Tracking photosynthetic injury of Paraquat-treated crop using chlorophyll fluorescence from hyperspectral data

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Abstract
Three diurnal experiments were conducted on winter wheat to investigate how solar-induced chlorophyll fluorescence (SIF) responded to Paraquat treatments (a PSI herbicide). First, a slight increase in spectral reflectance in the red region and an obvious decrease in reflectance in the NIR regions was observed due to crop injury from Paraquat. Secondly, a significant increase in SIF at 688 nm, but a significant decrease at 760 nm was observed. The opposite response trends of SIF confirm the mechanism of PSI herbicides in photosynthetic injury. Finally, the sensitivity of SIF signals were compared to other vegetation indices, and the Fr688/760 ratio turned out to be a sensitive tool to rapidly detect herbicide injury.
Keywords: Chlorophyll fluorescence, Paraquat herbicide, hyperspectral, photosynthetic injury.

Introduction
Optical remote sensing provides a powerful tool for monitoring changes in crop canopy throughout the growing season. Radiometric measurements in the solar spectral domain contain important information pertinent to the health of vegetation. Adcock et al. [1990] used a ground-based radiometer to assess soybean injury from Paraquat and glyphosate and compared radiometer injury ratings with visual observations. Nelson and Renner [2001] reported an increase in the red (660 nm) to far-red light reflectance (740 nm) ratio in soybeans treated with lactofen when compared with glyphosate and bentazon treatments. Thelen et al. [2004] found that both digital aerial imagery and ground-based optical remote sensing were effective in detecting lactofen and imazethapyr injuries on soybeans. Robles et al. [2010] found that remote sensing can be used to monitor herbicide injuries and predict the ultimate mortality of water hyacinths treated with slow-acting herbicides, such as imazapyr and glyphosate.
Under optimal conditions, most of the light energy absorbed by chlorophyll is dissipated via chemical conversion, with a small proportion being emitted through heat and fluorescence. When photosynthetic electron transport is blocked, an increased proportion of the absorbed excitation energy is reemitted as fluorescence [Schreiber et al., 1977; Agati et al., 1995].
The analysis of changes in chlorophyll fluorescence (ChlF) offers possibilities not only to determine the site of electron transport inhibition by herbicides, but also to evaluate injuries from herbicides. Miles and Daniel [1973] detected changes in leaf fluorescence resulting from the inhibition of photosynthetic electron transport by several inhibitors, including the herbicides simazine and diuron. Szigeti et al. [1996] observed a difference in the chlorophyll fluorescence characteristics of the Paraquat-sensitive horseweed (Conyza canadensis) biotypes using a multichannel chlorophyll spectrofluorometer. Richard et al. [1983] detected an increase in the terminal level of fluorescence in plants following the foliar application of herbicides, which inhibit photosynthetic electron transport. Schreiber et al. [1977] found that ozone-induced injury could be detected by the fluorescence assay at least 20 hours before any visible signs of leaf injury. It is also known that the F684/F735 ratio, reflecting PSII and PSI activities, can be used as a tool to assess performance of the photosynthetic apparatus [Agati et al., 1995; Lichtenthaler et al., 1996]. Eullaffroy and Vernet [2003] found the F684/F735 ratio was a sensitive tool for detecting trace quantities of photosynthesis-inhibiting herbicides in water.

A characteristic spectral emission known as solar-induced chlorophyll fluorescence (SIF) has been observed in vegetation chlorophyll under excitation by solar radiation, occurring in red and near-infrared spectral regions. The SIF signal is less than 1% of the incident energy, but in principle it can be separated from the reflectance signal. The effect of chlorophyll fluorescence emissions on the apparent vegetation reflectance spectrum has recently been investigated [Zarco-Tejada et al., 2001; Liu et al., 2005; Amoros-Lopez et al., 2008]. Although the observed vegetation reflectance inevitably includes contributions from both reflected and fluoresced radiation, many researchers have proved that it is possible to separate SIF radiation from observed apparent vegetation reflectance. For example, the SIF signals at 656 nm, 687 nm, and 760 nm were successfully separated from the observed apparent vegetation reflectance based on the Fraunhofer-line principle [Mcfarlane et al., 1980; Carter et al., 1996; Liu et al., 2005; Amoros-Lopez et al., 2008]. Furthermore, Guanter et al. [2007] proved for the first time that spaceborne estimation of chlorophyll fluorescence is feasible by coupling an atmospheric correction scheme during the SIF retrieval.

