, [b] increment of linear regression, [r] correlation coefficient. * and ** indicate $P < 0.05$ and 0.01, respectively.

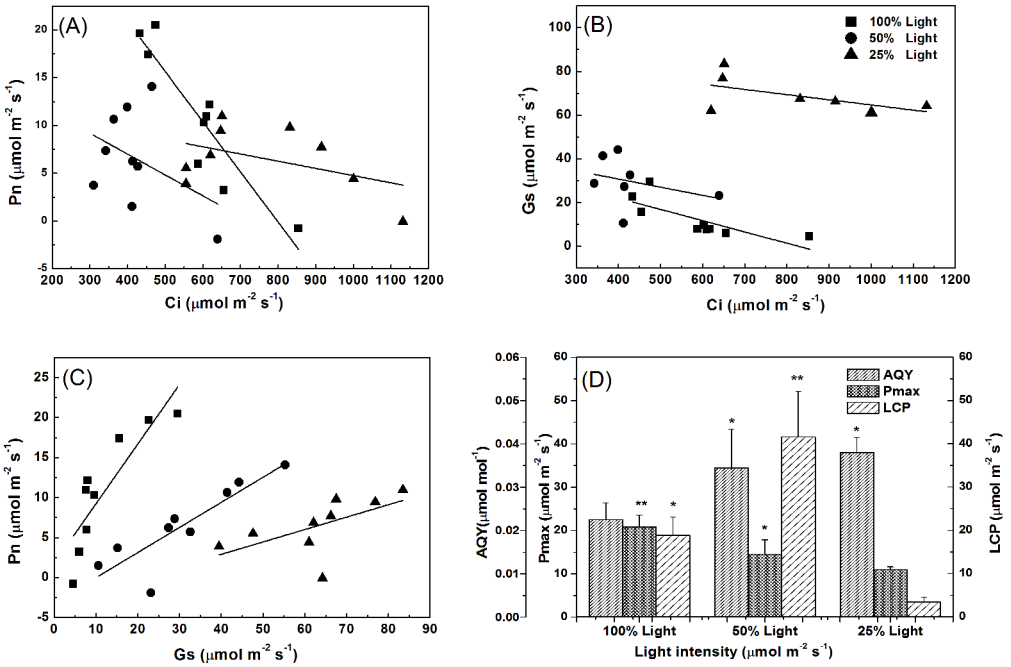

Fig 1. Relationship between net photosynthetic rate (Pn) and intercellular CO_2 concentration (Ci), stomatal conductance (Gs) and Ci, and Pn and Gs of ageratum grown under 100%, 50% and 25% light (A, B and C). Linear regression was performed for each treatment. The apparent quantum yield (AQY), maximum net photosynthesis rate (Pmax) and light compensation point (LCP) is shown in figure D. * and ** indicate $P < 0.05$ and 0.01, respectively. Bars indicate standard deviation.

suggested that all treatments and their interactions had significant effects on the levels of Chl a, Chl b and Chl a+b, but there were only significant main effects of TP and light with TP-salinity and three way interactions affecting Chl a/b levels (Table 3). The enhanced chlorophyll content in the shaded plants under 0 mM NaCl treatment clearly shows that ageratum could adapt to the reduced irradiance by changing chlorophyll contents (Dai et al., 2009b). Besides the changes in chlorophyll content, change of chloroplast structure also modifies the adaptation of plants under different light conditions. A much greater quantity of light-harvesting Chl a/b proteins (LHC II) and a fewer number of reaction centers on a total Chl basis affect the high irradiance adaptation response of the photosynthetic pigment apparatus in shaded leaves (Laisk et al., 2005). Meanwhile, the loss of chlorophyll is often considered as a marker of cellular component of salt stress (Singh and Dubey, 1995). Our results clearly show chlorophyll content was reduced by salinity treatments. Soluble sugar (SS), soluble protein (SP) and proline (Pro) almost increased positively with the increasing light intensity at the beginning of salinity treatments (Table 2). After 10 d, MDA, SS and Pro increased with increasing salinity (Fig. 5A, 5B and 5D). The SS was significantly higher in 75% shade at all salinity levels ($P < 0.05$). Full sunlight increased MDA and made it significantly higher than shaded plants at all salinity levels ($P < 0.05$). Meanwhile, MDA increased with increasing salinity, and the highest was in 0% shade and 150 mM NaCl treatment. SP decreased significantly with increasing salinity under full sunlight, but the trend was reversed in 50% shade ($P < 0.05$, Fig. 5C). The contents of proline increased significantly with increasing salinity, and the highest proline was in 0% shade and 150 mM NaCl treatment ($P < 0.05$). After 20 days, MDA, SS, SP and Pro still increased with increasing salinity (Fig. 5E, 5F, 5G and 5H); especially, SS increased with either high light or salinity, and the highest was in 0% shade and 100 mM salinity treatments ($P < 0.05$). The MDA was significantly higher in 50 mM NaCl treatment than in other salinity treatments ($P < 0.05$). MDA decreased as shade increased, but no significant differences could be found. SP increased when NaCl concentration increased, and decreased as the light intensity decreased. The Pro increased as salinity increased, and the highest proline was at 150 mM salinity

