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Effect of Foliar Ferrous Sulphate Application on Alfalfa (*Medicago sativa* L.) Leaf Fe Content, Photosynthetic Capacity and Yield

Li Chun-xia*(**), Cai Tie*(**), Guo Hai-yan*(**), Han Qing-fang*(**)†, Zong Yu-zheng*(**), Ding Rui-xia*(**), Jia Zhi-kuan*(**), Kamran Muhammad*(**) and Melville J. L.*(**)

*The Chinese Institute of Water-saving Agriculture, Northwest A&F University, Yangling 712100, Shaanxi, China

**Key Laboratory of Crop Physi-ecology and Tillage Science in Northwestern Loess Plateau, Ministry of Agriculture, Northwest A&F University, Yangling 712100, Shaanxi, China

†Corresponding author: Han Qing-fang

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ABSTRACT

Alfalfa (*Medicago sativa* L.) is a vitally important forage crop. Due to its perennial characteristics, a gradual decline of soil nutrients (especially micronutrients) leads to a reduction of grass quality. Numerous studies have reported a direct relationship between ferrite levels and physiological function of plants. The present study investigates the impact of ferrite levels on alfalfa and tests five ferrous sulphate concentrations and three spraying frequencies. To investigate the influence of iron on alfalfa photosynthesis and iron uptake, we measured photosynthetic capacity and hay yield of alfalfa on iron deficient soil. We furthermore investigated the effects of iron on alfalfa yield and explored its mechanism of action. The results revealed that the wiron fertilizer sprayed at the appropriate concentration (0.6%-0.8%) increased the F_v/F_m and F_v/F_0 of the photosystem II complex. Spraying furthermore increased electron transport rate and photochemical quantum yield, reduced non-irradiative energy dissipation, and hence increased the photosynthetic rate and hay yield of alfalfa. Two applications of 0.6% ferrous sulphate spray increased the chlorophyll concentration of alfalfa by 26%. Three applications increased hay yield by 22% compared with check. Two applications of 0.8% spray increased photosynthetic rate by 42% and three applications increased the iron content of leaves by 117%. These results indicated that applying Fe (foliar-applied) from seedling to squaring stage improved the photosynthetic capacity and yield of alfalfa.

INTRODUCTION

Alfalfa (*Medicago sativa.* L.) orlucerne, is an important forage legume throughout the world and is known as “the king of forages” (Li et al. 2014). Alfalfa is often considered to be a speciality fodder crop, requiring careful management to achieve high levels of production and persistence. The grass production and quality will decline steadily after continuous planting without fertilization in consideration of soil nutrient depletion. Currently, this is the key problem of alfalfa production. This is closely associated with photosynthetic capacity and affected both by genetic factors and external environmental conditions, with a special emphasis on fertilization and water conditions. The amount of soil trace elements is closely related to the production and the quality of alfalfa, and iron is the main trace element for alfalfa production. The northwest arid and semi-arid areas of China are part of China’s iron deficient soil region. Thus, iron fertilization is important to enable good yield in the main planting area of alfalfa in the northwestern region. As a perennial plant, alfalfa absorbs high levels of soil N, P and K (Razmioo & Henderlong 1997), but cannot always obtain sufficient supplies of micronutrients from calcareous soils. Recent research has focused on maximizing yield and the efficiency of N, P and K utilization (Sions & Grant 1995, Raun & Johnson 1999, Huo & Wang 2013). Micro-element nutrient research has focused mainly on B, Mo and Zn (Zong et al. 2010, Liu et al. 2004), and a variety of microelement fertilizer applications (Liu et al. 2008, Hu et al. 2008). However, there has been little research on iron nutrition in alfalfa.

Iron is an essential element for plants because it is crucial for photosynthesis and it is involved in many physiological actions such as chlorophyll synthesis, redox reactions, and respiration. It affects many physiological processes including N and carbohydrate metabolism and protoplasm properties (Su & Miller 1961, Tong et al. 1986). Of the plant leaf iron, 60% is bound to the thylakoid membrane of chloroplasts and 20% can be found in the chloroplast stroma (Terry & Low 1982). Iron deficiency reduces the amount of all membrane components, including electron carriers of the photosynthetic electron transport chain (Spiller & Terry 1980, Sandmann & Malkin 1983) and light-harvesting pigments (Terry & Abadia 1986). About 40% of the soils in the world are iron deficient, leading to a potential worldwide malnutrition problem. Iron chlorosis is one of the leading factors limiting crop productivity (Terry & Abadia 1986). Researchers exhibited great interest to understand the factors and processes that lead to iron chlorosis in plants (Spiller & Terry 1980, Terry & Abadia 1986). Nevertheless, the causes of iron chlorosis are not completely clear, and the available means for treating iron chlorosis are not yet satisfactory. Soil applications of iron are generally ineffective due to the fact that inorganic iron sources that are applied to soils rapidly form compounds. This makes the iron unavailable to plants (Mortvedt 1991). Numerous studies have shown that foliar sprays can effectively correct iron chlorosis (Kadman & Gazit 1984, Raese et al. 1986). Alfalfa yield is predominantly determined by photosynthetic activity. Studies on photosynthesis in alfalfa have mainly concentrated on changes of CO_2 concentration (Sanz-Sáez et al. 2010, Aranjuelo et al. 2005, Sanz-Sáez et al. 2013), abiotic stresses such as salt and drought stress (Fan et al. 2013, Han et al. 2007, Kou et al. 2013), P and water availability (Sun & Wang 2012), and B availability (Zong et al. 2010).

