



# Cotton under leaves – where do they go?

Pope Paul VI College  
4B Margaret Tse  
Priscilla Tse  
Rachel Lo  
Carrie Wong  
4C Stephanie Lam  
Teacher: Miss Kristy Chung

## Contents

Introduction .....	3
Our observation leading to the current study.....	3
Background information of <i>Icerya Aegyptiaca</i> .....	3
Synopsis .....	3
Features.....	4
Geographical distribution .....	4
Host.....	4
Life stages .....	4
Objective of the study .....	4
Materials and methods.....	5
Record of field trips: .....	5
Procedures and principles of experiments.....	6
Results .....	12
Discussion .....	16
Conclusion .....	17
Bibliography.....	18

## **Abstract**

In this study, the factors affecting the distribution of *Icerya Aegyptiaca* were investigated. The field study was carried out in the roadside outside our school in Shek Lei and the countryside in Tuen Mun, Hung Shui Kiu. Some leaves from Tuen Mun were also collected and put into different conditions. It was found that *I. Aegyptiaca* preferred countryside to roadside, upper side of the leaves. The density of the distribution of them decreased when the distance from the leaf increased. They preferred the leaf with more green, reducing sugar and thinner cuticle. Light intensity had no effect on their distribution. The occurrence of *I. Aegyptiaca* is more in countryside than roadside. The white wax secretion is hydrophobic which may probably use for excretion of reducing sugar.

## **Introduction**

## **Our observation leading to the current study**

When we joined this competition, we walked around the street to see if we could get some inspiration from the nature. Then we observed some white ‘cotton-like’ things on the leaves in the street and found them strange. Before that we think they are the secretions of some animals but when we look at them more carefully, we find that they are moving! They appeared on the trees outside our school. However, we could not find any of them in the school. We discover that they only appear in some kinds of tree and even some parts of a tree only. We want to know the factors that affect their occurrence.

## **Background information of *Icerya Aegyptiaca***

### **Synopsis**

*Icerya Aegyptiaca* (fig. 1) is a kind of large scale insects under Section *Icerya* scale insects. Their geographical distribution is extensive, especially in tropical regions. Their size is usually small. All of them are herbivorous. They rely on their mouthparts piercing in plants for saps as food to live. They live on the dorsal of leaf, usually scatter along the vein. They have a big ethnic to live collectively. Most of them cluster on the vein and stem inhaling sap. This action reduces plant vigor, lead to poor growth, dead branches, leaf drop, and even plant death.

(fig. 1)



Because of this, they are defined as pests. Some types of scale insect can spread plant diseases which makes plants suffer greater damages. (羅幹成，2003)

## **Features**

Adults are oval-shaped, their front-ended are slightly pointed, and their skin is membranous. There are clusters of long wax filaments on their body side, their body length are around 2.8-5.3 mm, width 2.0-3.7 mm (fig. 2). Their body color is red-browned but covered by white wax powder. There are strips of white wax wire on their ventral side.

(fig. 2)



## **Geographical distribution**

Distributed in the plains to low and medium Elevation Mountain Area, Asia (Taiwan, China, Hong Kong, Japan, India, Sri Lanka); Africa (Egypt); Oceania (Australia)

## **Host**

Mainly mulberry, peach, pear, apple, cherry, grapes, persimmons, figs, loquat, coconut, mango, eggplant son, tea, roses and other crops. (王文哲，2000)

## **Life stages**

### *Egg*

Oval-shaped. Orange, the size about 0.65x0.29 mm

### *Nymphs*

When newborn, flat oval-shaped. Their color is dull red and not covered by white wax.

About 0.6mm to 0.7mm. In the growth process, they are covered with white wax powder gradually.

### *Pupa*

Male pupae grows into oblong shape. Their bodies are in pale red color, about 3mm to 4mm. They are staying in a spongiform white cocoon.

### *Adult*

Female adults have no wing, their bellies are flat, back are swelled. Their bodies are in tortoiseshell and structures are soft. The color is yellowish. Some black short hair are found on their body densely, also their body are covered by some white waxy substance which is used to secrete flocculent wax line.

