

Different causes of photosynthetic decline and water status in different stages of girdling in *Alhagi sparsifolia* Shap. (Fabaceae)

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Abstract Phloem girdling can cause decline of photosynthetic rate (Pn), and the reason for Pn decline had been attributed to the reduction of stomatal conductance (Gs) and end-product feedback inhibition. In order to explore the reason for Pn decline, the different stages of girdling—control, semi-girdling (SG), and full-girdling (FG)—were performed on *Alhagi sparsifolia* Shap. (Fabaceae) on the southern rim of the Taklamakan Desert. Our results showed that on the 1st day, abscisic acid (ABA) content and water use efficiency (WUE) increased, and Gs, Pn, and transpiration rate (Tr) decreased in the full-girdled leaf. On the 30th day, leaf ABA content, leaf starch content, and leaf soluble sugar content increased in the full-girdled leaf, and Gs, Pn, Tr, WUE, root starch content, root soluble sugar content, chlorophyll (Chl) content, maximum photochemical efficiency (F_v/F_m), and leaf water potential (Ψ_{leaf}) all decreased in the full-girdled leaf. SG showed no physiological change on the 1st day, whereas on the 30th day, the change was similar to FG, although the degree was less.

The result of the present work implied that the reason for Pn decline in girdling may depend on time. In the short term, girdling (FG)-induced Pn decline was due to ABA accumulation, which resulted in the reduction of Gs. In the long term, however, Pn decline caused by girdling was due to many factors, including Gs reduction, which resulted from ABA accumulation, carbohydrate feedback inhibition, degradation of Chl content, decreasing of F_v/F_m , and deterioration of Ψ_{leaf} . In addition, a portion (half) of the phloem cannot undertake the transport work conducted by the whole phloem, and thus the girdled half circle of the phloem would lead to a similar effect to FG in the long term, although the degree was less.

Keywords Chlorophyll · Carbohydrate feedback · Girdling · Phloem transport · Stomatal conductance · Water potential

Introduction

Photosynthesis is vital for life on earth. Specifically, it makes use of sunlight to convert carbon dioxide into useful biomass, and provides chemical energy for almost all life on earth (Cheng and Fleming 2009; Collini et al. 2010). However, photosynthesis is a complex process that comprises light-driven electron transport, carbon reduction cycle reactions, and end-product (primarily starch and sucrose) synthesis (Woodrow and Berry 1988; Pammeter et al. 1993). Photosynthesis can be influenced by many factors, including radiation (Pallozzi et al. 2013), nitrogen content (Urban et al. 2004), temperature (Saxe et al. 2001), water condition (Bréda et al. 2006), stomatal conductance (DaMatta et al. 2008), CO₂ content (Leakey et al. 2012), etc.

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In addition, recent study has also shown that photosynthesis is affected by phloem properties (Mencuccini et al. 2012). As a central component of the plant's vascular system, phloem plays an essential role in moving photoassimilates from sites of primary acquisition to the heterotrophic tissues and organs of the plant (Ainsworth and Bush 2011). Because photosynthesis is tightly coordinated with the carbohydrate content in source organs and its utilization in sink (Paul and Foyer 2001; Kaschuk et al. 2010; Ainsworth and Bush 2011), the effect of phloem properties on photosynthesis may be due to the change of source–sink ratio and their interaction (Layne and Flore 1995; Ainsworth and Bush 2011; Nikinmaa et al. 2013), which is called end-product feedback inhibition (Nebauer et al. 2011). The hypothesis of feedback inhibition asserts that high photosynthetic rates in the leaves are not sustained unless photoassimilates are transported to other parts of the plant continuously, so that photoassimilates in the leaves can always maintain a low level (Ainsworth and Bush 2011; Nebauer et al. 2011; López et al. 2015). Although end-product downregulation has been found in a number of studies (Ainsworth and Bush 2011; Nebauer et al. 2011), the signal to which downregulation responds is not yet understood (Nikinmaa et al. 2013). Moreover, end-product inhibition may be quite different, depending on species (Goldschmidt and Huber 1992). One of the reasons that photosynthesis downregulation that occurs prior to any sugar build-up may be to guarantee that no harmful accumulation of carbohydrates would take place in the phloem (Nikinmaa et al. 2013).

When the phloem was girdled, carbohydrates, such as soluble sugar and starch, cannot export from source organs (leaf), which subsequently cause an accumulation of carbohydrates above the girdle and reduction of carbohydrates below the girdle (Nebauer et al. 2011; Yang et al. 2013; Tang et al. 2015a). As girdling can easily manipulate carbohydrate contents above and below the girdle, it has also been used as a research tool to identify the response dynamics of a specific tissue or organ, such as photosynthesis in leaf (Paul and Foyer 2001; Paul and Pellny 2003; Tang et al. 2015b). Thus, it may provide an excellent system to study the effects of changes in source/sink balances on photosynthesis in leaves (Ben Mimoun et al. 1996; Génard et al. 1998; Urban et al. 2004). However, girdling-induced soluble sugar and starch accumulation in leaves did not lead to downregulation of the photosynthetic rate in citrus (Nebauer et al. 2011). DaMatta et al. (2008) showed that decline of photosynthesis in defruited coffee was largely unrelated to direct feedback mechanisms mediated by end-product accumulation, and they attribute the decline of photosynthesis to the change of stomatal conductance. The most reasonable explanation for this

phenomenon is that end-product-induced photosynthesis inhibition may differ among different species (Goldschmidt and Huber 1992).

In the girdling system, some previous studies demonstrated that the decline of photosynthesis may result from abscisic acid (ABA) accumulation in girdled leaf, which subsequently led to the reduction of stomatal conductance (Setter et al. 1980). However, present studies showed that ABA and photoassimilates were transported in a similar manner to leaves (Hoad 1995; Nikinmaa et al. 2013). It is still unclear why most studies found that either ABA-induced stomatal conductance decrease or photoassimilates-induced feedback inhibition is the reason for photosynthesis decline in girdled leaf, even though they were both transported in a similar manner to phloem (Hoad 1995; Nikinmaa et al. 2013).

