# Effect of gender, leaf morphology, and seasonality on Specific Leaf Area (SLA) of *Ginkgo biloba* leaves

## Biology

(3907 words)

# Table of Context

**Research Question:** How does the functional ecological unit (SLA; Specific Leaf Area) give an insight into *Ginkgo biloba* growth in terms of gender, leaf morphology, and phenology?

## Introduction

*Ginkgo biloba*, also known as maidenhair tree, is dioecious–having individual male and female organs on separate individuals. This species, native to China, is known as living fossil due to its long presence since the Permian period of the Paleozoic Era–about 300 to 250 million years ago–with not much change in its characteristics, then and now. (Crane 2013) The other species in the order Ginkgoales are extinct today and *G. biloba* is the only remaining for many reasons including the species' natural resilience. (Earle, 2016 ; Crane, 2013) In this investigation, the impact of gender, leaf morphology, and season on the tree was studied using Specific Leaf Area (SLA) The SLA, being the ratio of surface area of one side of leaf to dry mass, may indicate numerous aspects of the leaf; this functional index scales positively with photosynthetic rate and with nitrogen concentration, and negatively with leaf longevity and C investment in compounds such as tannins and lignin–substances used in defending the plant from pathogens. (Pérez-Harguindeguy et al., 2013 ; Vanholme et al., 2010 ; Galloway, 1989) Thus by investigating and analyzing the SLA trend of *G. biloba*, even over some months may yield much information.

$$SLA = \frac{surface\ area\ of\ one\ side}{dry\ mass}\ (\mathrm{cm^2\ g^{-1}})$$

(Pérez-Harguindeguy et al., 2013)

To find the SLA values of different types of leaves on *G. biloba*, three variables were considered: gender of the tree, position of the leaf, and season in which the leaf was collected. Gender is chosen as one of the variable since *G. biloba*, as mentioned above, is a dioecious plant and the difference in the gender of the trees could affect the growth of the leaves for the purpose of reproduction. The leaf position was chosen according to the shoot it has grown on. On a *G. biloba* tree, there are distinctive parts of branch: short shoot and long shoot. The short shoot–brown part of the branch–is closer to the trunk of the tree, and the long shoot–new green branch that sprouts every year, elongating the length of the branch–is farer from the trunk. Since the shoots differ significantly, from the location to the age of the tissues, it is probable that the growth rate of the trees vary. In the experiment, the number of collecting periods was five in total, according to its season. As the season determines the time of the sprouting of new leaves, optimum period of leaf growth, and the falling of the leaves, it is a considerable factor that determines the growth rate of leaves. The collection starts from May 15$^{th}$ to July 15$^{th}$, because before May 15$^{th}$, the long shoot has not yet sprouted, and after July 15$^{th}$, the long shoots have turned into short shoots. The leaves were collected every 15 days, in order to see the change in SLA values. Initially, height was included as one of the variables, but a fast-forward video filming the early life of *G. biloba*, and available in the public domain, revealed that branches grow sporadically on the stem, implying little relationship between the growth and the height. (Claessen, 2011) Thus, the height was excluded from the list of variables. In order to keep the other variables constant, the trees, provided by Beijing Institute of Landscape Architecture, were all from Shandong, with almost identical genes apart from sex, and had the age of approximately thirty years.

## Hypothesis

For this study, a null hypothesis may be assumed, as this hypothesis will either will rejected or fail to be rejected through a statistical test called t-test. There are two null hypotheses for this study. The first hypothesis is that the SLA of the leaves of different gender of *G. biloba* will be the same, even over an extended time period. The second premise is that the SLA of the leaves on varying location of branches, long shoot and short shoot, of *G. biloba* will be the same.

<u>**Methodology**</u>

1. **Material**
   a. a laboratory precision balance: accuracy to 1mg
   b. a pair of scissor
   c. a permanent marker
   d. a tree branch cutter
   e. 3 to 4 newspapers for every collection (every 15 days)
2. **Variables**
   a. <u>Independent:</u> seasonality (May 15th, June 1st, 15th, July 1st, 15th), morphology of branch (long shoot, short shoot), gender of the tree (male, female)
   b. <u>Dependent:</u> SLA (specific leaf area) of the leaves
   c. <u>Controlled:</u>
      i. Tree used for collecting samples: one for male, and one female
         1. To control this variable, a specific place was designated for the collection of leaves and each of the trees were taken pictures with it surroundings.
      ii. Height of the branch: 250cm
         1. The height was controlled using the labelled on the tree branch cutter.
      iii. How the leaves are treated: petiole is cut off, and the leaves are dried for 1 month, covered with newspaper
         1. It was made sure in the process of treating each collection of leaves that all the leaves are treated in the same way.
3. **Method**
   1. Choose a tree for sampling the leaves each for male and female.
   2. Every 15 days, starting on May 15th, collect two branches from each of the trees chosen, male and female.
      a. Make sure the branches are collected at the height of 250cm.
      b. Keep in mind to collect branches with relatively long long-shoots.
   3. Label the branches as A or B; one A and one B for each gender.
   4. Leave the branches in shade for a night.
   5. On the consecutive day, pluck 10 leaves each from the short and long shoots of the 4 branches collected.
   6. Using a permanent marker, label the leaves as following: M/F for gender, S/L for short and long shoot, and A/B for branch A and branch B of the same gender, and .
   7. Then, using a normal pair of scissors, the petioles of the leaves were cut off
   8. Sandwich the leaves on newspaper neatly, and leave them in a shady place for a month to dry.
   9. After dried, weigh the leaves using a laboratory precision balance and note them down.
      a. When setting the balance up, it was made sure that the balance was on a flat horizontal surface.
   10. Then, scan the leaves to calculate their surface area.
   11. Using Adobe Photoshop, find the surface area of each of the leaves.
   12. With the surface area and dry mass of the leaves, calculate the SLA for each of the leaves using the function mentioned in the above

4. **Safety and Ethics**

There were not much safety implications to be considered, as the whole experiment did not include any dangerous chemical or sharp materials. The tree branch cutter was made safe to avoid any cuts; therefore, the only thing needed to be careful was when using normal pair of scissors to cut the petioles of the leaves. In order to be avoid any cuts, the fingers holding the leaf was made sure that it was out of the scissor's way.

## Data collection

*Table 1: Sample of* ***raw data*** *of SLA value of Ginkgo biloba leaves (May 15th 2017)*

This table is an extract from the raw data set of the SLA of *Ginkgo biloba*. The SLA was calculated according to the equation of SLA stated in the **Introduction**. The leaves were collected according to the gender, location on branch, and seasonality. This specific table is data collected on May 15th 2017. Two branches were collected from each gender and were labelled A and B. Ten leaves were collected from each gender and location for both branch A and branch B, and the leaves were identified by numbering them from 1 to 10. The SLA is uncertain to ±1 $cm^2\ g^{-1}$ as there could have been error caused in the process of counting the pixels, measuring the dry mass of the leaves, or estimating the values. The full raw data is presented in the **Appendix**.