Table 2. Effects of long time shade cover (unshaded versus shaded) that was immediately followed by two periods of salt treatment (0, 50, 100 and 150 mM) on plant height (PH, cm), leaf area (LA, cm^2), chlorophyll a (Chl a, mg g^{-1}), chlorophyll b (Chl b, mg g^{-1}), total chlorophyll (Chl a+b, mg g^{-1}), chlorophyll a:b ratio (Chl a/b), soluble sugar (SS, mg g^{-1}), malondialdehyde (MDA, μmol g^{-1}), soluble proteins (SP, mg g^{-1}), proline (Pro, mg g^{-1}) of ageratum [a].

Light intensity	100%	50%	25%
PH	20.3±6.36	20.6±4.36	22.4±5.25
LA	24.87±0.068	27.58±0.12*	30.24±0.13**
Chl a	0.634±0.048	0.758±0.077	0.770±0.016
Chl b	0.249±0.030	0.318±0.015	0.285±0.025
Chl a+b	0.882±0.079	1.076±0.092	1.055±0.041
Chl a/b	2.549±0.118	2.384±0.127	2.698±0.182
SS	0.0998±0.0062*	0.0609±0.0058*	0.0597±0.0083
MDA	0.0226±0.0002	0.0158±0.0067	0.0183±0.0003
SP	0.334±0.0147	0.321±0.0577	0.302±0.0111
Pro	0.1425±0.0023*	0.0808±0.0105	0.0797±0.0004

[a] data with * and ** are significant differences at $P < 0.05$ and 0.01, respectively.