The SIF signal is a promising indicator of vegetation conditions. Zarco-Tejada et al. [2009] found that the canopy chlorophyll fluorescence from airborne, narrow-band multispectral data could be used to assess vegetation stress. Damm et al. [2010] reported on remotely sensing sun-induced fluorescence to improve the modeling of diurnal courses of gross primary production (GPP). Joiner et al. [2011] found that the chlorophyll fluorescence retrieved from TANSO-FTS GOSAT satellite data could reflect a regional-scale measure of instantaneous, dynamic photosynthetic activity, and the retrieved fluorescence could be used to augment important information on global productivity and seasonal dynamics. Although there have been many reports on detecting herbicide injury by using active ChlF or the passive optical reflectance method, very few studies have focused on assessing crop injury with the passive SIF method. The ability to use SIF to detect herbicide injury would also be valuable because SIF could be remotely sensed from space-borne or airborne hyperspectral imagery.

Paraquat is an active ingredient in several herbicide preparations and is extensively used in agriculture as a non-selective contact herbicide, which is the secondly most widely used herbicide in world, and still widely used in most developing countries. Non-selective
herbicides act by siphoning electrons from photosystem I (PSI), and donating them to O2, producing superoxide and other active oxygen species [Purba et al., 1995]. Application in bright sunlight can lead to rapid necrosis of affected leaves without death of the plant [Baldwin, 1963]. A bipyridylum herbicide, Paraquat disrupts photosynthetic electron transfer by accepting electrons from PSI. In addition, reaction of the Paraquat radical with molecular oxygen leads to the production of superoxide radicals and eventually to the far more damaging hydroxyl radicals, which in turn disrupt cellular membranes [Babbs et al., 1989; Preston et al., 1991]. The objective of this study was to investigate the possibility of using solar-induced chlorophyll fluorescence (SIF) to determine the site of electron transport inhibition by herbicides, and also to determine whether SIF could be used to assess herbicide injury on winter wheat (Triticum aestivum).

Materials and Methods

Study area and herbicide treatments
In this study, three paraquat herbicide rates were tested on winter wheat, and the paraquat percentage of the liquid herbicide is 42%. The measurements were made on May 11, 2010, May 7, and June 1, 2011 at the National Precision Agriculture Demonstration Base, which is located in the town of Xiao Tangshan, China, north of Beijing (40°11′ N, 116°27′ E). All of the winter wheat in the experiment had conventional fertilizer management and uniform growth, and the variety in the experiment site was Jing 9428. The experiment treatments and crop growth information were summarized in table 1, and the leaf area index (LAI) was measured by a SunScan sensor (Dynamax Inc., Houston, TX, USA). For each experiment, a zero rate was also used as a non-stressed reference in the above three treatments. In each case the herbicide was sprayed on a 2 m² uniform area.

| Growth stage | LAI   | Herbicide treatment                          | Spray time             |
|--------------|-------|---------------------------------------------|------------------------|
| 1 shooting   | 4.5   | 5 kg/ha, ten times the normal level         | 11:09 AM, May 11, 2010 |
| 2 shooting   | 3.9   | 2 kg/ha, four times the normal level        | 11:10 AM, May 7, 2011  |
| 3 grain-filling | 3.5   | 1 kg/ha, twice the normal level             | 10:10 AM, June 1, 2011 |

Photosynthetic injury from Paraquat is strongly temperature dependent [Preston et al., 1991]. The daily variation curves of total solar radiation and temperature (measured by a DYNAMET Weather Station) in the three experiments are given in Figure 1. The DYNAMET Weather Station (Dynamax Inc., Houston, TX, USA) was set in a field close to the study site, and the radiation and temperature sensor was fixed at a height of 2 m above the ground. The weather was sunny on the three experiment dates, and the herbicide was sprayed when the temperature was above 20°C. Due to differences in the daily temperature changes in the three experiments, the crop injury from Paraquat was not linearly linked to the herbicide rates.
Spectral measurements