The effects of foliar-applied ferrous sulphate on photosynthesis for alfalfa growth are currently not clearly understood and the effect of iron on photosynthesis needs further investigation. In this report, we determined the effects of iron spraying at different concentrations and different frequencies on alfalfa (*Medicago sativa* L.). We measure leaf iron content, photosynthetic capacity and yield, in order to determine the action of iron on alfalfa photosynthesis and the optimal rate and timing of iron fertilization.

MATERIALS AND METHODS

Experiment Location Profile

We conducted the field experiment on the No. 1 experiment farm of the Northwest A &F University (NWAFU, Yangling, Shaanxi, China, 34°21' N and 108°10' E). This farm is located in a semiarid climate with an average annual light regime of 2,150 hours, an average annual temperature of 12-14°C, and a low temperature extreme between -15 and -21°C. The soil is Lou soil (Earth-cumuli-Orthic Anthrosols) with an organic matter content of 15.9 g kg^{-1}, total N content of 1.12 g kg^{-1}, and available iron content in 0-20 cm soil layer of 3.67 mg kg^{-1} (compared to a critical iron concentration standard of 5 mg kg^{-1}). No fertilizer was applied during the growth period of alfalfa.

Materials

Alfalfa, cv Algonquin (introduced from Canada) has been growing for five years, which was sowed at 15 kg seeds ha^{-1}. During the whole growing period, dryland farming practices were employed and no agricultural pesticides were applied. Hand weeding was done as appropriate.

Experimental Design

The experimental design was a randomized block and we used six $FeSO_4 \cdot 7H_2O$ fertilizer concentrations in w/w (C0: 0.0, C1: 0.2, C_2: 0.4; C3: 0.6; C4: 0.8; C5: 1.0 g $FeSO_4 \cdot 7H_2O$ 100 mL^{-1}). Iron as foliar fertilizer sprayed at the six rates prior to the first cutting with spraying frequencies of each concentration as one (S1), two (S2) or three (S3) times. Stages of application were: S1 at the seedling establishment stage; S2 at both the seedling establishments and the branching stages; and S3 at the seedling establishment, the branching and the squaring stages. Each treatment was replicated three times and plots were 3 m × 3 m (9 m^2). Iron fertilizer volume for each plot was 1 L. Thus, six concentrations of iron were applied in each of three application regimes.

Methods of Measurement

The photosynthetic activities of alfalfa treated with the different amounts of Fe were simultaneously measured three days after alfalfa squaring (between the sixth and the ninth day after the third Fe fertilizer spray).

Gas Exchange Parameters

We measured photosynthetic rate (P_n), intercellular carbon dioxide concentration (C_i) and stomatal conductance (C_d) of the middle leaflets of the second fully expanded leaves of alfalfa branches from the top. For measurements, we used a Li-6400 portable photosynthesis system (LI-COR, USA) and conducted all measurements between 9:00 h and 11:00 h on a sunny morning. The LED actinic light was set at 600 µmol m^{-2} s^{-1}, flow velocity of 500 $mmols^{-1}$, external CO_2 concentration of about 380 µmol CO_2 mol^{-1}, leaf temperature between 23°C and 24°C, relative humidity (RH) of about 14.4-19.4%, and each replicated treatment was measured five times.

Chlorophyll Concentration

Two tenths of the middle leaflets of the second fully expanded leaves of alfalfa branches (from top) were cut for further analysis. We extracted total chlorophyll content using a mixture of acetone, ethanol and water (V/V: 4.5:4.5:1) and centrifuged at 1600 rpm for 10 min. The extraction was done in the dark with all samples kept on ice. The extraction volume was 10 mL. The absorbance of the supernatant was measured using a dual-wavelength/double beam (UV-3000) spectrophotometer. Chlorophyll *a* and *b* were measured at 663.2 and 646.8 nm, respectively. The results were

Table 1: The method of different ferrous sulfate level in this experiment.