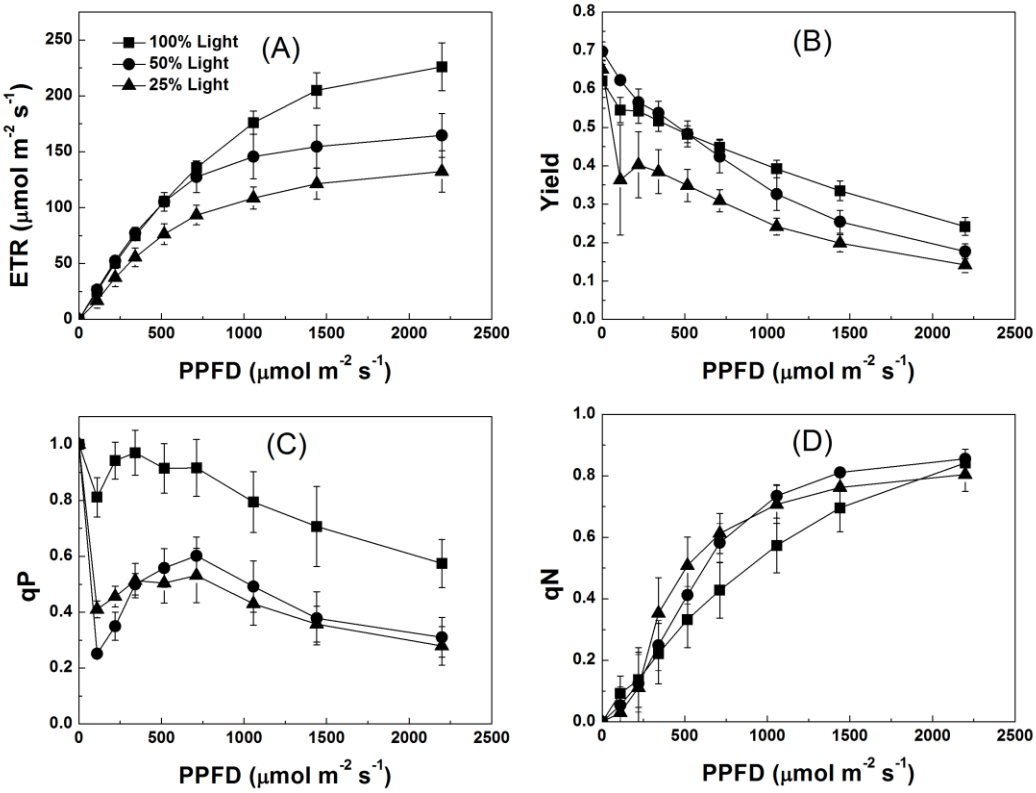

Fig 2. Photosynthetic irradiance-response curves of chlorophyll fluorescence parameters of ageratum grown under 100%, 50% and 25% shade. These include electron transport rate (ETR), quantum yield (Yield), photochemical quenching (qP) and non-photochemical quenching (qN). Bars indicate standard deviation.

level in 50% shade. All treatments and their interactions significantly affect the levels of SS, SP and Pro. While, there were significant main effects of light and salinity with TP-light, TP-salinity and light-salinity interactions affecting MDA levels ($P < 0.05$, Table 3). It is already known that free radical-induced peroxidation of lipid membranes is a reflection of stress-induced damage (such as salt stress) at the cellular level and therefore used as an indicator of oxidative damage (Jain et al., 2001). MDA increased markedly with increasing light intensity and salinity as indicated by some authors (Havaux et al., 2000; Mandhania et al., 2006; Xue and Liu, 2008). The increased proline is positively correlated with salt stress and described as an important osmolyte used by plants to adapt to saline conditions (Qasim et al., 2003). Soluble sugar plays a central role in the biosynthetic pathways of primary and secondary metabolites that are used to control the developmental processes (Price et al., 2004) and salt defense mechanisms (Arbona et al., 2005). The main role of soluble sugar accumulation in salt-stressed tropic ageratum was possibly for osmotic adjustment (Khelil et al., 2007). In some salt-tolerant plants, the increase in total soluble proteins was mainly from the synthesis of stress-specific proteins (Demiral and Türkan, 2006). In this study, the soluble proteins reduced markedly in 0% shaded leaves, which implied that the RuBPCO content (the major soluble protein of leaf) of plants grown under full sunlight reduced more than the increase in stress-specific proteins (Bertamini and Nedunchezhian, 2001). Thus, the increased soluble

Table 3. Multivariate general linear model function (MGLM) analysis of treatment main effects (light, salinity and treatment period) and their interactions on PH, LA, Chl a, Chl b, Chl a+b, Chl a/b, SS, MDA, SP, Pro of ageratum grown under different light and salinity levels [a].

	Effects							MS error	Whole model R^2
	TP	L	S	TP×L	TP×S	L×S	TP×L×S		
PH	2.43	3.76*	1.18	0.62	0.4	0.96	0.48	49.99	0.022
LA	4162.62****	275.94****	16.62****	31.18****	6.38***	1.29	0.76	1.05	0.986
Chl a	117.48****	304.01****	189.13****	54.65****	35.23****	31.82****	16.42****	0.0011	0.962
Chl b	54.07****	13.62****	53.17****	12.01****	17.99****	5.32***	5.84***	0.0008	0.836
Chl a+b	8.10****	155.25****	148.40****	39.73****	24.92****	19.98****	13.09****	0.0030	0.939
Chl a/b	334.32****	68.95****	2.35	0.11	13.70****	1.8	2.47*	0.0376	0.881
SS	282.47****	15.10****	52.50****	45.33****	11.31****	4.76***	3.58**	9.14×10^{-5}	0.898
MDA	0.1	113.40****	20.26****	10.68****	8.51****	3.49**	0.9	1.45×10^{-5}	0.826
SP	104.84****	24.65****	42.06****	26.90****	47.30****	18.67****	17.91****	7.71×10^{-5}	0.905
Pro	86.85****	362.11****	2351.8****	108.50****	60.89****	124.85****	73.42****	0.0003	0.993

[a] Data are expressed as F values. *, **, *** and **** indicate $P < 0.05$, 0.01, 0.001 and 0.0001, respectively. Abbreviations are: treatment period (TP), light (L), salinity (S), plant height (PH), leaf area (LA), chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl a+b), chlorophyll a:b ratio (Chl a/b), soluble sugar (SS), malondialdehyde (MDA), soluble proteins (SP), proline (Pro).