The canopy spectral measurements were taken by an ASD FieldSpec Pro spectrometer (Analytical Spectral Devices, Boulder, CO, USA) fitted with 25° field of view fibre optics, which function in the 350-2500 nm spectral range with spectral resolution of 3 nm at 700 nm and 10 nm at 1400-2500 nm. The sampling interval was 1.4 nm between 350 and 1050 nm, and 2 nm between 1050 and 2500 nm. The sensor was held at a height of 1.0 m above the winter wheat canopy for nadir viewing throughout data collection to obtain signatures from an area of about 1.0 m² for each signature. Canopy radiance measurements were taken by averaging 40 scans at an optimized integration time and had dark current correction at every spectral measurement. A 40 cm by 40 cm BaSO₄ reference panel was used to calculate reflectance and the solar irradiance spectrum. At the beginning and end of spectral measurement, the solar irradiance was measured by using the reference panel [Duggin, 1980], and the sensor was held about 0.3 m above the reference panel. The first canopy spectra were measured just before herbicide spray for both the non-stressed reference and the herbicide treated crop, then, the spectral measurement was carried out every half or one hour until 17:30 Beijing local time. All the canopy and panel radiance spectra were taken under clear blue skies condition.

The canopy spectral reflectance can be derived from the reflected spectral signals from both canopy and reference panel, listed as [Duggin, 1980]:

$$\rho(\lambda) = \frac{Rad_{Canopy}(\lambda)}{Rad_{Panel}(\lambda)} \rho_{Panel}(\lambda)$$  \[1\]

where $\rho(\lambda)$ and $\rho_{Panel}(\lambda)$ is the spectral reflectance of canopy and calibration panel,
respectively; $\text{Rad}_{\text{Canopy}}(\lambda)$ and $\text{Rad}_{\text{Panel}}(\lambda)$ is the spectral radiance of canopy and calibration panel, respectively; $\lambda$ is wavelength.

**Solar-induced chlorophyll fluorescence and vegetation indices**

The amount of chlorophyll fluorescence emitted by a leaf under natural sunlight only accounts for anywhere from less than 1% to greater than 10% [Liu et al., 2005; Campbell et al., 2008]. Although the fluorescence signal is obscured by the reflected light, it can be separated by the Fraunhofer line discrimination (FLD) method. The two oxygen absorption bands (688 nm and 760 nm) located close to the chlorophyll fluorescence peaks have been selected to monitor the chlorophyll fluorescence emission under daylight excitation [Carter et al., 1996; Moya et al., 2004; Liu et al., 2005].

The canopy chlorophyll fluorescence emission $f$ can be calculated as [Plascyk 1975; Liu et al., 2005]:

$$f = \frac{(a \times d - c \times b)}{(a - b)}$$  \[2\]

where $a$ and $b$ respectively represent the reference panel radiance in and out of the oxygen-absorption feature, and $c$ and $d$ respectively represent the target radiance in and out of the oxygen-absorption feature. The solar-induced fluorescence at 688 nm and 760 nm was calculated according to equation [2]. For the 688 nm O$_2$ Fraunhofer line, the bands in and out of the oxygen absorption feature were set at the 684 nm and 688 nm sampling bands of the ASD spectrometer [Liu et al., 2005]. Similarly, the bands in and out of the 760 nm oxygen-absorption feature were set at the 756 nm and 760 nm sampling bands [Liu et al., 2005].

SIF radiation is only a small part of the observed apparent vegetation reflectance, and the relative SIF intensity is defined as the ratio of SIF radiation to the light incident on the top of the canopy at the band outside of the Fraunhofer line. According to equation [2], the relative SIF intensity $F_r$ can be calculated as [Liu and Cheng 2010]:

$$F_r = \frac{f}{a} = \frac{(a \times d - c \times b)}{(a - b) \times a}$$  \[3\]

The chlorophyll fluorescence ratio of the red and NIR chlorophyll peaks reflects how energy was distributed between PSI and PSII [Lichtenthaler and Miehé 1997; Zhang et al., 2005]. Zhang et al. [2005] demonstrated that the ChlF peak ratio F685/F740 was closely related to leaf water content and fluorescence parameters. Lichtenthaler and Miehé [1997] have proposed that the fluorescence ratio at 685 nm and 740 nm was sensitive to environmental change and stress (such as uptake of herbicides, mineral deficiencies), and could be used to monitor the uptake of herbicides by plants. In this study, the SIF ratio of the red and NIR bands was calculated as:
where $F_{r688}$ and $F_{r760}$ are the relative SIF intensities at 688 nm and 760 nm, respectively.