Concentrations	Spraying frequency		
	One time (S1)	Two times (S2)	Three times (S3)
C0 (0%)	C0 S1	C0 S2	C0 S3
C1 (0.2%)	C1 S1	C1 S2	C1 S3
C2 (0.4%)	C2 S1	C2 S2	C2 S3
C3 (0.6%)	C3 S1	C3 S2	C3 S3
C4 (0.8%)	C4 S1	C4 S2	C4 S3
C5 (1.0%)	C5 S1	C5 S2	C5 S3

analysed based on the equations of Wellburn (1994) and then normalized to leaf dry weight. The chlorophyll concentration was calculated by Lichtenthaler's formula (Lichtenthaler &Wellburn 1983).

Chlorophyll Fluorescence Parameters

The chlorophyll fluorescence parameters of the middle leaflets of the second fully expanded leaves of alfalfa branches from top were measured with the PAM-2100 portable chlorophyll fluorometer (Heinz Walz GmbH company, Effeltrich, Germany) and with five replicates. Before the measurements, the leaflets were set under dark adaptation for two hours. The measurements were done at 23-24°C. Plants were first adapted for 30 min in total darkness. The initial fluorescence (F0) was determined by the measuring beam of the PAM fluorometer. The maximal fluorescence (Fm) was determined at the beginning of each measurement using a saturating pulse (2000 µmol m^{-2} s^{-1}). Actinic light was obtained from an LED (70 µmol m^{-2} s^{-1}). The variable fluorescence (Fv) was taken from the formula, Fv=Fm-F0 and the following fluorescence quenching parameters were used:

NPQ = (Fm-Fm')/Fm' = Fm/Fm'-1;

Photochemical quantum yield, E-Yield = (Fm'-Ft)/Fm'

Fm and F0 were measured after dark-adaptation, while Fm' was measured on an illuminated sample. Ft is the fluorescence yield at a given time (Horton & Bowyer 1990, Schreiber & Bilger 1987).

Hay Yield and Active Fe Concentration

At the early flowering stage, a 1 m × 1 m (1 m^2) sample area of alfalfa was chosen in each replication of each treatment and alfalfa was cut close to the ground as a fresh sample. Fresh replicate samples for each treatment were weighed separately and then mixed well. Three 100 g sub-samples (fresh weight) were taken from the mixture and these were brought to the laboratory. Samples were then deactivated at 105°C for 15 minutes, dried at 65°C to constant weight, cooled and weighed. The dry percentage weight and the hay yield (kg ha^{-1}) were calculated. Dried leaves from each treatment were triturated and incinerated, before extracting the active Fe using 0.1 mol L^{-1} HCl. Each 1.0 g dried sample was extracted in 10 mL HCl for 5 h with sequential oscillation (Pierson & Clark 1984, Takkar & Kaur 1984), before determining the active Fe using an atomic absorption spectrophotometer (Pye, UK). The active Fe concentration (mg kg^{-1}) = (Fe mass concentration, ¼ g mL^{-1}) × (the solution to be tested, mL) / the mass (g) of the dried sample.

Statistical Analysis

We performed Duncan's multiple range test and regression analysis, using SAS 8.0 (SAS Institute Inc., Cary, North Carolina, USA).

Data were analysed by using Statistical Analysis System 8.0 software (SAS Institute Inc., Cary, North Carolina, USA). Results were analysed using one way analysis of variance (ANOVA) followed by Duncan's multiple range test. Significance was determined by different letters are significantly difference at $P<0.05$ probability levels, respectively, which represent multiple comparisons among different concentrates in the same spraying time. The results are expressed as mean values and standard deviation (SD) and all assays were carried out in replicates (three sets of each analysis).

RESULTS AND ANALYSIS

Active Fe Concentration of Alfalfa Leaves

The active iron concentration of alfalfa leaves rose significantly ($P< 0.05$) with increasing concentration of iron fertilization (Fig. 1). With one (S1) and two (S2) iron fertilizer treatments, the active iron concentration of alfalfa leaves significantly increased as iron fertilizer concentration increased ($P< 0.05$) with a peak at C5 (S1 and S2). With three iron fertilizer sprays (S3), the active iron concentration increased from C0-C4 and peaked at C4 (367 mg kg^{-1}). However, the active iron concentration declined at C5, although it still remained much higher than that of the control. The average active iron concentration in alfalfa leaves at lower concentrations (C1-C4) with different spraying frequencies varied in the order S3>S2>S1, and all were higher than the control (P <0.05).