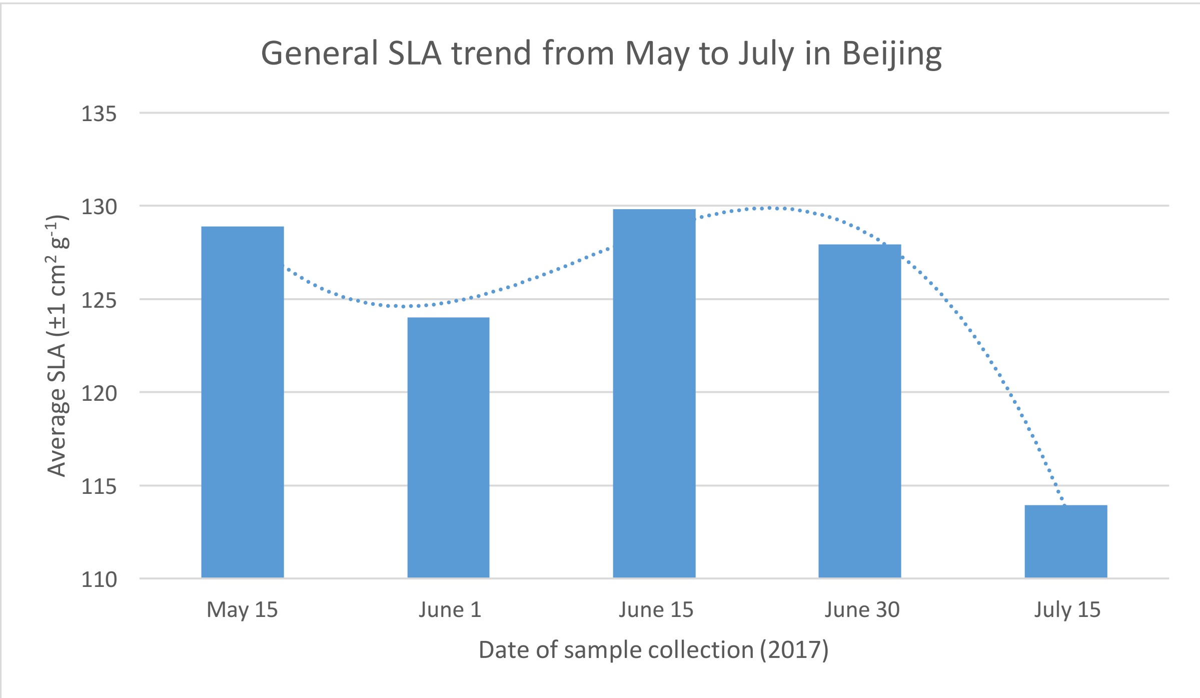
| Leaf No. | Female SLA ($cm^2\ g^{-1}$) (±1 $cm^2\ g^{-1}$) | | | | Male SLA ($cm^2\ g^{-1}$) (±1 $cm^2\ g^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Short shoot | | Long shoot | | Short shoot | | Long shoot | |
| | A | B | A | B | A | B | A | B |
| 1 | 114.50 | 132.06 | 111.27 | 115.34 | 134.92 | 159.88 | 122.78 | 142.88 |
| 2 | 115.49 | 129.41 | 117.70 | 127.76 | 94.01 | 144.42 | 127.25 | 120.63 |
| 3 | 115.19 | 129.27 | 117.77 | 115.58 | 149.34 | 87.65 | 127.97 | 120.92 |
| 4 | 130.49 | 150.33 | 111.60 | 119.61 | 131.70 | 168.30 | 124.12 | 125.28 |
| 5 | 142.88 | 130.34 | 110.71 | 120.03 | 161.74 | 150.50 | 118.02 | 107.34 |
| 6 | 117.74 | 132.23 | 107.92 | 119.10 | 130.64 | 146.57 | 121.54 | 136.77 |
| 7 | 148.93 | 127.36 | 103.02 | 113.28 | 152.11 | 169.56 | 102.04 | 154.11 |
| 8 | 116.28 | 151.47 | 107.93 | 117.30 | 136.14 | 159.16 | 135.13 | 126.96 |
| 9 | 132.01 | 132.16 | 113.41 | 120.70 | 150.63 | 165.49 | 129.75 | 128.81 |
| 10 | 152.64 | 147.76 | 109.59 | 131.75 | 136.22 | 141.64 | 89.86 | 118.75 |

## Data presentation

*Table 2 and Graph 1: Mean and standard deviation of SLA of Ginkgo biloba leaves by seasonality: May 15th, June 1st, June 15th, June 30th, and July 15th of 2017.*

This data set represents the mean and the standard deviation of SLA of *G. biloba* leaves, both according to the dates samples were collected. The leaves were collected 5 times, with 15-day interval. The leaves were left to dry between newspapers and their dry mass was measured one month after the collection using a laboratory precision balance with the accuracy of 0.001g. Using the dry mass and surface area obtained, the SLA was calculated using the equation given above. The data is sorted out according to the dates they were collected, neglecting the other variables. Yet, variables, such as height, that might have effect on the data was controlled using tree branch cutter which each segment has the length of 1 meter. The mean and standard deviation of the data were calculated using Microsoft Excel. The mean SLA is uncertain to ±1 $cm^2\ g^{-1}$ as there might be error in the process of counting the pixels, measuring the dry mass of the leaves, or estimating the values.

| Date | Mean SLA ($cm^2\ g^{-1}$) (±1 $cm^2\ g^{-1}$) | Standard Deviation |
|---|---|---|
| May 15th | 128.89 | 17.84 |
| June 1st | 124.02 | 23.63 |
| June 15th | 129.83 | 22.44 |
| June 30th | 127.94 | 22.96 |
| July 15th | 113.92 | 18.85 |