Photochemical reflectance index (PRI) and NDVI obtained in an easy, rapid, non-intrusive manner can provide an instantaneous assessment of photosynthetic function or biological status for eco-physiological studies. Thus, the two vegetation indices were also selected for this study to analyze the spectral response to herbicide injury.

PRI is sensitive to the relative concentration of xanthophyll cycle pigments, and thus is closely related to PSII efficiency as well as photosynthetic light-use efficiency (LUE) in conditions when photoelectron transport and carboxylation are well coupled [Gamon et al., 1997; Peñuelas et al., 1997]. PRI is expressed as [Gamon et al., 1992],

$$PRI = \frac{R_{531} - R_{570}}{R_{531} + R_{570}}$$

where R refers to the narrow-band (±2 nm) reflectance centered on the stated wavelength.

Herbicide had a significant effect on NDVI values derived from multispectral or hyperspectral data. Thelen et al. [2004] found significant differences among herbicides and herbicide rates by calculating the NDVI from digital aerial images of soybeans. Huang et al. [2012] indicated that NDVI from canopy spectral measurement could be used to differentiate crop stress from glyphosate at 24 hours after spray and to identify the effect of herbicide in each treatment. NDVI is calculated as [Tucker, 1979]:

$$NDVI = \frac{R_{850} - R_{680}}{R_{850} + R_{680}}$$

where R refers to the narrow-band reflectance on the stated wavelength.

The red edge spectral feature is sensitive to vegetation stress [Holer et al., 1983; Miller et al., 1991], and the red edge position (REP) will shift to a shorter wavelength when a plant is under stress, a process widely known as the “blue shift” [Miller et al., 1991]. The red edge position is defined by the wavelength of the maximum in the first derivative spectrum. However, the first derivative of the red-edge is subject to noise [Smith et al., 2004; Cho and Skidmore 2006]. The Savitzky-Golay smoothing filter was employed to remove noise in the crude reflectance spectra [Savitsky and Golay, 1964], and the window size of the filter was set as 5 bands. These smoothed reflectance spectra were then analyzed to derive REP by the maximum derivative method, as introduced in Smith et al. [2004].

**Sensitivity analysis for herbicide treatments**

The response amplitude of the spectral parameters to Paraquat herbicide was evaluated
with a normalized distance method. Two types of normalized distance indicators between the non-stressed reference and the Paraquat-treated crop were defined to eliminate the effect of the units of spectral parameters.

\[
Dis_1 = \frac{Average(|REF - PQ|)}{Range} \quad [7]
\]

\[
Dis_2 = \frac{Max(|REF - PQ|)}{Range} \quad [8]
\]

Dis\(_1\) and Dis\(_2\) are the normalized distances of two groups, REF and PQ, which are the spectral parameters’ data group for the non-stressed reference and Paraquat herbicide in each diurnal experiment. Range is the maximum variation value of the spectral parameter in all three diurnal experiments (only the non-stressed reference samples), Max is the function to calculate the maximum value of a data group, and Average is the function to calculate the average value of a data group.

**RESULTS AND DISCUSSION**

*Canopy spectral responses to herbicide treatments*

Figure 2 shows the reflectance spectra of the Paraquat-treated canopy and the non-stressed reference at about four hours after herbicide spray (15:23, May 11, 2011). Crop injury from the Paraquat, such as destroyed chloroplast, dehydration, and leaf curl [Babbs et al., 1989], caused a noticeable increase in the canopy spectral reflectance in the red region (600-690 nm) and a noticeable decrease in the NIR region.

![Canopy spectra of winter wheat about four hours after herbicide spray.](image)
NDVI was sensitive to canopy coverage measures such as LAI and chlorophyll content [Broge and Mortensen, 2002], and maybe also vary with herbicide injury. Figure 3a shows the daily changes in NDVI in the three experiments. Compared to the non-stressed reference, the NDVI of the canopy spectra with Paraquat injury noticeably decreased about two hours after the herbicide spray.