Table 4. Results expressed in terms of Pearson correlation coefficient of the morphological and biochemical parameters [a].

	PH	LA	CHL a	CHL b	CHL a+b	CHL a/b	SS	MDA	SP
LA	-0.113	1.000							
CHL a	0.451*	0.468*	1.000						
CHL b	0.508*	-0.018	0.806**	1.000					
CHL a+b	0.488*	0.244	0.954**	0.921**	1.000				
CHL a/b	0.034	0.887**	0.579**	0.002	0.335	1.000			
SS	0.045	-0.712**	-0.491*	-0.283	-0.35	-0.541**	1.000		
MDA	-0.518**	-0.215	-0.551**	-0.486*	-0.553**	-0.321	0.218	1.000	
SP	-0.124	0.349	0.121	-0.273	-0.008	0.547**	-0.062	0.061	1.000
PRO	-0.374	-0.06	-0.576	-0.68**	-0.614**	-0.092	0.382	0.454*	0.357

[a] data with *, ** and *** are significant differences at $P < 0.05$, 0.01, and 0.001, respectively.

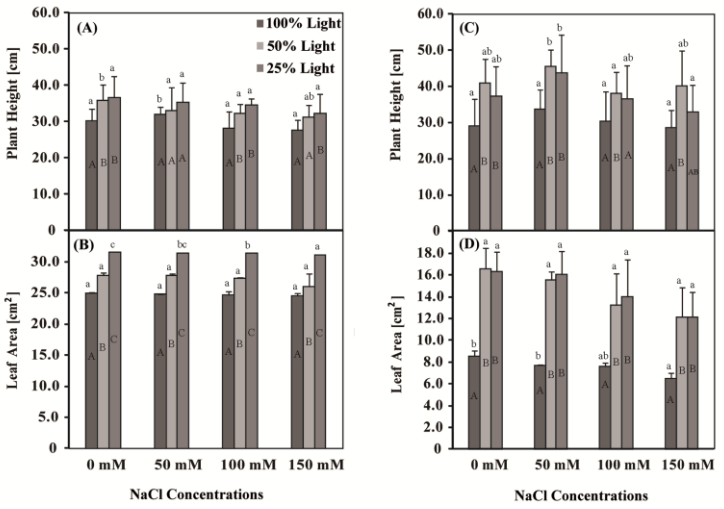

Fig 3. Effect of salinity and light on plant height (PH) and leaf area (LA) of ageratum. Figure A and B show the results of 10 days of salinity treatment, while figure C and D show the results of 20 days of salinity treatment. Bars indicate standard deviation. Different capital letters and lowercases indicate significant differences at 0.05% confidence interval among shade treatments and salinity treatments, respectively.

proteins implied that the synthesis of stress-specific proteins was greater than the degradation of RuBPCO.

Factor analysis of morphological and biochemical parameters of tropic ageratum grown in different shade and salinity treatments