We conducted an experiment in the inland sand in Xinjiang, China, in which *Alhagi sparsifolia* Shap. (Fabaceae) within $4 \times 4 \text{ m}^2$ quadrats was girdled. We choose this species because *A. sparsifolia* is a typical eremophyte, and thus the effect of girdling on photosynthesis in *A. sparsifolia* may be interesting. *Alhagi sparsifolia* may have a stronger tolerance for the accumulation of carbohydrate, and it may at least adapt to the carbohydrate-accumulation-induced low water potential (osmotic potential), *Alhagi sparsifolia* Shap. (Fabaceae) have a unique and interesting response to phloem girdling. We hypothesize that the reason for girdling-induced photosynthesis decline may constitute not one, but many factors, and it may change with time. Hence, we investigated the responses of photosynthesis in leaf of *A. sparsifolia* for girdling in the short term (1 day) and long term (30 days), and explored which factor leads to variation of photosynthesis in these two different stages. In addition, we added a treatment, semi-girdling (girdled semicircle of the phloem), which has not been previously conducted. This treatment is to study whether a portion of the phloem (e.g., half of the phloem) can undertake the whole work of phloem, and whether portion girdling had the same effect as full-girdling (girdled full-circle of the phloem).

Materials and methods

Plant materials

Alhagi sparsifolia is a kind of prickly, clonal, and perennial leguminous caudex in arid and semiarid regions, with a height of about 1 m (Zeng et al. 2008; Tang et al. 2015c). It is widely distributed in the oasis–desert transitional zone at the southern rim of the Taklamakan Desert in northwestern China, and is one of the most important plant species in the area (Xue et al. 2011).

Study site

The study was carried out at the Desert Experimental Area in the Cele National Field Research Station for Desert Steppe Ecosystems, Chinese Academy of Sciences. The research area is located in the Taklimakan Desert at an oasis–desert transitional zone on the southern rim of the Taklamakan Desert (35°17'55"–39°30'00"N, 80°03'24"–82°10'34"E).

Experimental design

On 15 August 2013, nine quadrats, each $4 \times 4 \text{ m}^2$ and with 10–12 plants, were set up on flat land. Of these, each quadrat was randomly selected for three different degrees of girdling treatments. Three different degrees of girdling treatments are control (plants without girdling, CK), semigirdling (girdled semicircle of the phloem, SG) and full-girdling (girdled full-circle of the phloem, FG), and every treatment comprised three squares (each $4 \times 4 \text{ m}^2$ and with 10–12 plants). Girdling consisted of removing a 10–12 mm wide band of bark at the bottom of the main stem of each branch, and the girdled regions were immediately covered with silicon grease and wrapped in adhesive plastic tape to prevent desiccation. The treatment of girdling was between 8:00 and 9:30 am, temperature was between 25 and 34 °C, and the photosynthetic active radiation was 1250–1580 $\mu\text{mol m}^{-2} \text{ s}^{-1}$. One and thirty days after girdling, stomatal conductance (Gs), net photosynthesis rate (Pn), transpiration rate (Tr), water use efficiency (WUE), soluble sugar content, starch content, abscisic acid (ABA) content, chlorophyll (Chl) content, Chl fluorescence, and leaf water potential (Ψ_{leaf}) were measured.

Measurement of photosynthetic parameters

Net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), and water use efficiency (WUE) were measured in the field as described by Mittler et al. (2001) using a Portable Photosynthesis System (LI-6400 Inc., Lincoln, NE, USA). Specifically, the parameters were measured every 2 h from 06:00 am to 18:00 pm (GMT + 6) on August 16 and September 14, 2013. As the measured leaves did not reach the standard size of the leaf chamber (2 cm \times 3 cm), a scanner was used to scan the measured leaves. In addition, Image Pro Plus 6.0 software (Media Cybernetics, Silver Springs, MD, USA) read the surface area of the measured leaves, and was later employed to calculate the actual values of Pn, Gs, Tr, and WUE (the value read from the apparatus divided by the area of leaf which we measured and then multiplied by 6 cm² are the actual values of Pn, Gs, Tr, and WUE when leaf fills the leaf chamber). Four replicate measurements were performed for each treatment.

Measurement of Chl content

All leaves were of a similar size and selected from the second to fifth leaves on each of the branches. We collected five leaves from each *A. sparsifolia*, so that each quadrat had 50 leaves (5 \times 10), for a total of 150 leaves (50 \times 3). Measurement of photosynthetic pigment content was as described by Lichtenthaler (1987). Specifically, for every 30 leaves (a total of five groups and 150 leaves), the Chl were extracted with mortar and pestle in 10 mL 80 % chilled acetone plus 50 mg MgCO₃ and purified sea sand. After centrifugation at 2500 rpm for 2 min, the resultant solution of Chl (Chl *a* + *b*) was determined spectrophotometrically at 663 nm (Chl *a*) and 647 nm (Chl *b*) (Jenway 6400, Krackeler Scientific, London, UK). The concentration was calculated as described by Lichtenthaler (1987). Five replicates were performed at each treatment.