PRI is sensitive to xanthophyll cycle pigments and has been found to be strongly correlated with photosynthetic LUE at the canopy or ecosystem scale [Rahman et al., 2004; Liu and Cheng 2010]. Aside from xanthophyll components, PRI is also affected by many other factors such as pigments, canopy structure parameters, background, and
the viewing and illumination configurations [Grace et al., 2007]. Rahman et al. [2004] suggested that PRI data would be most useful in heavily vegetated areas subjected to periodic stress. Figure 3b shows the daily changes in PRI in the three treatments. Compared to the non-stressed reference site, the PRI of the canopy spectra with Paraquat injury decreased immediately after the herbicide spray, except for one abnormal record at 12:50 PM on May 11, 2010. The results also confirm that PRI was a better indicator to detect herbicide stress than NDVI, which was consistent with the research by Rahman et al. [2004].

The red edge spectra at 650-800 nm are sensitive to vegetation stress [Holer et al., 1983; Miller et al., 1991], and as mentioned the REP will move to a shorter wavelength (a blue shift) in stressed vegetation. Figure 3c shows the daily changes in REP in the three treatments. The blue shift was observed in the three treatments, with an average amplitude of about 3.2 nm, and a maximum amplitude of 12 nm in the experiment on May 7, 2011. The results indicate that REP can track serious photosynthetic injury from heavy herbicide usage.

**SIF responses to herbicide treatments**

Paraquat does not inhibit photosynthetic electron transport at the O$_{B}$-binding site of photosystem II, but causes electrons to deviate on the reducing side of photosystem I. In this case, the chlorophyll fluorescence of Paraquat-treated plants is partially quenched and thus the chlorophyll fluorescence would increase at red band (684 nm or 690 nm) but decrease at far-red (735 nm) [Szigeti et al., 1996; Eullaffroy and Vernet, 2003]. Therefore the solar-induced chlorophyll fluorescence method would be very useful to detect photosynthetic injury from Paraquat and also to determine the site of electron transport inhibition.

Figure 4a and 4b show the daily changes in SIF at 688 nm and 760 nm in the three treatments. The results illustrated that the relative SIF intensities at 688 nm increase noticeably about one hour after spraying the herbicide. However, the relative SIF intensities at 760 nm decrease under herbicide stress. The opposite variation trends of the relative SIF intensities at 688 nm and 760 nm confirm the mechanism of PSI herbicides (Paraquat) in photosystems. The decrease in chlorophyll fluorescence in the far-red band was also observed by Eullaffroy and Vernet [2003] for algae under PSI herbicide treatment, which was measured by an active fluorometer (LS50B-PerkinElmer) equipped with a red-sensitive photomultiplier. Therefore, the inverse variation of chlorophyll fluorescence was consistent with physiological knowledge of energy distribution among the photosystems due to paraquat herbicide treatment.

Since PSII and PSI contribute principally to the fluorescence emissions in red and far-red bands, respectively [Agati et al., 1995], the relative intensities of these chlorophyll fluorescence bands reflect changes in activity of the two photosystems. Variation of the relative SIF ratio at 688 nm and 760 nm (Fr688/760) was indicative of the process of photosynthetic quantum conversion, thus providing information on the functioning of the photosynthetic apparatus under herbicide treatments. The Fr688/760 ratio of the relative SIF intensities was also employed to evaluate crop injury from Paraquat herbicide, as shown in Figure 4c. The Fr688/760 ratio increased noticeably about one hour after spraying the herbicide, with a maximum value of 5.8, 3.0, and 3.4 for the
experiments in May 2010, May 2011, and June 2011, respectively. A large increase in the Fr688/760 ratio was also observed by Eullaffroy and Vernet [2003] for different herbicide treatments on algae.

**Figure 4 - Daily changes in SIF values at 688 nm, 760 nm, and its ratios in the three diurnal experiments.** The spraying time was 11:09 AM, 11:10 AM, and 10:10 AM for experiments on May 11, 2011, May 7, 2011, and June 1, 2011, respectively (as marked by the dotted line), and the corresponding treatment amount was 5 kg/ha, 2 kg/ha, and 1 kg/ha.

**Comparing SIF to spectral indices’ sensitivity under herbicide treatments**
The significance of the effect of herbicides on spectral response was determined using a one-way analysis of variance (ANOVA). The p-values of the one-way ANOVA of the spectral parameters are summarized in Table 2. The results showed that the relative SIF intensity at 688 nm, the Fr688/760 ratio, NDVI and PRI were sensitive to Paraquat herbicide for all
three treatments.