Correlations between all parameters (Table 4) revealed that MDA was significantly negatively correlated with PH, Chl a, Chl b and Chl a+b. Pro was significantly negatively correlated with Chl b and Chl a+b. SS was significantly negatively correlated with LA, Chl a and Chl a/b. Finally, SP was significantly negatively correlated with Chl a/b. Three factors explained 82% of the total variance caused in the parameters (Table 5). Factor 1, 2 and 3 explained 38%, 29% and 15% of total variance. The factor scores represented the growth status of ageratum at various light and salinity levels (Fig. 6). The lower value meant that the plants suffered more severe stress and vice versa. In figure 6A, the scores increased as PPFD decreased in 0 mM NaCl treatment, and the scores of 50% shaded plants were highest when salinity increased from 50 to 150 mM. In figure 6B, the scores increased as PPFD decreased, and then the scores of 50% shaded plants were highest at all salinity levels. The clustering pattern of parameters, according to the three factors, was further used to explain the plausible hidden functions, such as (a) the stress responses sourced from peroxidation and osmotic damage and (b) the effects of stresses on the metabolism of substance, including photosynthate and protein. The factor 1 indicates that plants grown in abiotic stress conditions (including salinity and high light intensity) suffer oxidative stress, especially the formation of reactive oxygen species (ROS) that cause extensive cellular damage (increase in MDA and Pro) and inhibition of photosynthesis (decrease in Chl; Allen, 1995). Reduced photosynthesis in salt-stressed plants has been associated with the toxicity of Cl^- and/or Na^+, and osmotic stress (Levy and Syvertsen, 2010). The factor 2 and 3 could be interpreted as the responses of carbon and nitrogen

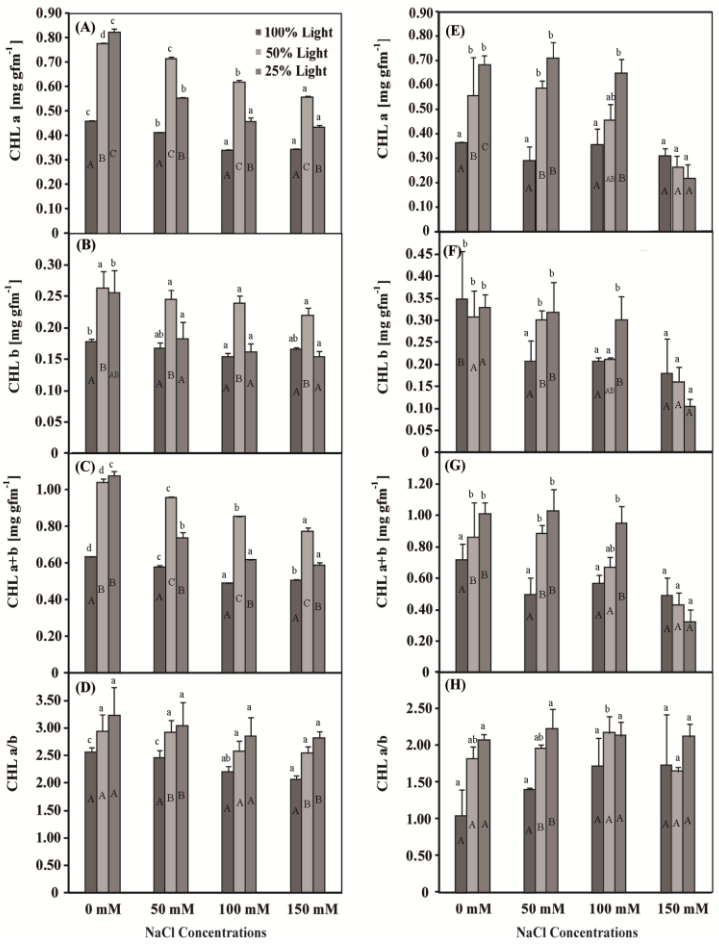

Fig 4. Effect of salinity and light on chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl a+b) and chlorophyll a:b ratio (Chl a/b) of ageratum. Figure A, B, C and D show the results of 10 days of salinity treatment, while figure E, F, G and H show the results of 20 days of salinity treatment. Bars indicate standard deviation. Different capital letters and lowercases indicate significant differences at 0.05% confidence interval among shade treatments and salinity treatments, respectively

metabolisms under salt and light stress, such as reduction of photosynthate, accumulation of stress-specific proteins and decrease of RuBPCO (Bertamini and Nedunchezhian, 2001; Demiral and Türkan, 2006). As expected, the suppression of whole-plant growth by high salinity was stronger at high than low light condition. In other words, shade could alleviate the damage of salinity stress, although moderate and heavy shade had different effects on the growth status of this weed. Salinity limited water uptake by plants and required lower leaf water potentials to drive transpiration. Low leaf water potentials led to reduced stomatal conductance, causing lower leaf intracellular CO_2 concentrations, and decreased photosynthetic rates (Lin and Sternberg, 1992; Sobrado, 1999). Thus, the negative effects of salinity on leaves would be greater at high than at low light condition.