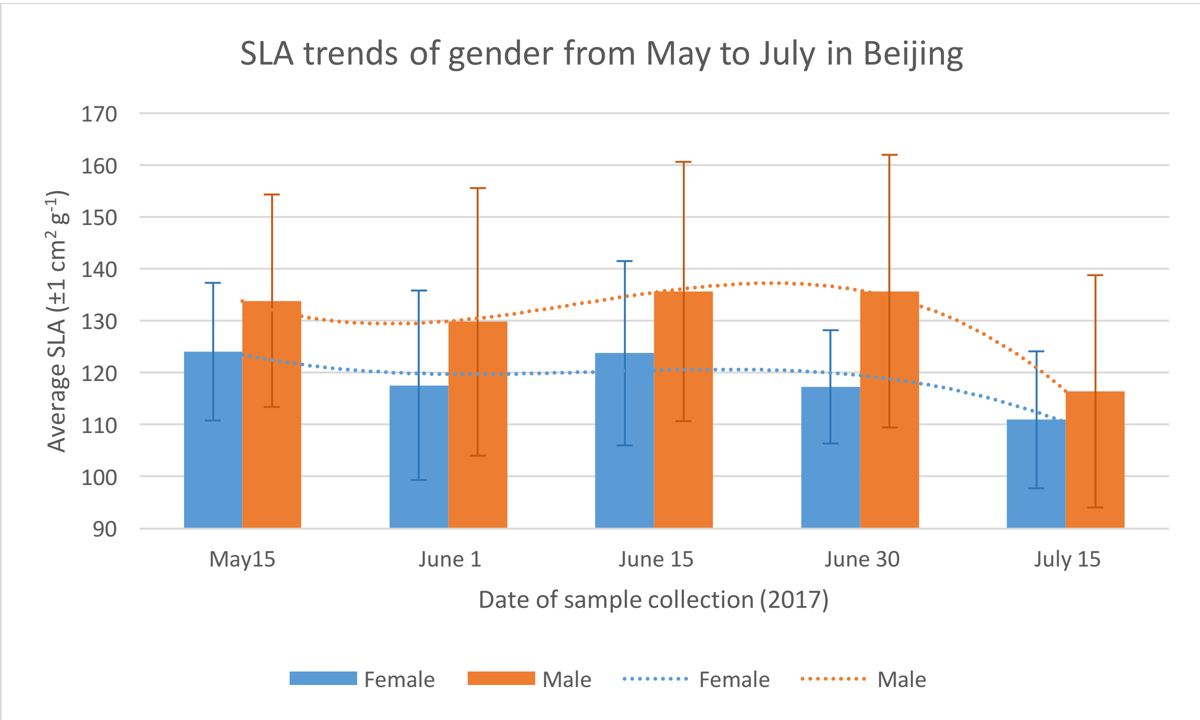



*Standard Deviation (SD) is less than one third or more than the data, indicating the data is normally distributed

*Table 3 and Graph 2: Mean and standard deviation of SLA of Ginkgo biloba leaves by gender (male and female) and seasonality (May 15$^{th}$, June 1$^{st}$, June 15$^{th}$, June 30$^{th}$, and July 15$^{th}$).*

This data set represents the mean and standard deviation of SLA of *G. biloba* leaves according to the gender of the tree that the samples were collected from and the date samples were collected. The leaves were collected 5 times in total, having 15-day interval. As mentioned, the leaves were left to dry between newspapers for a month in order for the leaves' dry mass to be measured. The surface areas of the leaves were measured by counting the pixels of the scanned image of the leaves using Adobe Photoshop. The data is organized according to the dates and the gender of the tree the samples were collected, neglecting other variables. The mean was calculated using Microsoft Excel. The mean SLA for both gender is uncertain to ±1 $cm^2\ g^{-1}$, as there could have been error in counting the surface area of the leaves, in measuring the mass of the leaves, or in estimating certain value.

| Date | Male | | Female | |
|---|---|---|---|---|
| | Mean SLA ($cm^2\ g^{-1}$) (±1 $cm^2\ g^{-1}$) | Standard Deviation | Mean SLA ($cm^2\ g^{-1}$) (±1 $cm^2\ g^{-1}$) | Standard Deviation |
| May 15$^{th}$ | 133.79 | 20.48 | 124.00 | 13.26 |
| June 1$^{st}$ | 129.78 | 25.75 | 117.53 | 18.26 |
| June 15$^{th}$ | 135.62 | 25.01 | 123.73 | 17.72 |
| June 30$^{th}$ | 135.68 | 26.21 | 117.26 | 10.95 |
| July 15$^{th}$ | 116.42 | 22.38 | 110.92 | 13.22 |

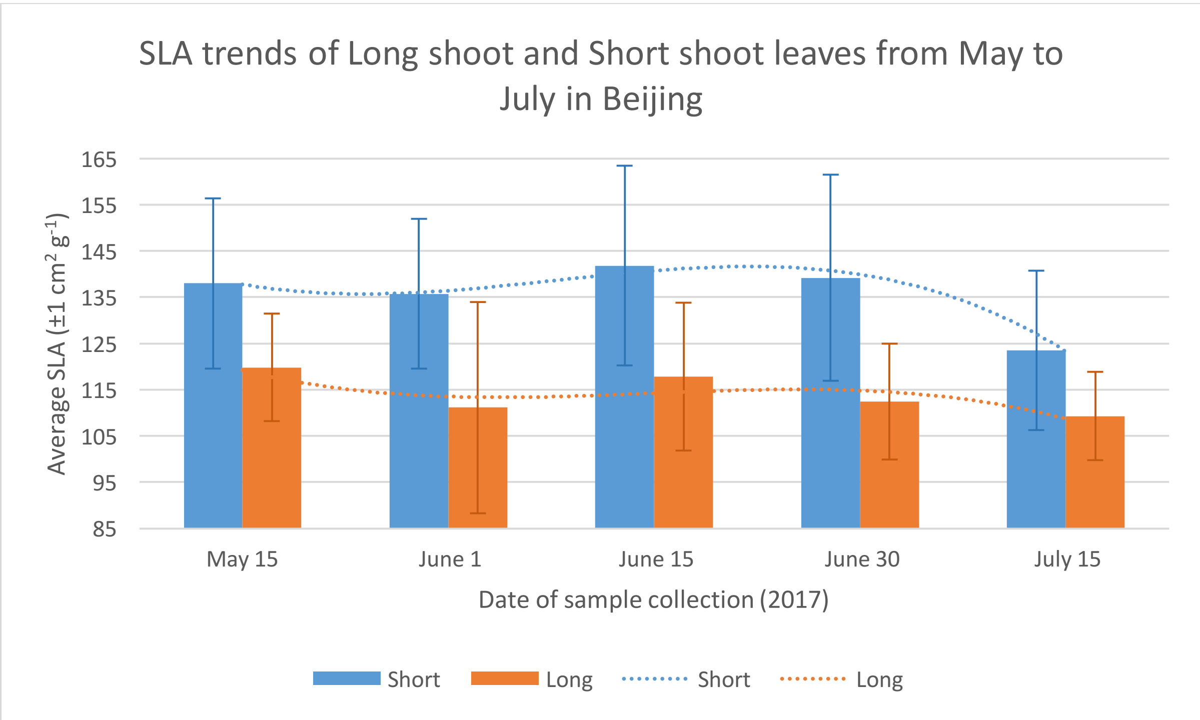



*Standard Deviation (SD) is less than one third or more than the data, indicating the data is normally distributed

*Table 4 and Graph 3: Mean and standard deviation of SLA of Ginkgo biloba leaves by the location of the leaves on the branch and seasonality (May 15$^{th}$, June 1$^{st}$, June 15$^{th}$, June 30$^{th}$, and July 15$^{th}$).*

This data set represents the mean and standard deviation of SLA of *G. biloba* leaves according to the location of the leaves on the branch and the dates the samples were collected. The samples were collected every 15-days, from May 15 until July 15, a total of 5 times. The leaves were collected from two different parts of the branch, long and short shoots. Each collection having 2 branches from each gender of the tree, the 10 leaves were collected from each long and short shoot of all 4 branches, a total of 80 leaves per collection. The mean and standard deviation were both calculated using Microsoft Excel. The uncertainty level both mean and standard deviation is ±1 $cm^2\ g^{-1}$ which might be due to error in the process of counting pixels when measuring surface area of leaf, when measuring the dry mass of the leaves, and when estimating the value.