Table 2 - Summary of p values from one-way ANOVA of spectral parameters for Paraquat treatments.

|                  | 20100511 | 20110507 | 20110601 |
|------------------|----------|----------|----------|
| Fr688(%)         | 0.050    | 0.018    | 0.038    |
| Fr760(%)         | 0.105    | 8.84E-05 | 9.02E-06 |
| Fr688/760        | 0.021    | 0.001    | 7.55E-07 |
| NDVI             | 0.023    | 0.000263 | 5.41E-05 |
| PRI              | 0.010    | 1.96E-06 | 1.93E-09 |
| REP              | 0.0489   | 0.128    | 2.17E06  |

The response amplitude of the spectral parameters to Paraquat herbicide was evaluated with a normalized distance method, as shown in equations [7] and [8]. The normalized distance values of different spectral parameters in the three diurnal experiments are listed in Table 3. The results showed that the Fr688/760 ratio was the most sensitive spectral parameter for Paraquat herbicide. The mean increase of Fr688/760 for Paraquat herbicide was about half of the largest natural variation of the non-stressed reference in the three diurnal experiments, including two different growth stages. The maximum increases of Fr688/760 for Paraquat were 1.12, 0.70, and 0.64 times the largest natural variation in May 2010, May 2011, and June 2011, respectively.

Table 3 - Response amplitude of the spectral parameters to Paraquat herbicide.

(a) Normalized distance for the average difference.

|                  | 20100511 | 20110507 | 20110601 |
|------------------|----------|----------|----------|
| Fr688(%)         | 0.47     | 0.58     | 0.18     |
| Fr760(%)         | 0.04     | 0.40     | 0.17     |
| Fr688/760        | 0.50     | 0.39     | 0.41     |
| NDVI             | 0.13     | 0.32     | 0.29     |
| PRI              | 0.18     | 0.43     | 0.37     |
| REP              | 0.12     | 0.22     | 0.21     |

(b) Normalized distance for the maximum difference.

|                  | 20100511 | 20110507 | 20110601 |
|------------------|----------|----------|----------|
| Fr688(%)         | 1.28     | 1.27     | 0.38     |
| Fr760(%)         | 0.07     | 0.53     | 0.31     |
| Fr688/760        | 1.12     | 0.70     | 0.64     |
| NDVI             | 0.32     | 0.58     | 0.40     |
| PRI              | 0.39     | 0.71     | 0.50     |
| REP              | 0.24     | 0.71     | 0.29     |

Conclusions

This study was conducted to investigate the spectral and SIF responses to winter wheat treated with Paraquat herbicide. The diurnal variations of spectral parameters under different
Paraquat treatments, including SIF at red and far-red bands, were compared to those of a non-stressed reference.

1) Canopy SIF was proved feasible to determine sites of electron transport inhibition under herbicide treatments. A significant increase (p value equals 0.050, 0.018, 0.038, respectively for the three experiments) in SIF at 688 nm was observed in the three diurnal experiments, which confirms the expected increased proportion of absorbed excitation energy reemitted as fluorescence, when photosynthetic electron transport is blocked by herbicide treatment. In contrast, a significant decrease (p value equals 0.105, 8.84E-05, 9.02E-06, respectively for the three experiments) in SIF at 760 nm was observed in the three treatments. The opposite response trends of the relative SIF intensities at 688 nm and 760 nm confirm the mechanism of PSI herbicides (including Paraquat) on photosynthetic inhibition;

2) Canopy SIF was useful to assess herbicide injury on winter wheat. Compared with other traditional spectral indices (such as NDVI, PRI, REP), the Fr688/760 ratio turned out to be a practical, sensitive tool for rapidly detecting herbicide injury in winter wheat. For each diurnal experiment, the mean increase in the Fr688/760 ratio under Paraquat treatment was about half of the largest natural variation in the three diurnal experiments, which covered two years and two different growth stages.

Acknowledgements
The authors gratefully acknowledge financial support provided for this research by the National Natural Science Foundation of China (41222008, 91125003) and the National High Tech R&D Program of China (2012AA12A30701).

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