Table 5. Factor analysis for the effects of light and salinity on growth of ageratum [a].

Parameters studied	Communalities	Factors		
		1	2	3
Factor 1				
Chl a+b	0.876	0.903	0.228	-0.092
Chl a	0.926	0.858	0.433	0.041
Chl b	0.876	0.856	0.04	-0.378
MDA	0.631	-0.782	0.018	-0.139
PH	0.628	0.729	-0.3	0.086
Pro	0.744	-0.666	-0.172	0.521
Factor 2				
LA	0.924	0.117	0.908	0.294
SS	0.794	-0.237	-0.838	0.189
Chl a/b	0.928	0.277	0.751	0.536
Factor 3				
SP	0.788	-0.042	0.219	0.859
variance	9.029	4.218	3.198	1.613
% Variance	82.074	38.343	29.07	14.661

[a] This form of analysis divided the ten parameters into three synthetical factors (factor 1, factor 2 and factor 3), and the 'Communalities' show the percentage of each parameter that can be explained using these three factors.

Materials and methods

Plant material and growing conditions

The mature seeds of tropic ageratum were sterilized with 0.01% $HgCl_2$ for 1 min and rinsed four times with deionized water. Seeds were then placed on two layers of filter paper in closed 10-cm-diameter Petri dish and moistened with 5 ml of deionized water. Germination trials were conducted in an air-conditioned, unlighted incubator under an approximate temperature range of 20 - 25 °C (Shen et al., 2008). After germination, the seedlings with similar elongation were transplanted into Hoagland's solution under three shade treatments (100, 50 and 25% of natural incident irradiance). After two months, plants with similar height and leaf numbers were selected and cultivated in Hoagland's solution with four salinity levels (0, 50, 100 and 150 mM NaCl) under the same shade conditions. Morphological and biochemical parameters were measured 0, 10, 20 days (d) after treatment. Measurements of photosynthesis and chlorophyll fluorescence were conducted at vegetative growth stage (four weeks after shade treatments) of ageratum before salinity treatments.

Photosynthetic gas exchange and chlorophyll fluorescence

Net photosynthetic rate (Pn), transpiration rate (E), stomatal conductance (Gs), and intercellular CO_2 concentration (Ci) were measured using a GFS-3000 portable photosynthesis system. Considering the leaf size of ageratum, the measurements were conducted on the 10th fully expanded mature leaves (whose size were large enough to fit the equipment) under light intensity of 0, 50, 100, 200, 300, 400, 800, 1200, 1600 and 2000 μmol (photon) m^{-2} s^{-1} (Liu et al., 2008). The air cuvette temperature and the air CO_2 concentration were maintained at 25 ^oC and 750 μl L^1. Assimilation was recorded at each of the 10 light levels following a 10 min acclimation period. Three plants were measured in each group and two replications were conducted for each plant. Chlorophyll fluorescence quenching analysis was carried out at room temperature with a MINI-PAM (pulse-amplitude modulation) fluorometer (WALZ, Effeltrich, Germany). Five randomly selected plants in each treatment group were kept in dark for 30 min and three fully expanded, mature leaves were chosen to measure the electron transport rates (ETR), effective quantum yield of photochemical energy conversion (Yield), photochemical (qP) and non-photochemical (qN) quenching of Chl fluorescence (Dai et al., 2009a, 2009b). Measurements were obtained over a range of PPFD between 0 and 2500 μmol m^{-2} s^{-1}. The quantum yield of PS II photochemistry can be determined from the yields of steady state (Fs) and maximal (Fm') fluorescence during steady state photosynthesis. ETR was calculated as Yield×PPFD×0.5, where PPFD was the absorbed light (μmol photons m^{-2} s^{-1}) by leaf. qP and qN was calculated with the Fluorescence Monitoring System (FMS) software (Genty et al., 1989).

Plants height and leaf area

Shoot elongation was measured on each plant 0, 10 and 20 days after salt treatments. All plants in each group were randomly selected and measured. The leaf area of all mature leaves in each plant was measured separately with a Li-Cor 3100 leaf area meter.