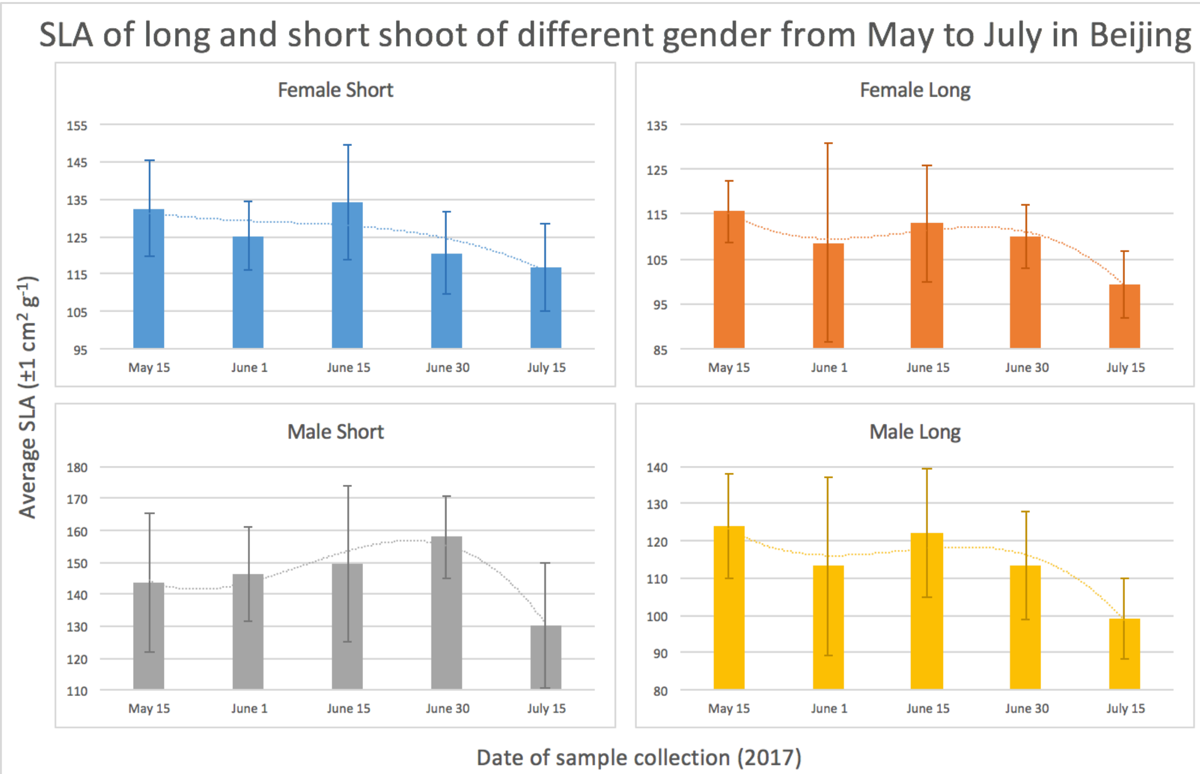
| Date | Short shoot | | Long shoot | |
|---|---|---|---|---|
| | Mean SLA ($cm^2\ g^{-1}$) (±1 $cm^2\ g^{-1}$) | Standard Deviation | Mean SLA ($cm^2\ g^{-1}$) (±1 $cm^2\ g^{-1}$) | Standard Deviation |
| May 15$^{th}$ | 137.98 | 18.45 | 119.81 | 11.61 |
| June 1$^{st}$ | 135.71 | 16.19 | 111.12 | 22.90 |
| June 15$^{th}$ | 141.83 | 21.64 | 117.83 | 16.01 |
| June 30$^{th}$ | 139.19 | 22.31 | 112.42 | 12.54 |
| July 15$^{th}$ | 123.46 | 17.24 | 109.28 | 9.52 |




*Standard Deviation (SD) is less than one third or more than the data, indicating the data is normally distributed

*Table 5 and Graph 4: Mean and standard deviation of SLA of Ginkgo biloba leaves by the location of the leaves on each gender.*
This data set represents the mean and standard deviation of SLA of *G. biloba* according to the location of the leaves (long shoot and short shoot) on each gender. The leaf samples were collected form May 15$^{th}$ to July 15$^{th}$ with 15-day interval, having a total of 5 collections. Leaf samples were collected from each male and female trees, 2 branches from each tree. From each of the branch, 10 leaves were collected each from long and short shoots. The leaves were left dry between newspapers for a month for the dry mass of the leaves were to be measured. The surface areas of the leaves were calculated by counting the number of pixels of the scanned image of the leaves using Adobe Photoshop. The mean and the standard deviation of the data was calculating using Microsoft Excel. The uncertainty level of both mean and standard deviation are ±1 cm$^2$ g$^{-1}$ as there could have been error in the process of measuring the mass of the leaves, measuring the surface area of the leaves, or estimating the values.

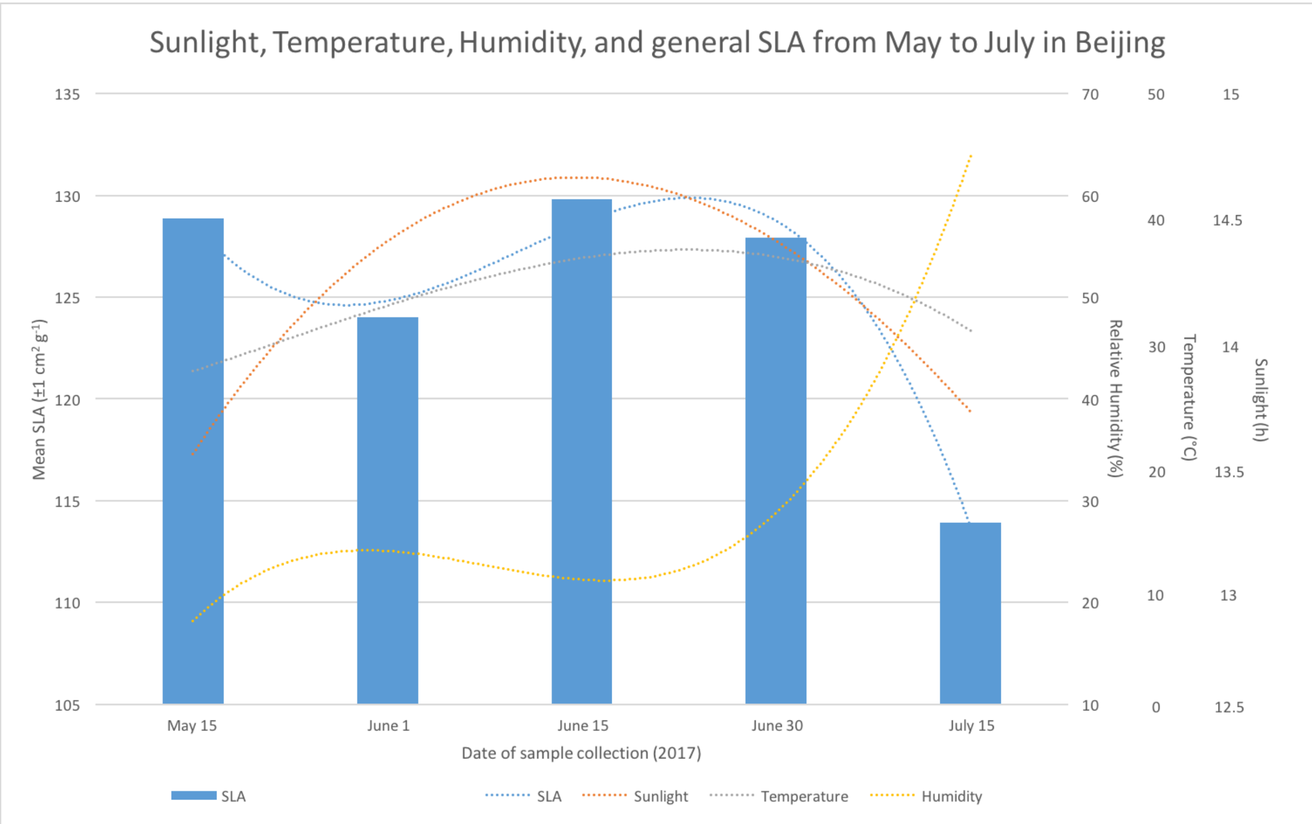
| Date | Mean SLA (cm$^2$ g$^{-1}$) (±1 cm$^2$ g$^{-1}$) | | | | Standard Deviation | | | |
|---|---|---|---|---|---|---|---|---|
| | Female | | Male | | Female | | Male | |
| | Short | Long | Short | Long | Short | Long | Short | Long |
| May 15$^{th}$ | 132.43 | 115.57 | 143.53 | 124.05 | 12.86 | 6.79 | 21.64 | 13.88 |
| June 1$^{st}$ | 125.17 | 108.54 | 146.26 | 113.30 | 9.09 | 22.20 | 14.88 | 23.83 |
| June 15$^{th}$ | 133.99 | 112.89 | 149.68 | 122.27 | 15.37 | 13.14 | 24.42 | 17.34 |
| June 30$^{th}$ | 120.49 | 110.07 | 157.89 | 113.48 | 10.99 | 6.99 | 12.84 | 14.40 |
| July 15$^{th}$ | 116.76 | 99.24 | 130.17 | 99.25 | 11.53 | 7.42 | 19.55 | 10.86 |