## **Objective of the study**

In this investigation, we are going to find out the factors affecting the distribution of *Icerya Aegyptiaca* and the function of the white wax secretion.

# Materials and methods

## Record of field trips:

Venue	Roadside (Shek Lei, outside the school) (fig. 3)			Countryside (Tuen Mun, Hung Shui Kiu) (fig. 4)		
Date	30/1 13:00-13:30	27/2 13:00-14:00	21/3 16:00-17:30	2/2 13:00-15:00	11/3 13:00-15:00	20/3 13:00-15:00
Temperature (°C)	13.2	18.3	23.6	20.1	17.9	24.7
Light intensity (lux)	224	301	267	332	250	344
Relative humidity	77.0%	80.0%	88.0%	78.0%	88.0%	62.0%
Wind speed (km/h)	3.1	4.2	7.6	2.2	4.7	11.2
Soil temperature (°C)	14	18	20	19	16	23
Air Quality Health Index (from Environmental Protection Department)	6	4	5	4	4	3

Table 1 shows the records and physical factors of our field visits



(fig. 3)



(fig. 4)

# Procedures and principles of experiments

During or after each field visit, we conducted our experiments into four main directions:

- i) Compare the distribution of *I. Aegyptiaca* on the parts of the tree in roadside with countryside
- ii) Compare the distribution of *I. Aegyptiaca* on leaves from countryside in different conditions
- iii) Compare the content of the leaf juice and the body fluid of *I. Aegyptiaca*
- iv) Attempt to explain the function of white wax secretion in the aspect of nutrition

- i) Compare the distribution of *I. Aegyptiaca* on the parts of the tree in roadside with countryside
- Principles

We compared the physical factors in table 1 between roadside and countryside and counted the number of *I. Aegyptiaca* on different parts of the tree. We could know the preferable living environment of *I. Aegyptiaca* afterwards.

## Materials

<u>Materials</u>	<u>quantity</u>
-trees with <i>I. Aegyptiaca</i> rely on	3
-forceps	2
-binocular	1

## Procedure

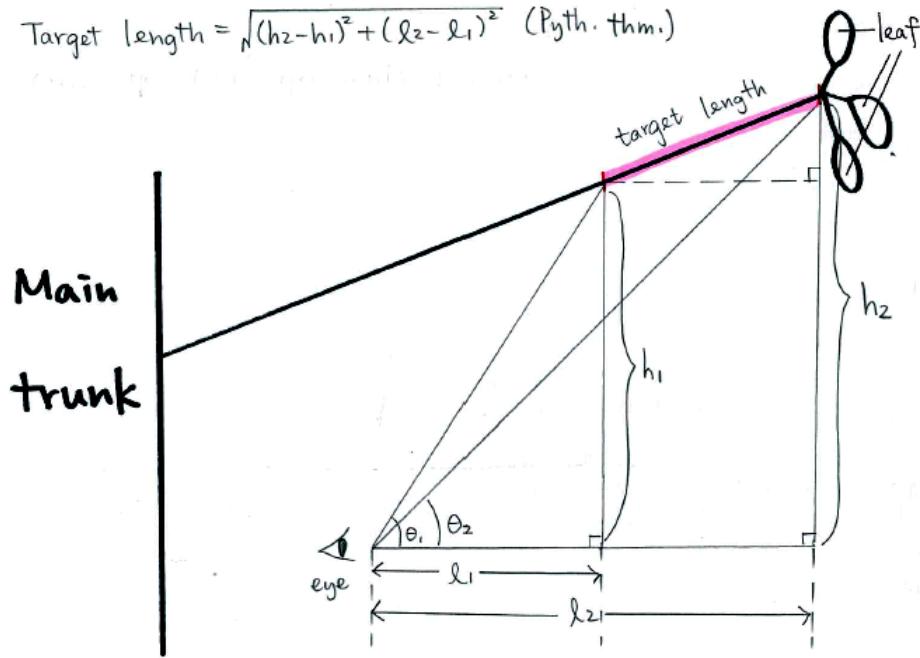
1. Three trees which had *I. Aegyptiaca* to rely on were selected randomly in roadside and countryside respectively.
2. The number of *I. Aegyptiaca* on 10 randomly selected leaves on a tree was counted and the average number was taken. If the leaf was too far away, binocular was used.
3. The region from the selected leaf to the main trunk was divided into three parts equally. The number of *I. Aegyptiaca* on the leaf stalk, the first part closer to leaf, the second part close to the leaf and the last part far away from the leaf was estimated. The method for estimating the length of the branch segment was shown in fig. 5 below.