Measurements of soluble sugar and starch content

Leaves for measurement of soluble sugar and starch were picked using the same way as that for the measurement of Chl content described above. The leaves were dried in 75 °C for 24 h until the mass maintained stability. Then, the dried *A. sparsifolia* leaves were ground to powder. The leaf powder (0.5 g) was extracted with 4 mL of 80 % ethanol at 75 °C for 40 min, followed by two extractions with 2 mL of 80 % ethanol. The supernatants were then combined and purified by 10 g activated carbon at 80 °C for 30 min. Ethanol (80 %) was added to the extract in order to maintain a constant volume (10 mL) for measuring the amount of soluble sugar. The remaining sample (after measurement of soluble sugar) was dried at 45 °C to remove the ethanol, and boiled for 10 min with 3 mL double-distilled water in 7.5 mL centrifuge tubes. The samples were then cooled to room temperature (28 °C), and four mL of HClO₄ was added to decompose the starch and hydrolyzed for 15 min. Soluble sugar (mainly glucose) was measured at 630 nm as described by Li et al. (2011). Starch content was calculated using the formula:

Starch content %

$$= G \text{ (glucose weight obtained from standard curve)} \\ \times 0.9 \text{ (coefficient of glucose converted to starch)} \\ /DW \text{ (dry weight)} \times 100 \%$$

Measurement of Ψ_{leaf}

Water potential readings were conducted according to the procedures of Williams and Araujo (2002) and Williams et al. (2012). Specifically, predawn water potential (Ψ_p)

measurements began at about 04:30 am (Greenwich Mean Time + 6) and were finished before sunrise using a pressure chamber (PMS instruments Co., Corvallis, Oregon, USA). Midday water potential (Ψ_M) measurements occurred between 11:30 am and 12:30 pm (GMT + 6). Leaf blades for Ψ_{leaf} determination were covered with a plastic bag, quickly sealed, and petioles were then cut within 1–2 s. The time between leaf excision and chamber pressurization was generally <10–15 s. These leaves were located on the south side of east–west rows and the west side of north–south rows. Five replicate measurements were performed at each treatment.

Measurement of ABA content

ABA extraction was determined as described by Veselov et al. (2008). All samples were taken in the morning at approximately 08:00 am (GMT + 6), weighed, and then frozen in liquid nitrogen. Frozen samples were ground to fine powder in liquid nitrogen. ABA was extracted in 80 % ethanol and incubated overnight at 4 °C. Distilled water was added to dilute the aqueous residue, then acidified with HCl (1 mol/L) to pH 2.5, then partitioned twice with peroxide-free diethyl ether (ratio of organic to aqueous phases was 1:3). The organic phase was transferred into 1 % sodium hydrocarbonate (pH 7–8, ratio of organic to aqueous phases was 3:1). The solution was re-extracted with diethyl ether, methylated with diazomethane, and immunoassayed using antibodies to ABA (Vysotskaya et al. 2004). For additional details and calculations, see Veselov et al. (2008).

Data analysis

The differences of *A. sparsifolia* between treatments of CK, SG, and FG were analyzed by one-way analysis of variance (ANOVA) using PASW Statistics 18.0 software (Macintosh, SPSS Inc., Chicago, IL, USA) for each parameter. Once a significant difference was detected, post hoc LSD multiple range tests at $P < 0.05$ were used to identify statistical significance. Results shown in graphs are presented as the mean value \pm standard deviation.

Results

Photosynthesis characteristics after phloem were girdled

Pn, Gs, Tr, and WUE were measured and summed up over all measurement time points in the whole day, and then their differences were compared. In the present study, each

type of treatment showed a bimodal distribution in Gs (Fig. 1a, b) and Pn (Fig. 1c, d) in *A. sparsifolia* leaves. On the 1st day compared with CK, Gs, Pn, Tr, and WUE in semi-girdled (SG) leaf changed very little (Fig. 1); while Gs, Pn, and Tr in full-girdled (FG) leaf decreased significantly ($p < 0.05$) (Fig. 1), and WUE in full-girdled (FG) leaf increased significantly (Fig. 1g). On the 30th day compared with CK, Gs, Pn, Tr, and WUE in both semi-girdled (SG) leaf and full-girdled (FG) leaf decreased significantly (Fig. 1), and the treatment of FG showed a much more decrease. On the 30th day, Gs, Pn, Tr, and WUE values in full-girdled (FG) leaf decreased by 72.1 % (Fig. 1b), 83.1 % (Fig. 1d), 65.7 % (Fig. 1f), and 69.5 % (Fig. 1h), respectively.

Photosynthetic end-product characteristics after phloem were girdled

In our study, on the 1st day, compared with CK, starch content in semi-girdled (SG) leaf increased by 1.3 % (Fig. 2a) and in semi-girdled (SG) root decreased by 0.9 % (Fig. 2b); starch content in full-girdled (FG) leaf increased by 1.7 % (Fig. 2a) and in full-girdled (FG) root increased by 1.4 % (Fig. 2b). On the 30th day, compared with CK, starch content in semi-girdled (SG) leaf increased by 35.3 % (Fig. 2a) and in semi-girdled (SG) root decreased by 25.5 % (Fig. 2b); starch content in full-girdled (FG) leaf increased by 46.0 % (Fig. 2a) and in full-girdled (FG) root decreased by 39.0 % (Fig. 2b).

On the 1st day, compared with CK, soluble sugar content in semi-girdled (SG) leaf decreased by 0.3 % (Fig. 3a) and in semi-girdled (SG) root increased by 2.8 % (Fig. 3b); soluble sugar content in full-girdled (FG) leaf increased by 0.3 % (Fig. 3a) and in full-girdled (FG) root decreased by 16.2 % (Fig. 3b). On the 30th day, compared with CK, soluble sugar content in semi-girdled (SG) leaf increased by 8.8 % (Fig. 3a) and in semi-girdled (SG) root decreased by 16.2 % (Fig. 3b); soluble sugar content in full-girdled (FG) leaf increased by 22.6 % (Fig. 3a) and in full-girdled (FG) root decreased by 21.0 % (Fig. 3b).