*Standard Deviation (SD) is less than one third or more than the data, indicating the data is normally distributed

*Graph 5: Relationship between sunlight hours, temperature, and humidity in Beijing on the dates of sample collection and SLA values*

This graph is the visual representation of general SLA from *Graph 1* and the chosen natural factors–including hours of sunlight, temperature, and relative humidity in Beijing on the dates of sample collection. The weather data comes from an Internet site. (freemeteo.co.uk, 2017) To estimate the time that *G. biloba* was exposed to sunlight, the time interval of sunlight from dawn to dusk on May 15th, June 1st, 15th, 30th, and July 15th were found, subtracting the times that rained in between. For temperature, 3 readings on May 15th, June 1st, 15th, 30th, and July 15th at 15:30, 16:00, and 16:30 were chosen to be calculated, as these dates and times were when the samples were collected. The humidity was measured as relative humidity, which does not show the absolute humidity but still suggests a reasonable trend of the humidity of the days. In order to get accurate data that affected the leaves' SLA values, 3 readings on May 15th, June 1st, 15th, 30th, and July 15th at 15:30, 16:00, and 16:30 were chosen to be calculated, as the samples were collected during these time periods. In order to show a clear relationship between the SLA and the natural factors, each data were given its own axes: SLA axis on the left and natural factors axes on the right. Although the natural factors data was not continuous, it was drawn using lines, as they elucidate the trend and also the relationship with other data. To make SLA trend clearly recognizable, the trendline was bolded. Also, to differentiate the lines of natural factors, each natural factor was given its distinct color.



**Data Processing** (Student's t-test)
To find out the significance of the difference in mean of SLA of the leaves of different genders or on different shoot morphs, Student's t-test has been used. The t-values and degree of freedoms were calculated using the formulas below.

t-value:

$$t = \frac{|\overline{X_1} - \overline{X_2}|}{\sqrt{\frac{S^2{}_1}{n_1} + \frac{S^2{}_2}{n_2}}}$$

degree of freedom:

$$d.f. = \frac{\left(\frac{S^2{}_1}{n_1} + \frac{S^2{}_2}{n_2}\right)^2}{\frac{\left(\frac{S^2{}_1}{n_1}\right)^2}{n_1 + 1} + \frac{\left(\frac{S^2{}_2}{n_2}\right)^2}{n_2 + 2}} - 2$$

$\overline{X_a}$ = mean of an arbitrary data set $a$
$S^2{}_a$ = variance of an arbitrary data set $a$
$n_a$= number of data in an arbitrary data set $a$

(Ambrose III and Ambrose, 1995)

Significance of gender on the SLA value of *G. biloba* leaves:

| Date | t-value | Degree of freedom | Result |
|---|---|---|---|
| 15 May | 2.54 | 70 | Gender has significant impact on SLA value |
| 1 June | 2.42 | 73 | Gender has significant impact on SLA value |
| 15 June | 2.40 | 70 | Gender has significant impact on SLA value |
| 30 June | 3.99 | 56 | Gender has significant impact on SLA value |
| 15 July | 1.24 | 61 | Gender has no significant impact on SLA value |

Significance of shoot morphs on the SLA value of *G. biloba* leaves:

| Date | t-value | Degree of freedom | Result |
|---|---|---|---|
| 15 May | 5.27 | 67 | Shoot morphs have significant impact on SLA value |
| 1 June | 5.40 | 67 | Shoot morphs have significant impact on SLA value |
| 15 June | 5.50 | 72 | Shoot morphs have significant impact on SLA value |
| 30 June | 6.33 | 68 | Shoot morphs have significant impact on SLA value |
| 15 July | 4.30 | 67 | Shoot morphs have significant impact on SLA value |

Significance of gender on the SLA of long shoot leaves of *G. biloba*:

| Date | t-value | Degree of freedom | Result |
|---|---|---|---|
| 15 May | 2.45 | 32 | Gender has significant impact on SLA of long shoot leaves |
| 1 June | 0.63 | 40 | Gender has no significant impact on SLA of long shoot leaves |
| 15 June | 1.89 | 40 | Gender has no significant impact on SLA of long shoot leaves |
| 30 June | 0.86 | 32 | Gender has no significant impact on SLA of long shoot leaves |
| 15 July | 1.95 | 30 | Gender has no significant impact on SLA of long shoot leaves |

The data was analyzed at 0.02 alpha level, as the laboratory balance used was a reliable material, and even though the surface area value might have some error, the errors are minor that it will not alter the trend of the SLA.

**Data Analysis**

*Graph 1*:

It is clear from the graph that the SLA of *G. biloba* leaves decreases over time. This is shown explicitly through the 3rd degree polynomial trendline. However, there still are some fluctuations, such as the leaves reaching maximum SLA on June 15th and experiencing a huge decrease on June 1st just to go up again in the next 15 days. The error bars of the graph were not drawn, as the variation of the standard deviation of each data was big. Not only that, the error bar was unnecessary, as this graph is only for a general trend in SLA of *G. biloba* over time, thus the data inevitably has a large standard deviation.

*Graph 2*:

From this graph, it is explicitly shown that the trend of SLA of each gender of *G. biloba* is decreasing over time; the 3rd degree polynomial trendlines of both male and female have a low end in July 15th. Yet, just like the general trend of SLA from *Graph 1*, both data have reached their local minimum on June 1st. Then, the female SLA reaches its peak on June 15th and decreases shortly after, while the male SLA reaches its peak on June 15th and stays high until June 30th. The male SLA is higher than that of female, but over time, the two converges. However, the standard deviation of the male is much higher than female standard deviation, suggesting that the data has a higher variation of leaves relative to the female data.