We can measure  $\theta_1$ ,  $\theta_2$ ,  $l_1$ ,  $l_2$ .

$$h_1 = l_1 \tan \theta_1$$

$$h_2 = l_2 \tan \theta_2$$

$$\text{Target length} = \sqrt{(h_2 - h_1)^2 + (l_2 - l_1)^2} \quad (\text{Pyth. thm.})$$



(fig. 5)

4. Repeat steps (2) and (3) on the two other trees in roadside and countryside respectively.
5. Repeat steps (1) to (4) on two other days.
6. The average number of *I. Aegyptiaca* was calculated and a graph of the distribution was plotted.

#### Remarks

1. More leaves were counted so as to make the result more reliable.
2. The experiment was repeated for three times with different trees to increase the representativeness of the result.
3. The experiment was repeated on three different days because we could not go to the roadside and countryside for field visit at the same time. We visited the place for three times to minimize the random errors due to the change in physical factors.

- ii) Compare the distribution of *I. Aegyptiaca* on leaves from countryside in different conditions

#### Principles

We observed that *I. Aegyptiaca* was most likely found on the leaves. We could know the conditions of leaves which was preferred by *I. Aegyptiaca* by using leaves with different surface area, degree of 'greenness', number of veins, amount of sugar. Degree of 'greenness' might indicate the amount of minerals in the leaves. Number of veins on leaf might indicate the easy accessibility of nutrients. Therefore, we could determine whether the distribution of *I. Aegyptiaca* was affected by the availability of several nutrients.

Meanwhile, the preference of *I. Aegyptiaca* on light intensity and wax layer could be known by putting them into different conditions.

## Materials

<u>Materials</u>	<u>quantity</u>
-leaves with <i>I. Aegyptiaca</i>	10
-filter funnel	1
-forceps	several
-scissors	1
-mortar and pestle	1
-syringe	1
-dropper	4
-muslin cloth	1
-test tube	several
-Benedict's solution	5cm <sup>3</sup>
-DCPIP solution	5cm <sup>3</sup>
-Albstix paper	1

## Procedure

1. Ten leaves with *I. Aegyptiaca* were collected randomly from the countryside.
2. The leaves were scattered in an area at the back of the biology laboratory with dorsal side facing upwards.
3. *I. Aegyptiaca* were redistributed evenly on the area with the leaves.
4. The number of *I. Aegyptiaca* on each piece of leaf was counted after two days. (fig. 6)
5. *I. Aegyptiaca* were removed from each leaf.
6. The surface area of each leaf was found by outlining the leaf on a piece of graph paper.
7. The greenness of each leaf was rated.
8. The number of veins of each leaf was counted
9. A portion of the leaf were cut out and squeezed into leaf juice. (fig. 7)



(fig. 6)



(fig. 7)