Chlorophyll content

The fully expanded leaves of tropic ageratum (0.5 g) in each treatment were randomly picked and extracted in 100% acetone. The absorption of the extracts was read at 645 nm and 662 nm by a spectrophotometer. The concentrations (mg g^{-1} fresh leaf mass) of chlorophyll a, chlorophyll b were calculated using the equations of Mitchell and Arnott (1995).

MDA, soluble sugar, soluble protein and proline concentrations

Fresh leaf samples of tropic ageratum (1 g) in each treatment group were randomly picked and homogenized in 10 ml 10%

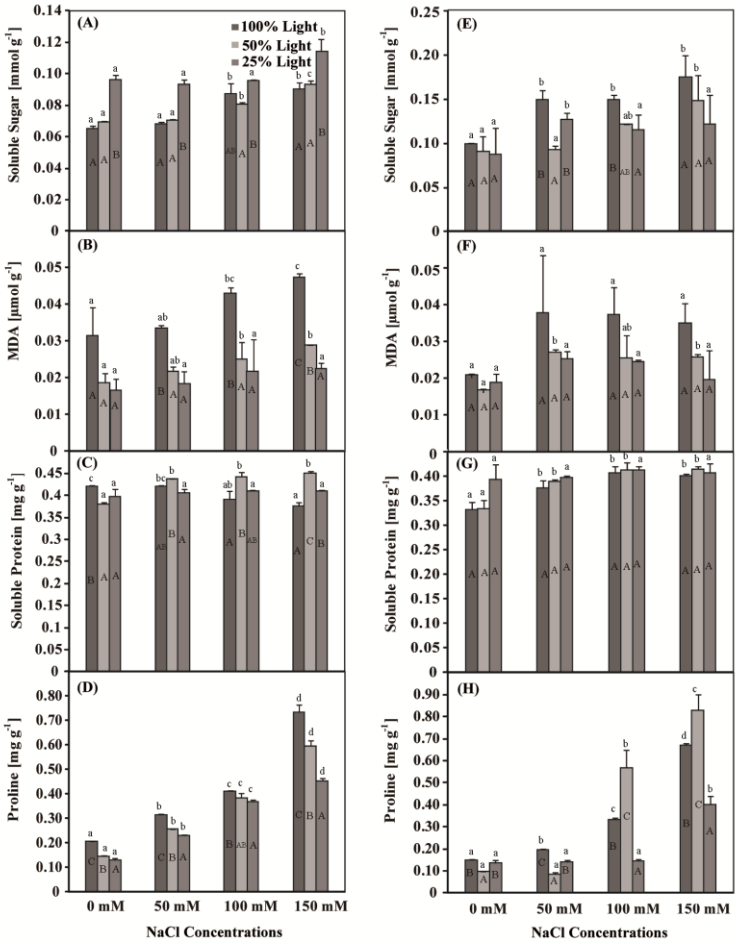

Fig 5. Effect of salinity and light on soluble sugar (SS), malondialdehyde (MDA), soluble protein (SP) and proline (Pro) of ageratum. Figure A, B, C and D show the results of 10 days of salinity treatment, while figure E, F, G and H show the results of 20 days of salinity treatment. Bars indicate standard deviation. Different capital letters and lowercases indicate significant differences at 0.05% confidence interval among shade treatments and salinity treatments, respectively.

trichloroacetic acid solution (TCA). The homogenate was centrifuged at 4,000 g for 10 min and 2 ml of the supernatant obtained was added to 2 ml 0.6% thiobarbituric acid (TBA) in 10% TCA (Liu et al., 2008). The mixture was incubated at 100 ^{o}C in a shaking water bath for 15 min, and the reaction was terminated by placing the reaction tubes in an ice-water bath. The samples were then centrifuged at 10,000 g for 10 min. The absorbance of the supernatant was read at 532 nm and 450 nm, and the value of non-specific absorption at 600 nm was subtracted (Liu et al., 2008). The amount of MDA (μmol g^{-1}) and soluble sugar (mmol g^{-1}) was calculated as described in Heath and Packer (1968). Total soluble protein content was measured according to Bradford (1976) using bovine serum albumin (BSA) as a protein standard. Fresh leaf samples (1 g) were homogenized with 4 ml Na-Phosphate buffer (pH 7.2) and then centrifuged at 4^{o}C. Supernatants and dye were pipetted in the spectrophotometer cuvettes and absorbance was measured by using a spectrophotometer at 595 nm. Fresh leaf samples (0.5 g) were extracted in 3% (w: v) salicylic acid and the proline