ABA characteristics after phloem were girdled

In our experiment, only a slight increase was found in semi-girdled (SG) leaf on the 1st day compared with CK (Fig. 4), whereas in full-girdled (FG) leaf, compared with CK, ABA content increased by 25.7 % on the 1st day (Fig. 4). On the 30th day, compared with CK, ABA content in semi-girdled (SG) leaf increased by 119.8 % (Fig. 4), whereas ABA content in full-girdled (FG) leaf increased by 213.3 % (Fig. 4).

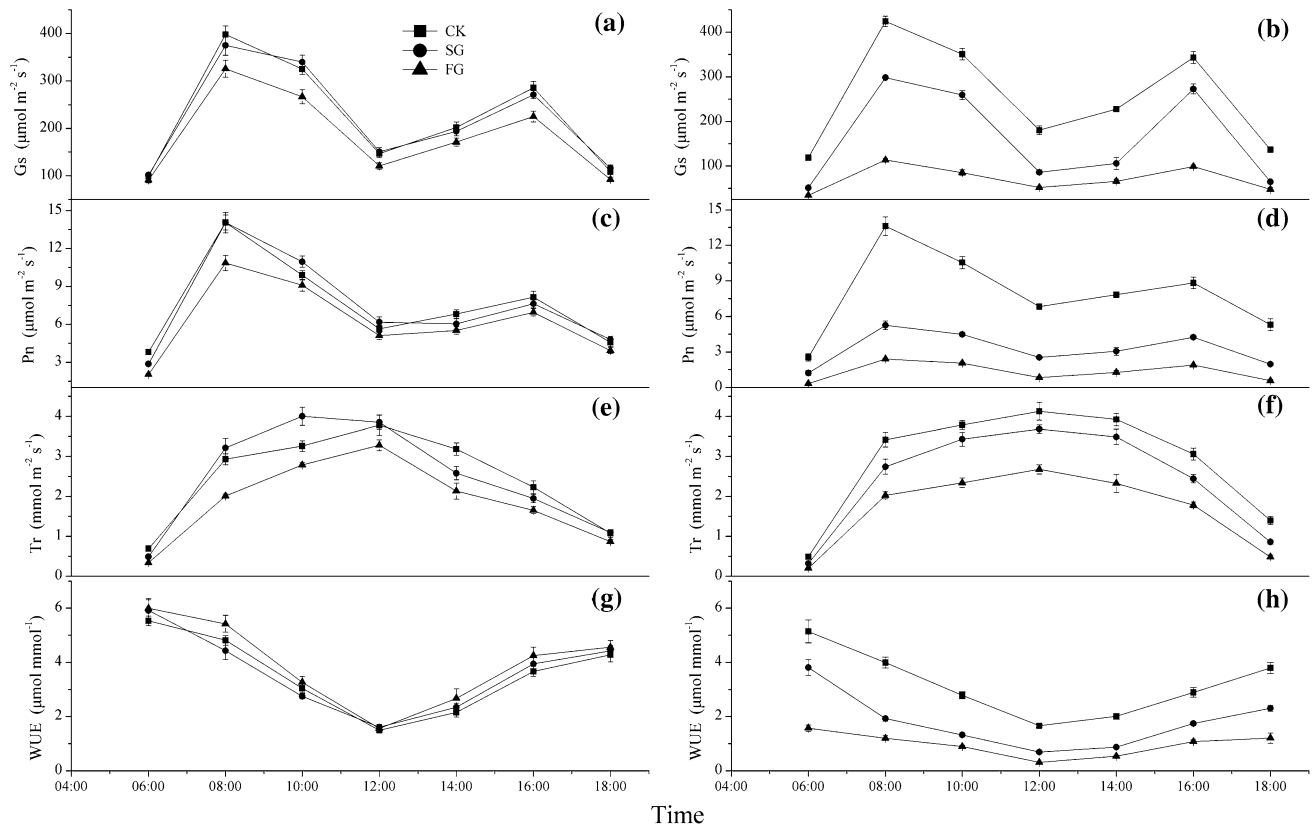


Fig. 1 Changes of stomatal conductance (Gs), net photosynthesis rate (Pn), transpiration rate (Tr), and water use efficiency (WUE) of *Alhagi sparsifolia* after girdling. **a** Gs, 1 day after girdling; **b** Gs, 30 days after girdling; **c** Pn, 1 day after girdling; **d** Pn, 30 days after girdling; **e** Tr, 1 day after girdling; **f** Tr, 30 days after girdling; **g** WUE, 1 day after girdling; **h** WUE, 30 days after girdling. Mean values \pm SE from 4 replicates