Chlorophyll Content of Alfalfa Leaves

With one, two, and three iron fertilizer sprays, the chlorophyll content of alfalfa leaves significantly increased with increasing iron spray concentration and was followed by a decline (Fig. 2). The differences in chlorophyll contents with different frequencies of iron fertilizer spray application were not significant (C1, C2 and C3). S by C interactions were also not significant (P < 0.05). A single bout of iron fertilizer spray resulted in peak chlorophyll content at C4 (increased by 28.1% compared to C0). Two bouts of spray resulted in a peak at C3 (reaching 3.4 mg g^{-1}), while three sprays resulted in a peak at C2. Iron fertilizer application above the concentration peaks ultimately led to a decrease in chlorophyll content. A regression analysis of chlorophyll concentrations on the active iron concentrations of alfalfa during the early flowering stage (Fig. 3) resulted in a significant linear relationship (r^2 = 0.4262, P < 0.01).

Photosynthetic Capacity of Alfalfa

Gas exchange parameters of alfalfa: With each of the three spray frequencies, the photosynthetic rate (P_n) of alfalfa led to an initial increase before declining with increasing iron fertilizer concentration (with S1 as exception; Fig. 4a). With the exception of C5S3, iron application resulted in a significantly higher P_n compared to the control concentration (C0) (P < 0.05), although we did not detect significant variation in P_n with different spraying frequencies (Fig. 4a). The S by C interaction was not significant (P < 0.05). P_n of alfalfa peaked at C3 with a single spray (increased by 35.53% compared to the control), at C4 with two sprays (increased by 41.7% compared to the control), and at C2 with three sprays (increased by 32.6% compared to the control).

With different spraying frequencies, the stomatal conductance (C_d) of alfalfa showed an initial increase before reaching a plateau (S1) or even decreasing with increasing iron concentration (S2 and S3; Fig. 4b). We found a similar trend to that for P_n. We did not detect significant differences for S by C interactions or for C_d at different spraying frequencies (P < 0.05).

Intercellular carbon dioxide (C_i) provides a measure for the dark reaction substrate during leaf photosynthesis. With each of the different spraying frequencies (S1, S2 and S3), the C_i of alfalfa increased initially with increasing frequency (C1), before declining to well below the control treatment (Fig. 4c). With the exception of the C1 level, compared to the control concentration, the iron C_i concentration decreased with all iron treatment concentrations to a variable extent. This result suggests that iron fertilizer sprays could indirectly raise the carbon dioxide assimilation efficiency. The C_i values were not significantly affected at different spraying frequencies and we did not detect a significant S by C interaction.

Chlorophyll Fluorescence Parameters

A frequently used standard for the occurrence of photo-in-

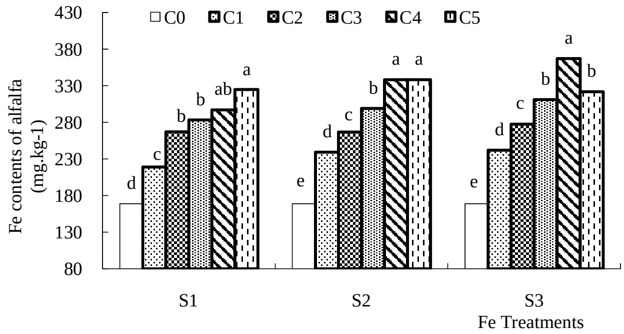

Fig. 1: Effect of three frequencies applications and six Fe concentrations on Fe content of alfalfa leaves.

Notes: Values followed by different letters are significantly difference at P<0.05 probability levels, respectively, which represent multiple comparisons among diferent concentrats in the same spraying time.

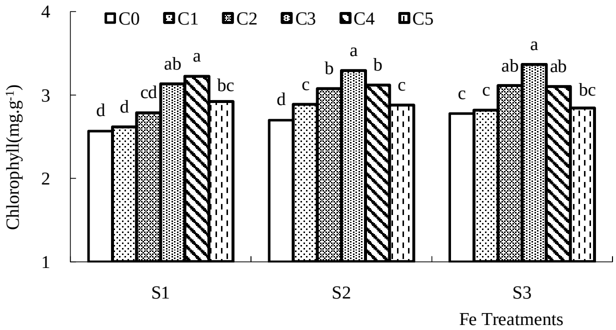

Fig. 2: Effect of three frequencies applications and six Fe concentrations on chlorophyll content of alfalfa leaves.