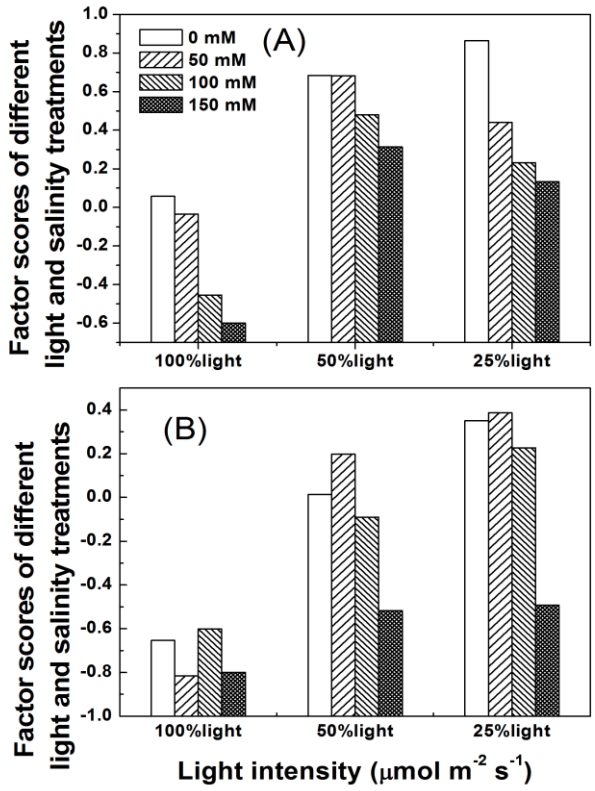

Fig 6. Factor scores of ageratum grown in different light and salinity treatments calculated from the results of factor analysis. The function: Factor Scores = Factor 1×38.343% + Factor 2×29.07% + Factor×14.661%. Figure A and B show the results of salinity treatment for 10 days and 20 days, respectively

concentration was estimated by ninhydrin reagent. The absorbance of the fraction with toluene aspired from the liquid phase was read at 520 nm. Proline concentration was expressed as μmol proline g^{-1} fresh weight.

Statistical analysis

Split-plot design was used in this experiment, and light (main-plot effect) and salinity (subplot effect) as treatment factors were analyzed in a 3×4 factorial design. The nine blocks to be used in the experiment were positioned side by side in three rows, and two blocks in each row were randomly covered with two kinds of shade clothes (50% and 25% of natural incident irradiance), respectively. Each block was in turn divided into four subplots (four salinity levels), and each subplots had 3 plants per replicate. All morphological and biochemical data were analyzed using Multivariate General Linear Model (MGLM; SAS Institute Inc., Cary, NC, USA) for testing differences in split-plot designs. Linear regression was used to describe relationships between selected variables and analysis of covariance was used to compare slopes of relationships. All morphological and biochemical data were subjected to factor analysis following FACTOR procedure, using principal components of SAS, to identify the contribution of individual parameters (based on their factor-loadings) to a treatment, and the inter-relationship among treatments (factor-scores). The factor-scores explained the spatial location of treatments, indicating

the relationships among treatments; where a positive factor-score was associated with a positive factor-loading for parameters.

Conclusion

Tropic ageratum now has widely spread in South China and Southeast Asia, and it grows abundantly among many annual and perennial crops in field or mudflat reclamated for agriculture. In these agro-ecosystems light intensity and salinity are the major limiting factors, and our study suggested that shade could alleviate the damage of salinity stress and exacerbate the invasion of tropic ageratum. Thus, appropriate agriculture management practices, such as rational close planting, inhibiting excessive growth of branches, developing cultivars with high salt-tolerance, are required to control the spread of this weed in croplands in the coast of South China and Southeast Asia. Further, this weed acclimatizes well to the various light and salinity environments, even high light intensity and severe salinity stresses. Thus, effective management should be taken to prevent further distribution and spread of this weed by environmentalists, ecologists and farmers.

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