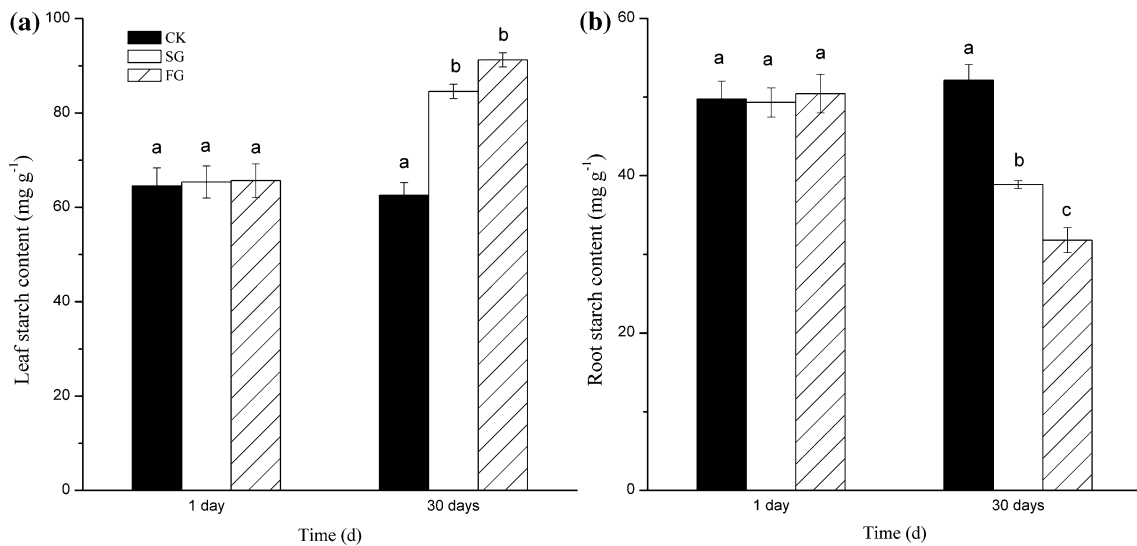


Fig. 2 Changes of starch in control (CK), semi-girdled (SG), full-girdled (FG) leaves of *Alhagi sparsifolia*, 1 and 30 days after girdling. **a** Leaf starch content; **b** root starch content. Different letters indicate a significant difference ($P < 0.05$). Mean values \pm SE from 5 replicates

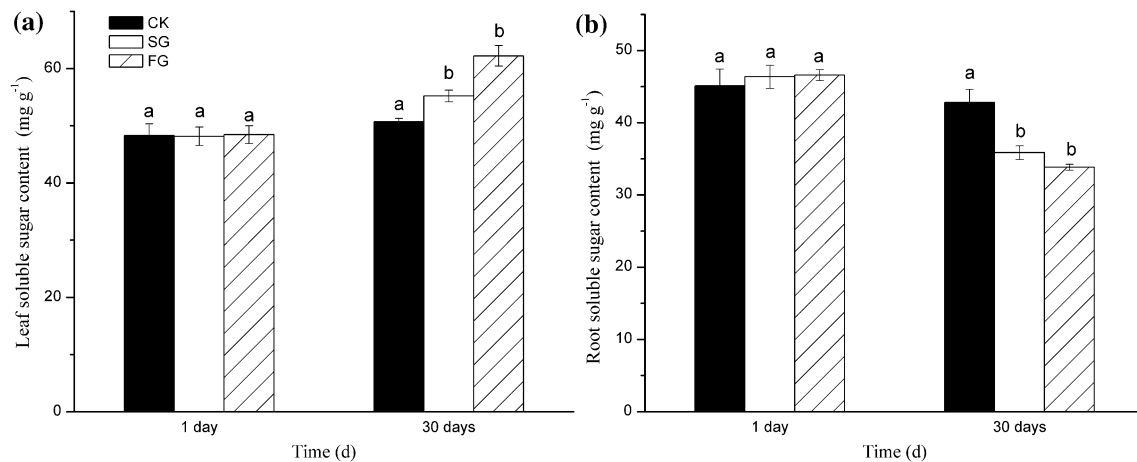


Fig. 3 Changes of soluble sugar in control (CK), semi-girdled (SG), full-girdled (FG) leaves of *Alhagi sparsifolia*, 1 and 30 days after girdling. **a** Leaf soluble sugar content; **b** root soluble sugar content. Different letters indicate a significant difference ($P < 0.05$). Mean values \pm SE from 5 replicates

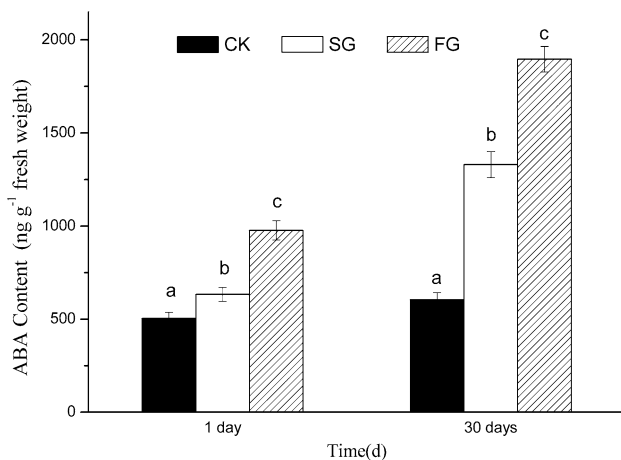


Fig. 4 Changes of ABA in control (CK), semi-girdled (SG), full-girdled (FG) leaves of *Alhagi sparsifolia*, 1 and 30 days after girdling. Different letters indicate a significant difference ($P < 0.05$). Mean values \pm SE from 5 replicates

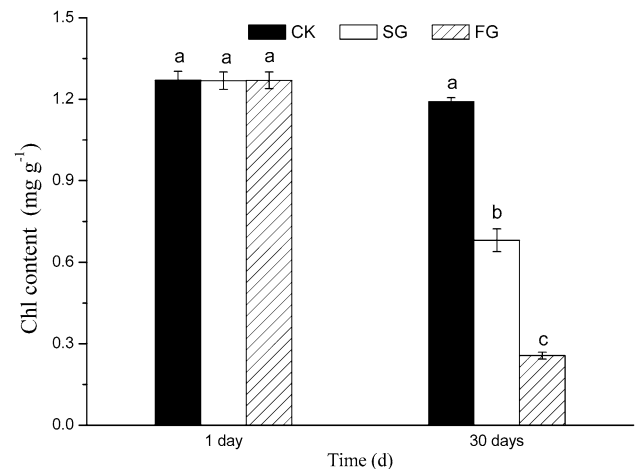


Fig. 5 Changes of chlorophyll (Chl) content in control (CK), semi-girdled (SG), full-girdled (FG) leaves of *Alhagi sparsifolia*, 1 and 30 days after girdling. Different letters indicate a significant difference ($P < 0.05$). Mean values \pm SE from 5 replicates

Chlorophyll content and chlorophyll fluorescence characteristics after phloem were girdled

In the present study, Chl (Chl $a + b$) content in semi-girdled (SG) leaf decreased by 1.5 % (Fig. 5) compared with CK on the 1st day, and decreased by 42.9 % (Fig. 5) on the 30th day. Compared with CK, Chl content in semi-girdled (SG) leaf decreased by 1.3 % (Fig. 5) on the 1st day, and the decline was 78.5 % (Fig. 5) on the 30th day.