*Graph 3*:

Just from *Graph 1*, it is evidently shown in the graph that the SLA of each location of the leaf of *G. biloba* decreases over time; the 3rd degree polynomial trendlines of both long shoot and short shoot exemplifies it. However, the data still follows the trend of all the other graphs above by having a local minimum on June 1st and a maximum on June 15th. Generally, the SLA of the leaves of short shoot is higher than the SLA of the leaves of long shoot, but on July 15th, the data of long shoot and short shoot converges. The standard deviation of this graph is smaller than those of *Graph 2*. This suggests that the data of *Graph 3* is a better way to interpret the SLA trends of *G. biloba* than *Graph 2*.

*Graph 4*:

The graph clearly indicates the SLA of leaves on each shoot morphs on each gender of *G. biloba* decreases over time; the 3rd degree polynomial trendlines of each type of leaves have a low tail on July 15th. Generally, the SLA of short shoots are higher than the long shoot, and the SLA of male leaves are higher than SLA of female leaves as depicted in *Graph 2* and *Graph 3*. However, the on July 15th, the SLA of both male and female converge. Even though the trendlines of male short shoots and female short shoots do not converge, the data on July 15th suggests a continuous convergence, since the divergence of the trendlines is due to the big increase in SLA of male short shoot on June 30th. The standard deviation of the graph is the smallest, suggesting that *Graph 4* is the best graph to analyze the data compared to the graphs above.

*Graph 5*:

To begin with sunlight hours, it is clear from the graph that the hour of sunlight increases as it reaches the mid of June, and decreases as it reaches the mid of July: the trendline of sunlight data is shaped like a 2nd degree polynomial. On the other hand, the trendline of SLA value tends to show a 3rd degree polynomial. Thus, through visual interpretation, it is suggested that sunlight and SLA values do not have a relation. Moving on to temperature, the graph indicates an increase in temperature around the mid of June, and a decrease towards the mid of July. Therefore, the trendline of temperature also shows a 2nd degree polynomial like sunlight, but skewed to the left. In other words, visually, temperature also does not have any relation to SLA value. Lastly, from the graph, it is noticeable from the graph humidity–unlike sunlight and temperature–has a 3rd degree polynomial, having the lowest value on May 15th and the highest value on July 15th. The trend does not have a clear 3rd degree polynomial, but is also negatively correlated to the general SLA value. Although there are some uncertainties on June 1st and June 30th, the error is small to the extent that it is negligible. Conclusively, this graph strongly suggests a negative correlation between the SLA values of *G. biloba* leave and relative humidity.

## Discussion

### Effect of natural conditions on SLA:

In *Graph 1*, it is shown that SLA of all *Ginkgo biloba* leaves tend to decrease over time. This decrease in SLA of the leaves indicates an increase in the dry mass of the leaves relative to the surface area according to the equation of SLA. As mentioned in the **Introduction**, SLA is an index indicating the growth of a leaf, and since *G. biloba* is a deciduous tree, the annual cycle of the SLA should be shown clearly. Therefore, as the season changes from spring to summer, and summer to fall, the SLA of the trees decrease in order to prepare for the coming fall and winter, the season when the leaves stop growing and start to fall. This is due to the increasing carbon content of the leaf throughout the season while the leaves' size stops growing. (Marron et al., 2003) Yet, how the trees detect the coming of fall and winter is still not shown clearly just by looking at the general trend. In order to investigate the actual factor that affects the change of SLA, the patterns of each individual data were speculated, considering other abiotic factors that could have affected the SLA values.

Some of the possible of abiotic factors that could have affected the SLA values are, temperature, humidity, altitude, soil nutritional values, pollution (such as $CO_2$ concentration), and sunlight. Although altitude, soil nutritional values, and pollution may affect the SLA of the leaves, these abiotic factors neither do not change seasonally, nor are difficult to measure with the technology provided. (Royal Society of Chemistry, 2013) Thus, altitude, soil nutritional values, and pollution were neglected in this study, and only temperature, humidity, and sunlight on May 15th, June 1st, 15th, 30th, and July 15th were speculated and graphed in *Graph 5*.

Sunlight and SLA:

Looking at *Graph 5*, there seems to be a low correlation between sunlight and SLA. Although sunlight does reach its maximum on the same date SLA reaches its maximum, on July 15th, when the SLA is lower than that of May 15th, the sunlight does not go below the sunlight data on May 15th. This result does not correspond with the study done by Meziane and Shipley, where the SLA of a plant is negatively correlated to the amount of light the leaf was exposed to. (Meziane and Shipley, 1999) The negative correlation between the light exposure and SLA is due to the tendency of shade leaves having a thinner palisade and spongy mesophyll layers, which is due to the increased photosynthetic rate under more exposure to light that increases the mass of the leaves. (Meziane and Shipley, 1999 ; Cooper and Wilson, 1969 ; Poorter et al., 2009 ; Lambers et al., 2008) However, the study only examined the difference between the sun leaves and shade leaves of an organism, thus the result does not directly indicate that sunlight is the abiotic factor that affected the seasonal trend in the SLA of *G. biloba*.

Temperature and SLA:
From *Graph 5* it can be known that temperature also has a low correlation with SLA values. According to a study done by Lee, Reich, and Bolstad, the mass of leaves tends to increase as the temperature increases. (Lee, Reich, and Bolstad, 2005) The increase in mass is due to the increase in the rate of photosynthesis, since the rate of photosynthesis increases with the temperature increase. (BBC, 2014) With the increase in mass, the SLA of the leaves will, therefore, increase. However, although *Graph 5* suggests a maximum value of both SLA and temperature on June 15$^{th}$; on May 15$^{th}$, the temperature is low, but the value of the SLA is relatively high; and on July 15$^{th}$, the temperature of the weather is higher than that of May 15$^{th}$, but the value of the SLA is lower than that of May 15$^{th}$. Thus, the result seems to not correlate with the findings of Lee, Reich, and Bolstad. This indicates that temperature is also not the factor that affects the value of SLA of *Ginkgo biloba*.

Humidity and SLA:
According to Rawson, Begg, and Woodward, humidity and SLA values should show a negative correlation. (Rawson, Begg, and Woodward, 1977) As the humidity increase, the photosynthetic rate increases, since more stomata open, and causes the leaves to become heavier, which leads to decrease SLA values. (Rawson, Begg, and Woodward, 1977) *Graph 5* thus suggests a negative correlation of humidity and SLA value. Relative humidity reaches its minimum on June 15$^{th}$, the date when SLA reaches its maximum. Not only that, the relative humidity even has a slight increase on June 1$^{st}$, when the SLA of *G. biloba* has the slight "dip", which makes it more clear that humidity has a significant effect on the value of SLA. Thus, to conclude, the trend in SLA of *G. biloba* was more related to the water use of the plant than any other reason.