10. The leaf juice was taken to perform Benedict's test.

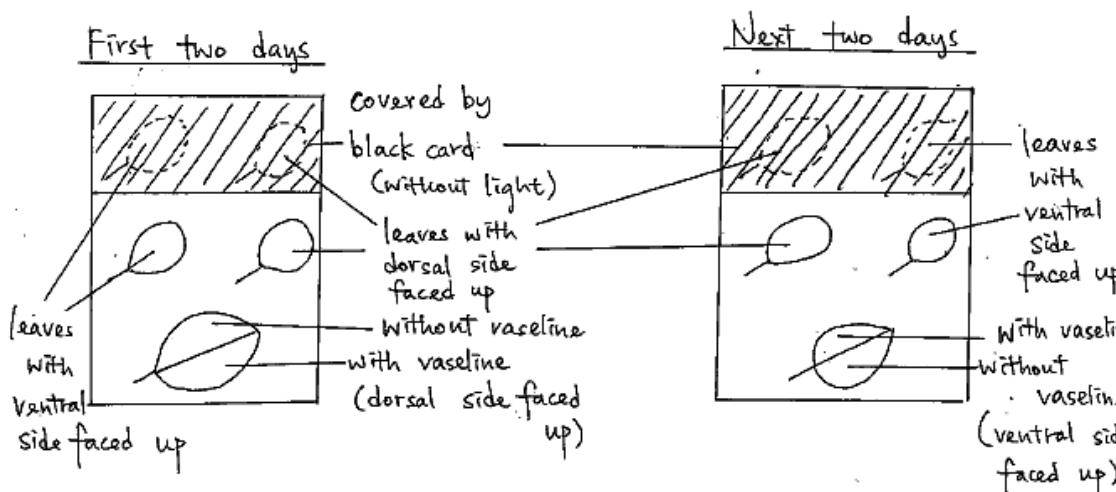
#### Remarks

1. *I. Aegyptiaca* should be isolated first and redistributed evenly to different area as they might have the tendency to stay on the original leaves. The initial number might affect the result.
2. *I. Aegyptiaca* might have preference on dorsal side of the leaves. All the leaves should have dorsal side facing upwards to make a fair test.

#### Procedure

11. Five pieces of leaves were collected randomly from the countryside.

12. The leaves were put in a tray with the conditions listed in fig. 8 below.



(fig. 8)

13. *I. Aegyptiaca* were redistributed evenly on the area with the leaves.

14. The number of *I. Aegyptiaca* on different regions was counted after two days.

15. All the leaves were turned over and *I. Aegyptiaca* were redistributed evenly on the area.

16. The number of *I. Aegyptiaca* on different regions was counted after two days.

#### Remarks

1. The leaves were turned over and the procedures were repeated so as to minimize the effect of random errors about individual leaves.

- iii) Compare the content of the leaf juice and the body fluid of *I. Aegyptiaca*

#### Principles

We assumed that the distribution of *I. Aegyptiaca* was related to the amount of nutrients which they could get. By comparing the content of the leaf juice and the body fluid of *I. Aegyptiaca*, we could understand the preference of the nutrients of *I. Aegyptiaca* and speculate the function of its white wax secretion.

#### Materials

<u>Materials</u>	<u>quantity</u>
<i>I. Aegyptiaca</i>	1g
leaves	1g
distilled water	20mL
mortar and pestle	1
filter funnel and muslin cloth	1
Benedict's solution	5cm <sup>3</sup>
iodine solution	5cm <sup>3</sup>

#### Procedure

1. One gram of *I. Aegyptiaca* was prepared.
2. The epithelium of *I. Aegyptiaca* was pierced and their body fluid was extracted with 10mL distilled water. (fig. 9)



(fig. 9)

3. Benedict's test and iodine test were carried out by using the body fluid of *I. Aegyptiaca*. Steps (1) and (2) were repeated when the fluid was not enough.
4. One gram of leaves was prepared.
5. The leaves were ground with 10mL distilled water.
6. The mixture was filtered to obtain the leaf juice.
7. Step (3) was repeated by using leaf juice.
8. The experiment was repeated for three times.

### Remarks

1. The weight of *I. Aegyptiaca* and leaves and the volume of distilled water for extracting the juice should be the same.
- iv) Attempt to explain the function of white wax secretion in the aspect of nutrition

### Principles

As we knew that *I. Aegyptiaca* might require sugar as nutrients, we provided *I. Aegyptiaca* with glucose, sucrose, starch and the leaves to see if *I. Aegyptiaca* prefer solely the sugar.

Moreover, we found that the content of reducing sugar in the body of *I. Aegyptiaca* was slightly more than that in the leaf. We speculated that *I. Aegyptiaca* needed to excrete the excess sugar. To make the sugar far away from its body, *I. Aegyptiaca* might need something hydrophobic in order to get rid of the water-soluble sugar. By dissolving the white wax into oil and water respectively, we knew more about the nature of the white wax. By collecting the substances on the tip of the white wax, we could check whether the sugar excreted was isolated by the white wax or not.