Chlorophyll fluorescence parameters can reflect a number of important regulatory processes inside the photosynthetic apparatus. The present study showed that the maximum photochemical efficiency from photosystem II

(F_v/F_m) in semi-girdled (SG) leaf decreased by 2.4 % compared with CK on the 1st day (Fig. 6), and on the 30th day, F_v/F_m in semi-girdled (SG) leaf decreased by 7.5 % (Fig. 6). Compared with CK, F_v/F_m in full-girdled (FG) leaf decreased by 1.1 % (Fig. 6) on the 1st day, and decreased by 53.8 % (Fig. 6) on the 30th day.

Leaf water potential characteristics after phloem were girdled

Water potential is one of the most important indicators of water status. In the present study, compared with CK, Ψ_p in semi-girdled (SG) leaf decreased by 9.1 % on the 1st day

(Fig. 7a), and decreased by 17.7 % on the 30th day (Fig. 7b); Ψ_P in full-girdled (FG) leaf decreased by 3.3 % on the 1st day (Fig. 7a), and decreased by 39.3 % on the

30th day (Fig. 7b). Compared with CK, Ψ_M in semi-girdled (SG) leaf increased by 6.4 % on the 1st day (Fig. 7c), and decreased by 7.8 % on the 30th day (Fig. 7d); Ψ_M in full-girdled (FG) leaf increased by 12.8 % on the 1st day (Fig. 7c), and decreased by 39.3 % on the 30th day (Fig. 7d).

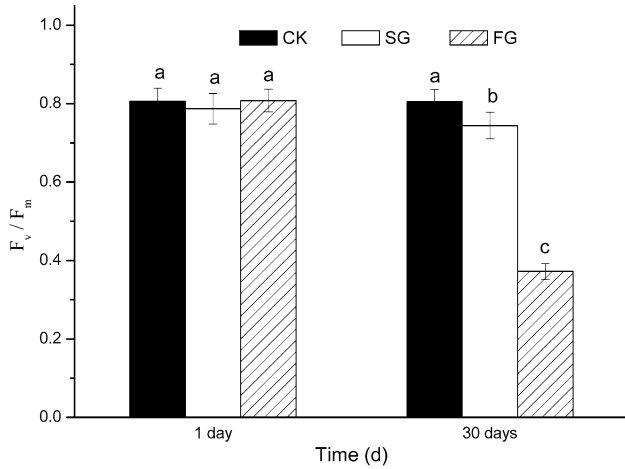


Fig. 6 Changes of chlorophylls fluorescence in control (CK), semi-girdled (SG), full-girdled (FG) leaves of *Alhagi sparsifolia*, 1 and 30 days after girdling. Different letters indicate a significant difference ($P < 0.05$). Mean values \pm SE from 5 replicates

Discussion

Photosynthesis is one of the most frequently studied indicators in girdling experiments. Our aim was not only to observe the change of Pn in *A. sparsifolia* leaf after the phloem was girdled, but also to explore the factor which led to the change of Pn. Previous studies showed that girdling-induced change of Pn may depend on species (Goldschmidt and Huber 1992); in a few species, such as spinach, Pn did not change in the girdled leaf. However, in many species, such as *Malus sylvestris* (L.) Mill. var. domestica (Borkh.) (Zhou and Quebedeaux 2003), *Vitis vinifera* L. (Roper and Williams 1989), *Prunus persica* var. nucipersica (Suckow) C.K. Schneid (Di Vaio et al. 2001), and *Mangifera indica* (Lu and Chacko 1998), girdling led