**Effect of gender on the value of SLA:**
Other than abiotic factors, the gender of the trees is also a significant independent variable to consider. (Sánchez Vilas, 2007) Looking at *Graph 2*, SLA values of male leaves are found to be higher than those of female leaves. In order to find out the whether gender has a significant impact on the SLA values of leaves or not, student's t-test done on the SLA of different gender (**Data Processing**), and it was found that all of the leaves from May 15$^{th}$ to June 30$^{th}$ rejected the null hypothesis. This suggests that the gender of *G. biloba* is significant in determining the SLA of the leaves, which is also shown in other species such as *Oemleria cerasiformis*. (Allen and Antos, 1988) However, according to a study done by Laporte and Delph, the photosynthetic rate was found to be higher in males. (Laporte and Delph, 1996) This study implies that the SLA values of males should be lower than those of females, which undermines the result of *Graph 2*. Yet, in another study published by New Zealand Journal of Botany, it was found that female's import of carbon should be greater than males, due to its reproductive structures such as fruits. (Hogan et al., 1998) It is also noted in a study done by the International Institute for Earth System Science, the net photosynthetic rate of female Ginkgo tree is significantly higher than that of male Ginkgo tree. (Jing et al., 2008) Thus, with higher biomass and higher photosynthetic rate, the female leaves will have lower SLA values than the male leaves. Therefore, the lower SLA values for female leaves are defined.

**Effect of leaf positioning on SLA:**
As done with the gender, to determine whether the difference of SLA values of short shoot and long shoot leaves, student's t-test was done, and all the t-values of the data rejected the null hypothesis by a significant amount. This, therefore, showed that leaf positioning is significant in determining the SLA value of the leaves. Looking at *Graph 3*, the SLA values of the short shoot leaves tend to be bigger. This suggests that short shoot leaves have a bigger surface area, and a low mass compared to the surface area, according to the SLA equation. As stated by Christianson and Niklas, the SLA of the long shoot leaves should be the same as the SLA values of the short shoot leaves, and the reproductive leaves (leaves on short shoot) should have larger SLA values than the non-reproductive leaves. (Christianson and Niklas, 2011) This finding corresponds to the result of this research where the long shoot leaves, the non-reproductive leaves, have lower SLA values than those of the reproductive leaves, the short shoot leaves. The reason that reproductive leaves have a larger SLA might be due to the bigger carbon usage of the reproductive organs. (Hogan et al., 1998) The usage of carbon decreases the carbon accumulation in the leaves, thus decreasing the biomass of the leaves. (Gross and Soule, 1981) This is further supported by July 15$^{th}$ of *Graph 4* and student's t-test done about the effect of gender on SLA fails to reject the null hypothesis, since both shows the insignificance of the gender on the SLA of long shoot leaves, indicating how the long shoot leaves are non-reproductive leaves and that they do follow the findings of Hogan. (Hogan et al., 1998)

## Evaluation

Through out the research, there were some unforeseen uncertainties and errors that could have altered the data either to a minimal or a maximal extent.

The first uncertainty is the insufficient amount of long shoot leaves collected. For each collection, two branches were collected, yet there were times when branches did not even have 10 leaves on long shoot. This error could have caused generalization, by focusing only on a small amount of samples. On the other hand, the data yielded a clear trend and a correlation with other researches found.

Another uncertainty is the inaccuracy of the surface area of the leaves. In the process of drying the leaves, some of the leaves were folded and had to be flattened when scanned. Yet, there still were some leaves that could not be flattened, resulting in some covered up area. In the future, before the leaves are left to dry, it should be made sure that they are flat, and the leaves should be pushed down by a relatively heavy material to keep the leaves' shape and flatness. Not only that, the size of leaves can alter more than 18%, but not necessarily for Ginkgo. (Juneau and Tarasoff, 2012) This also could have changed the actual ratio between the biomass and the surface area, but all the leaves would have simultaneously shrunk that it did not affect the trend.

One significant concern about the investigation is the degree of agreement with the other researches. The reason is that although it was concluded in the discussion that temperature does not have a correlation with the SLA value of *Gingko biloba*, there are studies that support the relationship between SLA and temperature, and that the increase in temperature leads to an increase in SLA value. (Kumar, Singh and Boote, 2012) It is difficult to know whether it was the inaccuracy of the data or simply an accurate result of the data, but the disagreement increases doubt on the accuracy of the data.

In the future, for further research, the age of the tree and the height of the tree could be used for independent variables. Although the height was neglected in this study due to the morphology of *G. biloba*, there still could be correlation between the SLA values. Also, due to the limitation of the technology given, the age of the tree could not be determined, and thus had to be excluded. Thus, if given the sufficient technology and information to include the age of the tree, investigating the relationship between the SLA values of the leaves and the age of the tree would be compelling. Not only that, but pH and nutritional values of soil could also be included as one of the independent variables, since pH value significantly determines the nutritional values of the soil by allowing the survival of bacteria crucial for nitrogen production in soil. (Ward, 2015)

Had this research be adopted to other areas, agriculture, such as *Ginkgo biloba* farming, would gain significantly from the research. For example, with the information of how *G. biloba* photosynthesizes faster under humid area, and the larger amount of carbon required for the reproductive area, the farmers could increase the humidity of the farm in order to increase the productivity of the fruit. This will not only raise their revenues, but will also enhance the quality of the fruit and increase the number of fruits produced, which are used for medicines.

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**Appendix**

1. Raw data tables: data in the cells are the SLA value of individual leaves collected May 15th 2017

| Leaf No. | Female SLA ($cm^2$ $g^{-1}$) (±1 $cm^2$ $g^{-1}$) | | | | Male SLA ($cm^2$ $g^{-1}$) (±1 $cm^2$ $g^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Short shoot | | Long shoot | | Short shoot | | Long shoot | |
| | A | B | A | B | A | B | A | B |
| 1 | 114.50 | 132.06 | 111.27 | 115.34 | 134.92 | 159.88 | 122.78 | 142.88 |
| 2 | 115.49 | 129.41 | 117.70 | 127.76 | 94.01 | 144.42 | 127.25 | 120.63 |
| 3 | 115.19 | 129.27 | 117.77 | 115.58 | 149.34 | 87.65 | 127.97 | 120.92 |
| 4 | 130.49 | 150.33 | 111.60 | 119.61 | 131.70 | 168.30 | 124.12 | 125.28 |
| 5 | 142.88 | 130.34 | 110.71 | 120.03 | 161.74 | 150.50 | 118.02 | 107.34 |
| 6 | 117.74 | 132.23 | 107.92 | 119.10 | 130.64 | 146.57 | 121.54 | 136.77 |
| 7 | 148.93 | 127.36 | 103.02 | 113.28 | 152.11 | 169.56 | 102.04 | 154.11 |
| 8 | 116.28 | 151.47 | 107.93 | 117.30 | 136.14 | 159.16 | 135.13 | 126.96 |
| 9 | 132.01 | 132.16 | 113.41 | 120.70 | 150.63 | 165.49 | 129.75 | 128.81 |
| 10 | 152.64 | 147.76 | 109.59 | 131.75 | 136.22 | 141.64 | 89.86 | 118.75 |