### Materials

<u>Materials</u>	<u>quantity</u>
White wax from the <i>I. Aegyptiaca</i>	Several
water	5mL
oil	5mL
dropper	3
test tubes	2
Benedict's solution	5cm <sup>3</sup>
hot water bath	1
Starch solution	5mL
Glucose solution	5mL
Sucrose solution	5mL
Leaves	3 pieces
<i>I. Aegyptiaca</i>	Several

### Procedure

1. *I. Aegyptiaca* was put into the middle of the tray with starch solution, glucose solution, sucrose solution and leaves at the four corners respectively. (fig. 10)



(fig. 10)

2. The number of *I. Aegyptiaca* at each corner was counted after 2 days.
3. White wax secretion from the *I. Aegyptiaca* was collected.
4. The white wax was put into 5mL of water and the degree of solubility was recorded.
5. The white wax was put into 5mL of oil and the degree of solubility was recorded. (fig. 11)
6. The substances on the tip of the white wax were collected by using a dropper.
7. Benedict's test was carried out by using the substances collected.



(fig. 11)

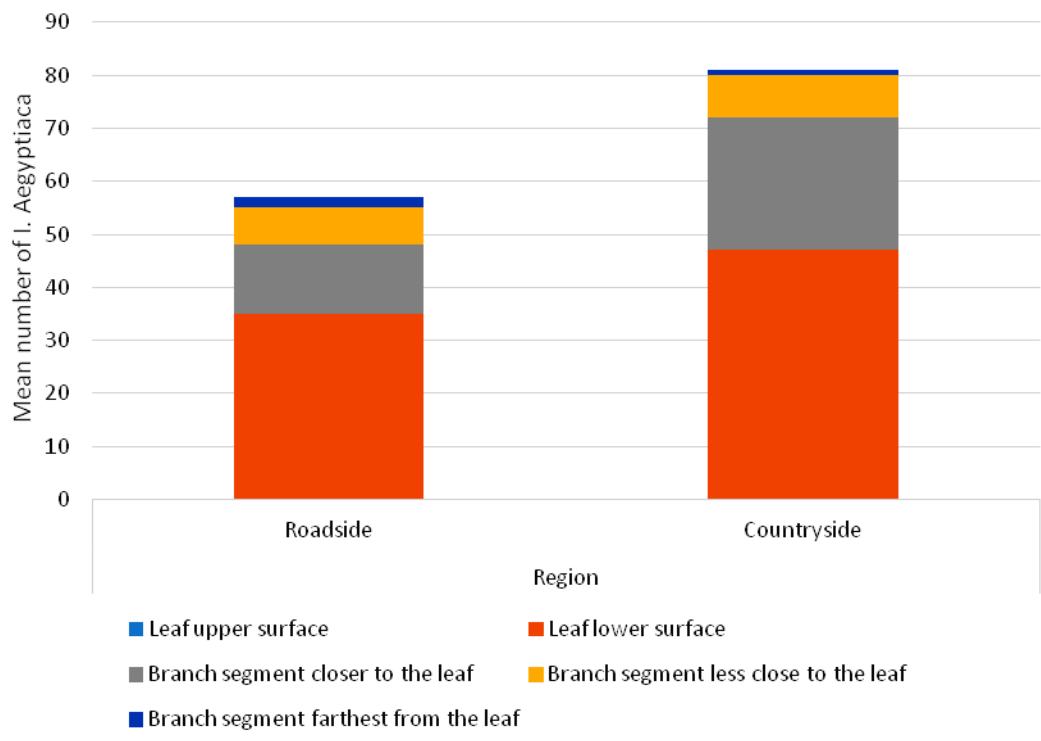
## Results

i) Compare the distribution of *I. Aegyptiaca* on the parts of the tree in roadside with countryside

Venue	Average number of <i>I. Aegyptiaca</i> on ten leaves on three trees on three different days
Roadside	35.94
Countryside	47.29

Table 2 shows the average number of *I. Aegyptiaca* on ten leaves on three trees on three different days in roadsides and countryside

Graph showing the distribution of *I. Aegyptiaca* in different parts of the leaf



Graph 1

According to table 2, the average number of *I. Aegyptiaca* on ten leaves in countryside was larger than that in roadside.

According to graph 1, we found that there was no *I. Aegyptiaca* on the upper surface of the leaf.