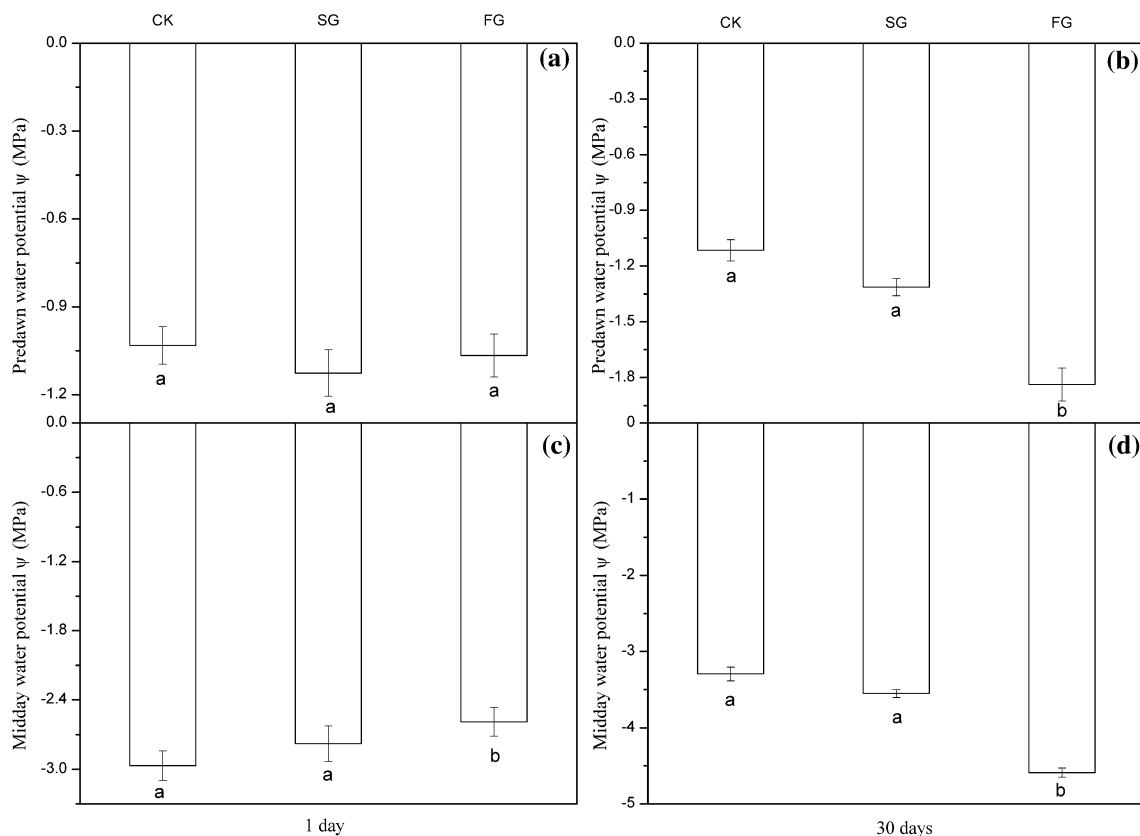


Fig. 7 Changes of water potential in control, semi-girdled, full-girdled leaves of *Alhagi sparsifolia*, 1 and 30 days after girdling. **a** predawn water potential, 1 day after girdling; **b** predawn water potential, 30 days after girdling; **c** midday water potential, 1 day after girdling; **d** midday water potential, 30 days after girdling. Different letters indicate a significant difference ($P < 0.05$). Mean values \pm SE from 5 replicates

to the decline of Pn. The present study showed that in *A. sparsifolia*, girdling decreased Pn (Fig. 1c, d). In the treatment of SG, decline of Pn appeared on the 30th day (Fig. 1d), whereas in the treatments of FG, both the 1st day (Fig. 1c) and the 30th day (Fig. 1d) showed a Pn decline.

It is well known that Pn is tightly connected to Gs (H eroult et al. 2013). We measured Gs in order to study whether Gs is the factor which leads to Pn decline in girdled leaf. Previous study showed that girdling led to the reduction of Gs (Setter et al. 1980; Ueda et al. 2014; Williams et al. 2000), and this was the reason for Pn decline in girdled leaf (Setter et al. 1980). The present study also showed that girdling can significantly decrease Gs on both the 1st day (Fig. 1a) and the 30th day (Fig. 1b). As we did not find any change of starch (Fig. 2) and soluble sugar (Fig. 3) contents on the 1st day, we attribute the Pn decline to the reduction of Gs, but not the end-product feedback inhibition on the 1st day. On the 30th day, the reduction of Gs is also an important factor for Pn decline; however, it cannot be the sole factor. One of the reasons for this is that the decline of Pn (Fig. 1d) was much greater than the decline of Gs (Fig. 1b) on the 30th day. An additional reason will be discussed later.

Most studies in the literature have shown that ABA content is tightly connected to the change of Gs (Speirs et al. 2013). In order to study the reason for the change of ABA in girdled leaf, we measured ABA content. Setter et al. (1980) found that ABA accumulated in girdled leaf, which subsequently led to the decline of Gs, and thus decline of Pn; this result was also found in our study. ABA increased in full-girdled (FG) leaf on the 1st day (Fig. 4), and this may be the key reason for Gs and Pn decline. On the 30th day, both the SG and FG treatments showed an ABA increase in *A. sparsifolia* leaf (Fig. 4). This is also an important reason for Gs and Pn decline in girdled leaf, although it may not be the only reason.

As is well known, stomata are the channels for the exchange of CO₂ and water between plants and the atmosphere (Rebetzke et al. 2013). Gs has a linear relationship with Tr, in that the reduction of Gs was always associated with the decrease of Tr (Medlyn et al. 2011; Bouranis et al. 2014a, b). This was also demonstrated in our study because the patterns of changes of Tr (Fig. 1e, f) and Gs (Fig. 1a, b) in the present study were almost identical. Specifically, each treatment (SG, FG) showed a Tr decline compared with CK on the 30th day (Fig. 1f), whereas only the treatment of FG showed a Tr decline on the 1st day (Fig. 1e), and this change was the same as that in Gs (Fig. 1a, b). The change of Tr was consistent with the result that girdling led to decreasing of Gs, which subsequently resulted in reduction of Tr (Williams et al. 2000).

WUE can be physiologically defined as the ratio between photosynthesis and transpiration (Blum 2005).

Reduced Gs can induce the increasing of WUE because when Gs is reduced, the decline of Tr is always greater than the decline of Pn (Blum 2005; Grant et al. 2012). This result was also found in our study. Specifically, WUE increased in the full-girdled (FG) leaf on the 1st day (Fig. 1g), which is consistent with the Gs decline on the 1st day (Fig. 1a). Additional evidence that Gs is not the only factor for Pn decline on the 30th day is the WUE reduction on the 30th day (Fig. 1h). If the Gs decline is the only reason for Pn decline, then WUE should increase just as it does on the 1st day (Fig. 1g); however, this was not found on the 30th day (Fig. 1h).

Pn can be affected by high carbohydrate levels, which is called end-product feedback inhibition (Layne and Flore 1995; Ainsworth and Bush 2011; Nebauer et al. 2011; Nikinmaa et al. 2013). Many studies have shown that girdling-induced leaf carbohydrate accumulation is the reason for Pn decline (Paul and Foyer 2001; Paul and Pellny 2003; Nebauer et al. 2011). The present study demonstrated that Pn decline on the 1st day was not due to carbohydrate accumulation because neither starch content (Fig. 2a) nor soluble sugar content (Fig. 3a) increased on the 1st day. On the other hand, on the 30th day, both starch content (Fig. 2a) and soluble sugar content (Fig. 3a) showed an obvious increase; this is one of the reasons for Pn decline in the girdled leaf.