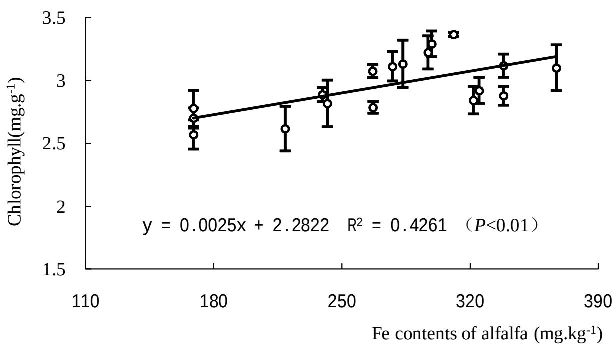

Fig. 3 Relationships between active Fe content and chlorophyll (±SD) of alfalfa leaves

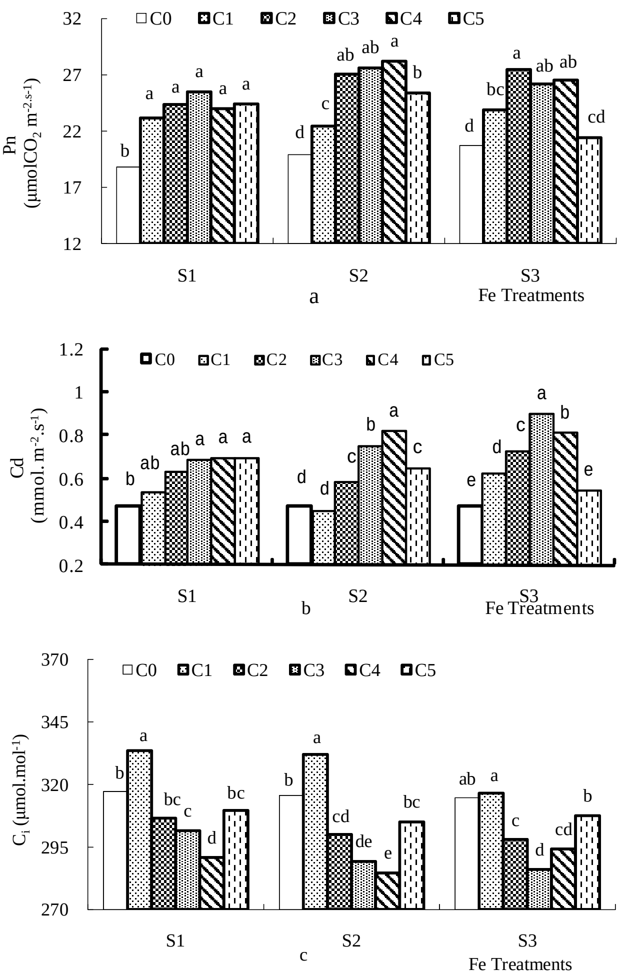

Fig. 4: Effect of three frequencies applications and six Fe concentrations on photosynthetic rates (P_n, a), stomatal conductance (C_d, b) and intercellular carbon dioxide (C_i, c) of alfalfa leaves.

hibition of photosynthesis is the decrease in maximum light energy conversion efficiency by the photosystem II reaction center (F_v/F_m). The potential chemical activity of photosystem II is F_v/F_0. The F_v/F_m and F_v/F_0 showed an identical response with the three spraying frequencies and the five iron concentrations (Fig. 5a and 5b). We measured an initial increase followed by a decline with increasing iron fertilizer concentrations (Fig. 5a and b). The F_v/F_m and F_v/F_0 did not differ significantly ($P < 0.01$) with the three spraying frequencies and the S by C interaction was nonsignificant.

With each spraying frequency, total photochemical quantum yield (E-Yield) of photosystem II increased initially and was followed by a decline with increasing Fe fertilizer concentration. E-Yield peaked at C2 and was significantly higher compared to that for the C0 and C1 iron fertilizer concentrations ($P < 0.01$) (Fig. 5c and d). With S1 and S2 spraying frequencies, the photosynthetic electron transport rate (ETR) of alfalfa peaked at C4 and was significantly higher compared to that of C0, C1, C2, and C3 iron treatments ($P < 0.01$). For S3 spraying frequency, the photosynthetic ETR of alfalfa peaked at C3 (reaching 113.23 μmol m^{-2} s^{-1}), which was significantly higher compared to all other treatments ($P < 0.01$). No differences were observed for the different spraying frequencies ($P < 0.01$), but the S×C interaction was significant ($P < 0.01$).

The non-photochemical quenching coefficient (NPQ) of alfalfa indicates the amount of the photosystem II antenna pigment-absorbed light energy that cannot be used for photochemical electron transport and that dissipates in the form of heat (Gilmore & Yamamoto 1996). With each spraying frequency, the NPQ was higher for both the low iron fertilizer concentrations (C0 and C1) and the high iron fertilizer concentration (C5), and was lowest at C2 (S2 and S3) and C3 (S1). Differences were not significant among spraying frequencies ($P < 0.01$), but the S×C interactions were significant.

Regression analysis showed that there was a highly significant quadratic linear relationship between the leaf active iron concentration at the squaring stage and F_v/F_0 ($r^2 = 0.47$, $P < 0.01$), and also with alfalfa Pn ($r^2 = 0.62$, $P < 0.01$).