*I. Aegyptiaca* seldom appeared on the upper surface of the leaf while lower surface of the leaf was the most popular place for *I. Aegyptiaca*. Furthermore, the farther away from the leaf, the parts of the branch segment contained fewer *I. Aegyptiaca*.

ii) Compare the distribution of *I. Aegyptiaca* on leaves from countryside in different conditions

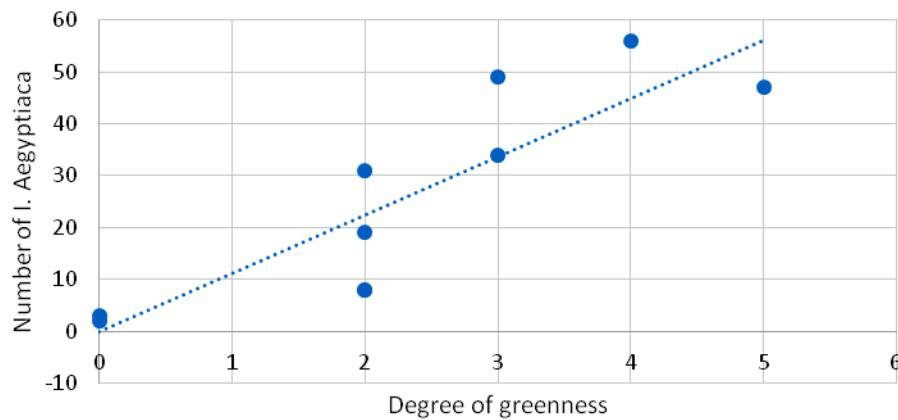
Leaf	Surface area (cm <sup>2</sup> )	Degree of greenness	Number of veins	Amount of reducing sugar	Number of <i>I. Aegyptiaca</i>
A	227	++	10	++	19
B	499	+++	12	++	49
C	327	++	12	++	34
D	573	++	12	+	31
E	394	++++	14	+++	56
F	491	+++++	14	+++	47
G	355	++	12	+	8
H	235	-	14	-	3
I	351	+++	12	++	8
J	378	-	14	-	2

Table 3 shows the number of *I. Aegyptiaca* on leaves with different conditions accordingly

Key: “-“ represents absence of the condition

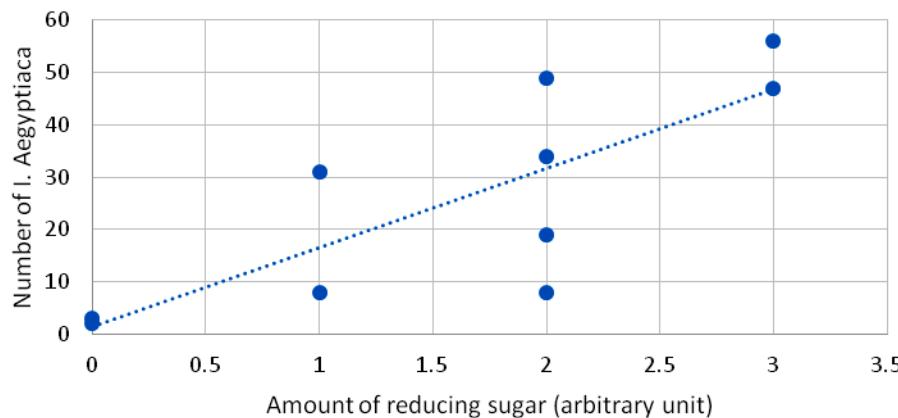
“+” to “+++++” represents from “low” to “high” abundance of the condition

Scatter diagram of number of *I. Aegyptiaca* in different degree of greenness



Graph 2

Graph showing number of *I. Aegyptiaca* on the leaf with different amount of reducing sugar



Graph 3

According to graph 2, we could see that when the leaf was more green, there were more *I. Aegyptiaca*.