However, the carbohydrate-accumulation-induced Pn decline may not completely result from end-product feedback inhibition because previous study also showed that high levels of carbohydrate can lead to leaf senescence which is characterized by degradation of Chl and Pn (Parrott et al. 2005, 2007; Shi et al. 2014). We investigate the change of Chl during the study to discover the other factor in Pn decline during girdling. The present study showed that Chl content did not change on the 1st day (Fig. 5) and decreased on the 30th day (Fig. 5). Thus, Chl degradation may be another reason for Pn decline on the 30th day. From the degradation of Chl and Pn combined with the yellow color of leaf which we observed on the 30th day, it can be concluded that girdling (both SG and FG) resulted in leaf senescence on the 30th day. However, we do not know whether the leaf senescence was induced by a high level of carbohydrates or ABA; it is possible that both factors play their important roles in leaf senescence. Chl *a* fluorescence has been widely used to evaluate vegetation photosynthetic capacity (Zhang et al. 2014). To this end, we also measured F_v/F_m , which is a key indicator of Chl *a* fluorescence. Some literature studies showed that high carbohydrate level (induced by branch girdling) did not change F_v/F_m (Yan et al. 2011); however, in other studies, carbohydrate accumulation resulted in the decrease of F_v/F_m (Adams III et al. 2014). Interestingly, both types were found in our study, i.e., on the 1st day, girdling

showed no change of F_v/F_m (Fig. 6), while on the 30th day, significant decline was found in the girdled (including SG and FG) leaf (Fig. 6). Moreover, the decline of F_v/F_m on the 30th day was another factor for Pn decline.

It is well known that Ψ_{leaf} is tightly connected to Pn (Ackerson et al. 1977; Zhou et al. 2014). In our experiment, we also measured Ψ_{leaf} in order to study the relationship between Ψ_{leaf} and Pn in the girdling system. Previous study showed that Ψ_{leaf} had a strong relationship with Gs (Jarvis 1976). Williams et al. (2000) found that girdling-induced reduction of Gs, which resulted in less leaf transpiration and, therefore, a more favorable vine water status. On the other hand, there are also some studies that showed that girdling had no effect on water potential (Kaufmann 1970). Interestingly, the present study not only showed a similar result to Williams et al. (2000), but also found another result that was different from the previous studies. Specifically, on the 1st day, girdling (FG) led to the reduction of Gs (Fig. 1a), which resulted in decline of Tr (Fig. 1e) and therefore resulted in the increasing of Ψ_M (Fig. 7c). However, on the 30th day, girdling (both SG and FG) induced declines of Ψ_P (Fig. 7b) and Ψ_M (Fig. 7d). One of the reasons for Ψ_{leaf} decline was girdling-induced leaf carbohydrate accumulation (Figs. 2a, 3a), which resulted in a lower osmotic potential in leaf. Another reason for Ψ_{leaf} decline was girdling-induced reduction of root carbohydrate (Figs. 2b, 3b), which resulted in the decreased respiration rates of root and water uptake. This result was consistent with previous study that demonstrated that girdling decreased the respiration rate of fine roots (Fumuro 1998), and thus decreased water uptake from soil to leaves (Ueda et al. 2014). The decline of Ψ_{leaf} may be also another reason for Pn decline on the 30th day.

In addition, our study found that the treatment of SG showed no significant change on the 1st day in Gs (Fig. 1a), Pn (Fig. 1c), Tr (Fig. 1e), WUE (Fig. 1g), starch content (Fig. 2), soluble sugar content (Fig. 3), ABA content (Fig. 4), Chl content (Fig. 5), F_v/F_m (Fig. 6), and Ψ_{leaf} (Fig. 7a, c). However, these parameters changed significantly on the 30th day, and the trend was consistent with that of FG. Therefore, in a short time (1 day), SG showed no significant change compared with CK, whereas over a long time (30 days), the trend of change of these parameters in SG was the same as FG, although the degree was less. This result suggested that a portion (such as half) of the phloem cannot undertake the transport work which is conducted by the whole phloem. In addition, after a period of treatment, SG would show a similar effect to that of FG.

In summary, full-girdling (FG) not only decreased Pn on the 1st day, but also decreased Pn on the 30th day (López et al. 2015). On the 1st day, the girdling (FG)-induced ABA accumulation in leaf which subsequently resulted in Gs decline was the key reason for reduction of Pn. On the

30th day, Pn in the girdled leaf decreased much more than that on the 1st day, and there are numerous factors that contributed to the decline of Pn. ABA-induced Gs decline was also an important reason for the reduction of Pn and girdling-induced carbohydrate accumulation, and thus led to end-product feedback inhibition. Moreover, girdling-induced leaf senescence subsequently resulted in the degradation of Chl and Pn. The decline of maximum photochemical efficiency from PSII (F_v/F_m) resulted from phloem girdling. In addition, the deterioration of leaf water status (showed as Ψ_{leaf}) caused by phloem girdling also played an important role in the decline of Pn. Semi-girdling only decreased Pn on the 30th day, and the reasons for Pn decline on the 30th day in semi-girdled (SG) leaf were the same as FG. Plant cannot sustain a regular physiological metabolism without a portion (e.g., half) of the phloem. The present study suggested that the mechanism for Pn decline induced by phloem girdling was simple at first, i.e., Gs restriction induced by accumulation of ABA. However, this mechanism became rather complicated after a few days. At that point in time, Gs, carbohydrate feedback, Chl content, PSII status, and water status played their respective important roles. To attribute the reason for Pn decline to only one of these factors would be incorrect. Since the present study did not explore the real reason for deterioration of leaf water status, other parameters, such as plant hydraulic properties, should be measured in future study. In addition, whether leaf senescence is induced by accumulation of ABA or carbohydrate, or both, should be elucidated in further study.

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