Alfalfa Yields

The yield response to iron fertilizer concentration varied depending on spraying frequency (Fig. 7). With S1, all yields were significantly higher compared to C0 treatment, whereas only the C4 yield was above the control for S2 ($P < 0.05$). With S3, yields increased up to C2 and C3 ($P < 0.05$), but declined below the control level (C0) at C5.

DISCUSSION

Iron fertilizer uptake and chlorophyll synthesis relative to iron fertilization rates: Soil iron primarily exists in insoluble forms (Loeppert & Hallmark 1985). Iron is transported to points of growth and expanding leaves mainly via the phloem subsequent to spray applications, whereas iron is immobile in lower and upper leaves (Stephan & Scholz 1993). Thus, the iron that is sprayed onto leaves is mobilized via the phloem (Robinson et al. 1997). We found that rapid alfalfa growth during seedling establishment in the spring (before the first cutting) requires comparatively large amounts of iron and new leaves were likely to suffer etiolation due to iron deficiency. Thus, a single spray application of iron fertilizer during this period promoted iron uptake and led to a significant increase in chlorophyll concentration. The iron concentration in alfalfa leaves increased with the iron fertilizer concentration.

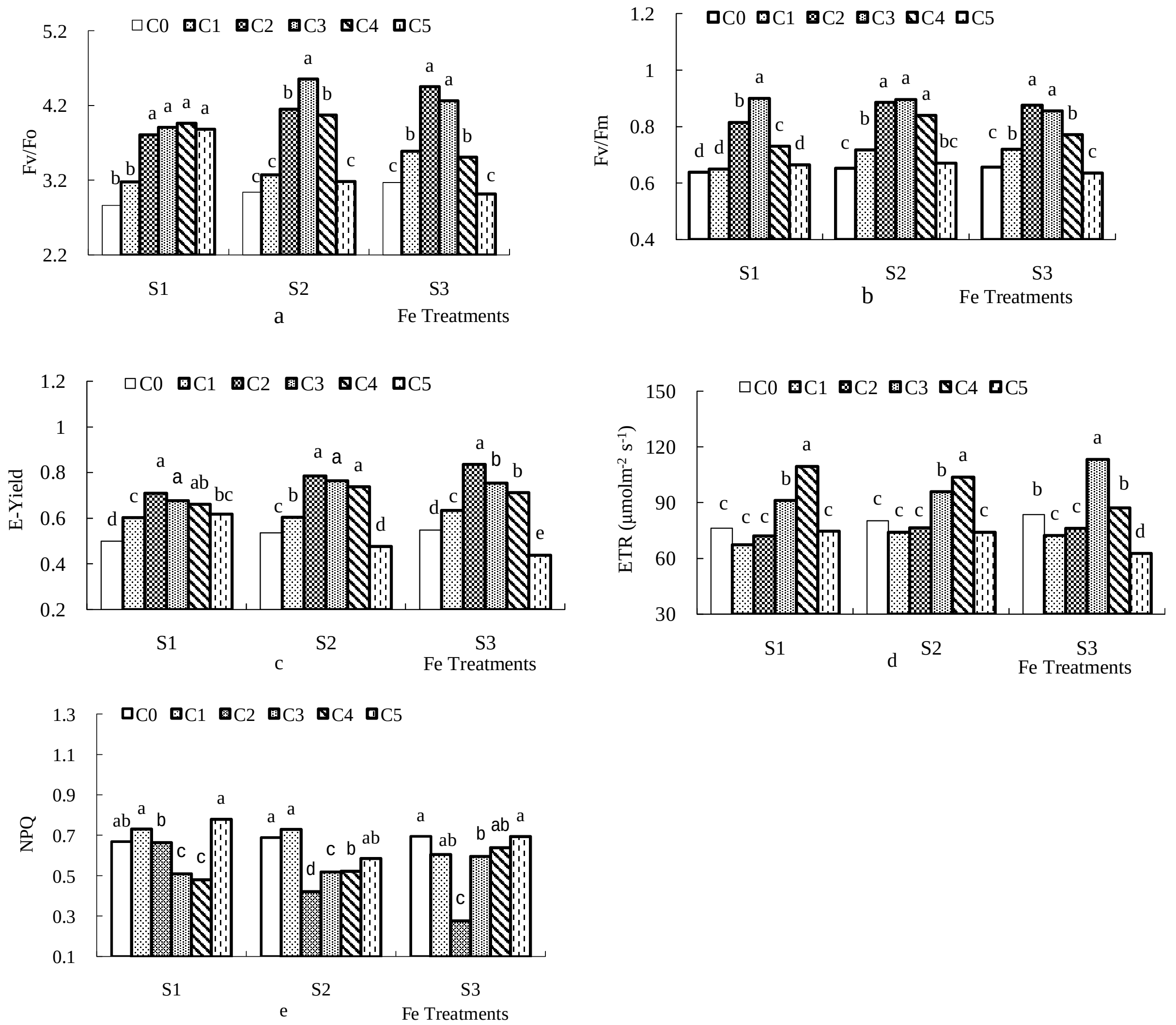

Fig. 5: Effect of three frequencies application and six Fe concentrations on chlorophyll fluorescence parameters of alfalfa leaves.