According to graph 3, we could see that when the leaf contained more reducing sugar, there were more *I. Aegyptiaca*. (fig. 12 and fig. 13)



(fig. 12)



(fig. 13)

Leaf	The side facing upwards	Light intensity	Number of <i>I. Aegyptiaca</i>	
1	Dorsal	With light	Upper surface: 1	Lower surface: 14
2	Ventral	With light	Upper surface: 2	Lower surface: 12
3	Dorsal	Without light	Upper surface: 0	Lower surface: 17
4	Ventral	Without light	Upper surface: 1	Lower surface: 13
1 (2 <sup>nd</sup> time)	Ventral	With light	Upper surface: 0	Lower surface: 15
2 (2 <sup>nd</sup> time)	Dorsal	With light	Upper surface: 0	Lower surface: 14
3 (2 <sup>nd</sup> time)	Ventral	Without light	Upper surface: 1	Lower surface: 12
4 (2 <sup>nd</sup> time)	Dorsal	Without light	Upper surface: 1	Lower surface: 16

Table 4 shows the number of *I. Aegyptiaca* in different conditions

According to table 4, we could see that the light intensity had no effect on the distribution of *I. Aegyptiaca*. No matter how the leaf was put, there were still more *I. Aegyptiaca* on the lower surface than the upper surface.

	Number of <i>I. Aegyptiaca</i>
With vaseline	5
Without vaseline	33

Table 5 shows the number of *I. Aegyptiaca* in the region with and without Vaseline

According to table 5, there was more *I. Aegyptiaca* in the region without Vaseline.

iii) Compare the content of the leaf juice and the body fluid of *I. Aegyptiaca*

Content	Body fluid of <i>I. Aegyptiaca</i>	Leaf juice
Reducing sugar (Average)	+++	++
Starch	Presence	Presence

Table 6 compares the content of the leaf juice and the body fluid of *I. Aegyptiaca*

Key: “+” to “++++” represents from “low” to “high” amount of reducing sugar accordingly

According to table 6, we found that there was slightly more reducing sugar in the body fluid of *I. Aegyptiaca* than leaf juice while both leaf juice and the body fluid of *I. Aegyptiaca* contained starch.

iv) Attempt to explain the function of white wax secretion in the aspect of nutrition

Content	Number of <i>I. Aegyptiaca</i>
Starch solution	0
Glucose solution	0
Sucrose solution	0
Leaves	16

Table 7 shows the number of *I. Aegyptiaca* in the regions with different content

According to table 7, we found that *I. Aegyptiaca* preferred leaves more than the nutrients solely. We also found that the white wax dissolves far more in oil than water. This showed that the white wax was hydrophobic.

However, we got negative result in Benedict's test as we might not collect enough substances from the tips of white wax for our analysis.

## Discussion

### Interpretation of the results

i) Compare the distribution of *I. Aegyptiaca* on the parts of the tree in roadside with countryside. We found that the average number of *I. Aegyptiaca* on ten leaves in countryside was larger than that in roadside. The physical factors we measured were similar in both roadside and countryside except that the Air Quality Health Index given by the Environment Protection Department was higher in roadside than the countryside. The number of *I. Aegyptiaca* was higher might be due to the fact that there is more human disturbance in the roadside. The leaf might be fallen more easily and the tree might have fewer nutrients which may affect the nutrition received by the *I. Aegyptiaca*.

We also found that *I. Aegyptiaca* seldom appeared on the upper surface of leaf but abundant on the lower surface. This might be because the upper surface of the leaf has thicker cuticle which may impede *I. Aegyptiaca* to obtain the nutrients from the leaves. Furthermore, the farther away from the leaf, the parts of the branch segment contained fewer *I. Aegyptiaca*. This might be because the lower the branch, the thicker the surface layer on the trunk. *I. Aegyptiaca* may find it more difficult to get the nutrients.

ii) Compare the distribution of *I. Aegyptiaca* on leaves from countryside in different conditions

We found that when the leaf was more green, there were more *I. Aegyptiaca*. As greenness may reflect the amount of minerals in the leaf, more *I. Aegyptiaca* may attach to the leaf with more minerals.

We also found that when the leaf contained more reducing sugar, there were more *I. Aegyptiaca*.

This shows that *I. Aegyptiaca* may prefer the place with more nutrients.