June 1st 2017

| Leaf No. | Female SLA ($cm^2\ g^{-1}$) ($\pm 1\ cm^2\ g^{-1}$) | | | | Male SLA ($cm^2\ g^{-1}$) ($\pm 1\ cm^2\ g^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Short shoot | | Long shoot | | Short shoot | | Long shoot | |
| | A | B | A | B | A | B | A | B |
| 1 | 140.96 | 117.19 | 112.99 | 115.33 | 134.92 | 159.88 | 122.78 | 142.88 |
| 2 | 141.29 | 129.86 | 107.67 | 144.95 | 94.01 | 144.42 | 127.25 | 120.63 |
| 3 | 114.50 | 123.14 | 107.99 | 118.43 | 149.34 | 87.65 | 127.97 | 120.92 |
| 4 | 129.73 | 117.11 | 108.13 | 111.81 | 131.70 | 168.30 | 124.12 | 125.28 |
| 5 | 122.91 | 118.47 | 30.43 | 113.65 | 161.74 | 150.50 | 118.02 | 107.34 |
| 6 | 118.77 | 126.17 | 112.53 | 116.80 | 130.64 | 146.57 | 121.54 | 136.77 |
| 7 | 116.59 | 124.85 | 105.40 | 107.06 | 152.11 | 169.56 | 102.04 | 154.11 |
| 8 | 134.58 | 137.29 | 119.14 | - | 136.14 | 159.16 | 135.13 | 126.96 |
| 9 | 116.71 | 137.49 | 111.12 | - | 150.63 | 165.49 | 129.75 | 128.81 |
| 10 | 113.38 | 122.34 | 101.79 | - | 136.22 | 141.64 | 89.86 | 118.75 |

June 15th 2017

| Leaf No. | Female SLA ($cm^2\ g^{-1}$) ($\pm 1\ cm^2\ g^{-1}$) | | | | Male SLA ($cm^2\ g^{-1}$) ($\pm 1\ cm^2\ g^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Short shoot | | Long shoot | | Short shoot | | Long shoot | |
| | A | B | A | B | A | B | A | B |
| 1 | 155.92 | 123.02 | 114.22 | 99.24 | 123.75 | 158.53 | 100.12 | 144.97 |
| 2 | 161.68 | 121.00 | 112.63 | 111.10 | 142.79 | 164.75 | 117.62 | 143.01 |
| 3 | 145.44 | 121.68 | 99.86 | 116.83 | 127.02 | 94.20 | 133.48 | 124.35 |
| 4 | 129.61 | - | 133.32 | 107.41 | 118.94 | 181.93 | 119.93 | 115.55 |
| 5 | 154.55 | 114.25 | - | 111.08 | - | 193.64 | 114.93 | 123.92 |
| 6 | 145.49 | 113.27 | 132.71 | 103.04 | 136.81 | 152.07 | 66.48 | 142.68 |
| 7 | 146.01 | 126.15 | 107.95 | 107.07 | 140.07 | 148.49 | 127.50 | 131.03 |
| 8 | 150.48 | 141.83 | 138.44 | 100.82 | 136.20 | 155.04 | 115.19 | 127.43 |
| 9 | 131.43 | 123.31 | - | 109.17 | 167.31 | 163.64 | 126.21 | 129.96 |
| 10 | 122.40 | 118.29 | 132.91 | 94.29 | 184.26 | 154.45 | 109.92 | 131.12 |

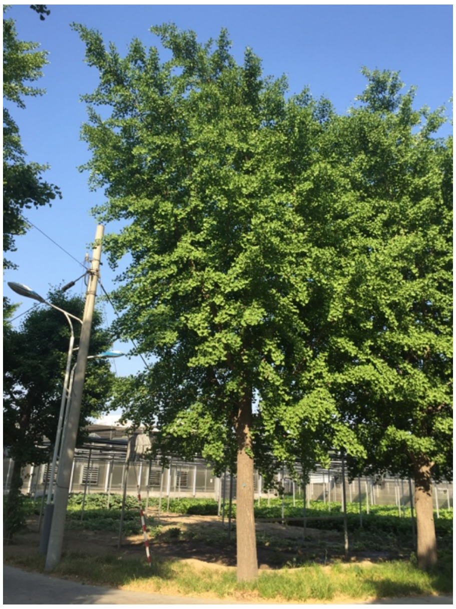
June 30th 2017

| Leaf No. | Female SLA ($cm^2\ g^{-1}$) ($\pm 1\ cm^2\ g^{-1}$) | | | | Male SLA ($cm^2\ g^{-1}$) ($\pm 1\ cm^2\ g^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Short shoot | | Long shoot | | Short shoot | | Long shoot | |
| | A | B | A | B | A | B | A | B |
| 1 | 122.55 | 118.60 | 116.23 | 99.81 | 170.01 | 146.17 | 96.50 | 121.31 |
| 2 | 109.06 | 125.81 | 108.70 | 119.69 | 152.66 | 174.84 | 122.32 | 121.91 |
| 3 | 135.79 | 140.49 | 114.19 | 115.67 | 167.10 | 144.90 | 93.06 | 119.02 |
| 4 | 111.84 | 112.12 | 100.59 | 106.23 | 161.57 | 147.62 | 125.03 | 114.07 |
| 5 | 131.45 | 108.94 | - | 109.56 | 175.93 | 167.32 | 95.95 | 118.50 |
| 6 | 147.89 | 113.92 | - | - | 148.95 | 164.97 | 107.11 | 134.64 |
| 7 | 121.24 | 116.46 | - | - | 158.89 | 141.34 | 102.98 | 143.97 |
| 8 | 120.47 | 118.72 | - | - | 143.44 | 145.34 | 105.14 | 132.15 |
| 9 | 107.70 | 111.59 | - | - | 177.03 | 176.62 | 97.46 | 115.45 |
| 10 | 120.92 | 114.32 | - | - | 147.28 | 145.74 | 97.26 | 105.72 |

July 15$^{th}$ 2017

| Leaf No. | Female SLA ($cm^2\ g^{-1}$) ($\pm 1\ cm^2\ g^{-1}$) | | | | Male SLA ($cm^2\ g^{-1}$) ($\pm 1\ cm^2\ g^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | Short shoot | | Long shoot | | Short shoot | | Long shoot | |
| | A | B | A | B | A | B | A | B |
| 1 | 117.56 | 119.39 | 97.50 | 102.82 | 159.17 | 109.34 | 102.49 | 86.50 |
| 2 | 109.49 | 117.02 | 100.13 | 98.01 | 138.20 | 109.53 | 95.14 | 87.17 |
| 3 | 133.57 | 118.90 | 100.12 | 104.26 | 153.40 | 140.34 | 88.63 | 103.29 |
| 4 | 102.66 | 143.01 | 101.38 | 79.47 | 153.63 | 96.46 | 87.97 | 93.89 |
| 5 | 105.95 | 112.93 | 103.00 | 105.72 | 108.67 | 112.03 | 86.35 | 100.35 |
| 6 | 129.14 | 122.03 | - | - | 118.10 | 135.33 | 94.15 | 104.83 |
| 7 | 112.16 | 135.51 | - | - | 143.98 | 100.75 | 113.96 | - |
| 8 | 105.06 | 121.44 | - | - | 144.59 | 139.92 | 112.91 | - |
| 9 | 113.14 | 106.10 | - | - | 146.95 | 111.60 | 112.37 | - |
| 10 | 100.39 | 109.66 | - | - | 140.92 | 140.42 | 117.94 | - |

Female *Ginkgo biloba* tree: from which all female leaf samples were collected



Male *Ginkgo biloba* tree: from which all male leaf samples were collected