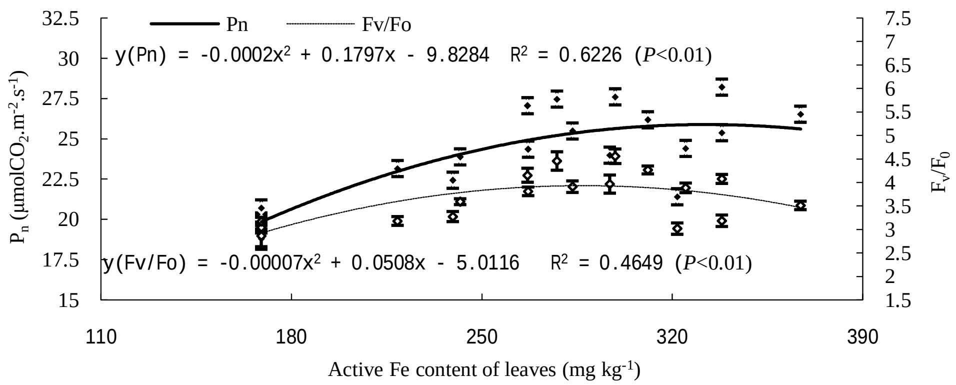

Fig. 6: Relationships between active Fe content and F_v/F_0 (±SD) and P_n (±SD) of alfalfa leaves.

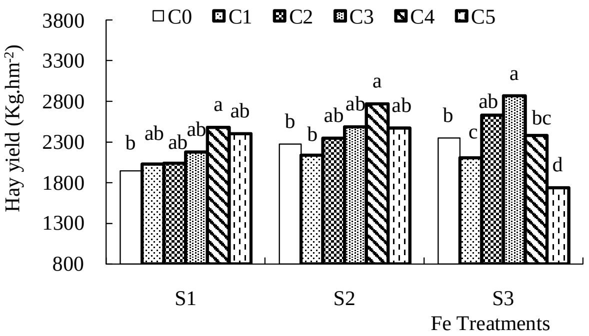

Fig. 7: Effect of three frequencies application and six Fe concentrations on hay yields of alfalfa.

In plants, iron is mainly distributed on the thylakoid membrane of chloroplasts and iron deficiency rapidly leads to a significant reduction in the activity of cis-aconitate and ferredoxin. This hinders the formation of pyrrole and porphyrin rings, which eventually damages chloroplast structure (Miller & Pushnik 1983). Mengel reported a very close correlation (r = -0.97) between the degree of iron chlorosis and leaf chlorophyll concentration (Mengel 1994).

We found that without ferrous sulphate treatment as well as with the 0.2% ferrous sulphate treatment the chlorophyll concentration was very low (*W/W*). However, chlorophyll concentration increased with higher concentrations of iron fertilizer treatment. The chlorophyll concentration decreased when 0.8% ferrous sulphate (*W/W*) was sprayed at any frequency, or when 0.6% ferrous sulphate (*W/W*) was sprayed on two or three occasions. The active iron concentration was significantly and positively correlated with chlorophyll concentration, which indicates that active iron is closely related to chlorophyll synthesis and that foliar ferrous sulphate application can significantly increase the chlorophyll concentration.

Gas exchange parameters during photosynthesis: Iron has a significant influence on plant photosynthesis, with ferritin, cytochrome, iron-sulphur protein, and ferrihaemoglobin all being involved in electron transfer and reductive biosynthesis. The concentrations of these substances and the activities of iron-containing enzymes are decreased due to iron deficiency, interfering with photosynthesis (Agarwala 1965, Lam & Malkin 1982).

In our study, photosynthetic rates were low without iron fertilizer or with only a small amount of ferrous sulphate sprayed. However, the net photosynthetic rate (P_n) of alfalfa increased dramatically with one spray treatment of 0.6% ferrous sulphate (*W/W*), two treatments of 0.8% ferrous sulphate (*W/W*), or three treatments of 0.4% ferrous sulphate (*W/W*). The stomatal conductance (C_d) was significantly and positively correlated with the P_n when the iron fertilizer spray treatment was applied at different concentrations, while the intercellular carbon dioxide (C_i) was negatively correlated with the P_n. This shows that the P_n increased and the C_i decreased with increasing iron fertilizer concentration, which in turn induced an increase in C_d. However, C_d was reduced when the iron fertilizer concentration was too high, which we suspect being due to light respiration resulting in a reduction of CO_2 utilization capacity.

Chlorophyll fluorescence parameters with iron fertilization: In normal conditions, the energy absorbed by chlorophyll is mainly consumed in three ways: photosynthetic electron transport, chlorophyll fluorescence and heat dissipation. The variations in photosynthesis and heat dissipation are capable of causing chlorophyll fluorescence to vary; thus variations in chlorophyll fluorescence can reflect variations in photosynthesis and heat dissipation (Petersonet al. 1998). In our study, lower maximum light energy conversion efficiency of photosystem (F_v/F_m) and potential chemical activity of photosystem (F_v/F_0) appeared in the iron deficient treatment and high iron treatments. We found higher F_v/F_m and F_v/F_0 in the treatments in which we administered iron fertilizer at a large enough concentration. This indicates that in iron deficiency, leaves cannot capture sufficient light energy. Over-supply of iron will likely lead to photo inhibition, reducing the potential chemical activity of the photosystem potential. Upon spraying of 0.6% *(W/W)* ferrous sulphate, the photochemical efficiency was promoted and thus alleviating photo-inhibition. This implies that iron deficiency reduced ETR and E-Yield, due to ferritin and iron-sulphur protein syntheses hindrance and consequently hindrance of the photosynthetic electron transport (Kampfenkel et al. 1995, Bertamini 2002). This is provided that the significantly lower photosynthetic electron transport rate in iron-deficient grape vine leaves is mainly due to the loss of photosystem II activity (Bertamini et al. 2002). We found photosynthetic electron transport improvement with spraying of 0.4% and 0.6% of ferrous sulphate. In our treatments, where iron was administered at a high concentration, the NPQ increased and the captured light energy was likely not effectively utilized and dissipated as heat. Where we utilized iron fertilization, the variations of alfalfa leaf F_v/F_0, F_v/F_m, NPQ, ETR and E-Yield indirectly supported the P_n results. Consequently, proper iron fertilization helped both capture and conversion of light energy of alfalfa, yet too much of an increase in iron fertilizer concentration turned out to be detrimental to raising P_n and F_v/F_0.

Alfalfa yield: A reduction in photosynthetic efficiency typically accompanies nutrient limitation, causing reduced production, due to impairment of synthesis of fully functional macromolecular assemblages. Several studies (Raese et al.

1986, Sanz-Sáez et al. 2010) indicate that iron fertilizer sprays are effective in correcting iron chlorosis in fruit trees. Heitholt et al. (2003) and Mostaghimi & Matocha (1988) reported that iron fertilizer sprays improved plant growth or yield. We found that, when iron fertilizer is sprayed at appropriate concentration, alfalfa yields significantly increased and are highest with 0.6% ferrous sulphate. This concentration is also related to the P_n results, indicating that iron fertilization influenced chlorophyll synthesis, light-capture capacity, light energy conversion efficiency and potential chemical activity of photosystem reaction center. This consequently increased the apparent photosynthetic transport rate and total photosystem photochemical quantum yield, and reduced the non-irradiative energy dissipation. Thus, leaves had a reduced capability of photosynthesis through full utilization of captured light energy via maximizing photosynthetic rate and yield. We conclude that proper iron fertilization is able to promote alfalfa and to increase its yield with the best performance for C3 (S3), C4 (S2) and C4 (S1).

CONCLUSIONS

1. The photosynthetic capacities and the productivity of alfalfa were effectively enhanced via three separate sprayings of 0.6% (*W/W*) ferrous sulphate: once at seedling establishment, once at branching and once at squaring. We observed the same enhancement for two separate sprayings of 0.8% (*W/W*) ferrous sulphate: once at seedling establishment and once at branching.
2. In iron deficient regions, spraying iron onto alfalfa leaves enables an effective increase of active iron and chlorophyll concentrations, enhances maximum light energy conversion efficiency and potential chemical activity of the photosystem reaction center. Furthermore, it leads to lower non-irradiative energy dissipation and a raise of both photosynthetic efficiency and yield.
3. Our results suggest that both the photosynthetic capacity and yield of alfalfa were raised with an increase of the concentration of ferrous sulphate and a decline of spraying times. We suggest that with ferrous sulphate concentrations of more than 0.8%, it will be possible to increase the photosynthetic capacity and yield of alfalfa by spraying ferrous sulphate only once in the appropriate period. However, this requires further detailed investigations.

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