We saw that the light intensity had no effect on the distribution of *I. Aegyptiaca* and the presence of vaseline might hamper the presence of *I. Aegyptiaca*. This shows that *I. Aegyptiaca* needs somewhere which is easily accessible to the inside of the vein. Vaseline and thick cuticle may block their ways. This shows the reason why *I. Aegyptiaca* prefer the lower surface of the leaf.

iii) Compare the content of the leaf juice and the body fluid of *I. Aegyptiaca*

We found that there was slightly more reducing sugar in the body fluid of *I. Aegyptiaca* than leaf juice. This was quite surprising as we believed that *I. Aegyptiaca* obtains reducing sugar from the leaf because they do not have enough. This may be because *I. Aegyptiaca* needs nutrients such as proteins, minerals etc. other than reducing sugar. However, those nutrients are in very small amount in the leaf juice. Therefore, *I. Aegyptiaca* needs to obtain a large amount of leaf juice which inevitably increases the amount of reducing sugar absorbed. As the reducing sugar obtained may be in excess, *I.*

*Aegyptiaca* needs to excrete the sugar.

iv) Attempt to explain the function of white wax secretion in the aspect of nutrition

We found that *I. Aegyptiaca* preferred leaves rather than starch, sucrose or glucose solution solely.

This shows that leaves contain other things which are essential to *I. Aegyptiaca*. This may match our speculation that *I. Aegyptiaca* needs other nutrients in the leaf juice other than reducing sugar.

We also found that the white wax dissolved far more completely in oil than water. This showed that the white wax was hydrophobic. This matches our speculation that *I. Aegyptiaca* needs hydrophobic white wax to eliminate the reducing sugar far away from their bodies as reducing sugar is water soluble.

However, we got negative result in Benedict's test as we might not collect enough substances from the tips of white wax for our analysis. Therefore, our hypothesis that *I. Aegyptiaca* excrete reducing sugar with the help of the white wax could not be further confirmed.

#### Implication of the results

As most people regarded *I. Aegyptiaca* as 'pest', by knowing the preferable conditions for *I. Aegyptiaca* to occur, we could know more about how to prevent the 'outburst' of the growth of *I. Aegyptiaca*.

#### Limitation of the experiment and suggestion for improvement

1. Our field study is carried out during late January to mid March which may not be the peak season of the growth of *I. Aegyptiaca*. The representativeness of the results about the distribution of *I. Aegyptiaca* was reduced because *I. Aegyptiaca* may have different growth pattern in other seasons with different physical factors. It is suggested that the study can extend throughout the year.

2. As our discussion involves the amount of sugar, we do not have an accurate measurement on this because Benedict's test just reflected a rough amount of reducing sugar. The determination of the colour and the amount of precipitates formed are subjective.

As the difference of the amount of sugar among the leaf juices and body fluid is very small, it is difficult to draw conclusion that the amount of sugar among them has significant differences. At the same time, the results could not directly show the amount of non-reducing sugar like sucrose. To improve, we may use more precise equipments such as light spectrometer or colorimeter instead of naked eye. We also may add hydrochloric acid to allow the hydrolysis of sucrose into reducing glucose and fructose first before doing Benedict's test.

#### Suggested further studies

1. We only study the amount of reducing sugar in leaf juice only. *I. Aegyptiaca* may obtain other nutrients such as proteins, lipids, vitamins etc. which may also affect their occurrences on the leaf. It is suggested that the content of other nutrients can also be included in the investigation.
2. In this study, we only focus on the dicotyledonous plant but not the monocotyledons. We can also include the monocotyledons and study why *I. Aegyptiaca* do not prefer parallel veins.

# Conclusion

1. The occurrence of *I. Aegyptiaca* is more in countryside than roadside.
2. *I. Aegyptiaca* prefer lower side of the leaf more than upper side. The density of *I. Aegyptiaca* on the trunk decreases when the distance away from the leaf increases.
3. *I. Aegyptiaca* prefer the leaf with more green and more reducing sugar.
4. Light intensity has no effect on the distribution of *I. Aegyptiaca* while they prefer the leaf with thinner cuticle.
5. The body fluid of *I. Aegyptiaca* contains more reducing sugar than the leaf juice. It is probable that they excrete the reducing sugar out of their bodies.
6. The white wax secretion is hydrophobic which may probably use for excretion of reducing sugar.

# Bibliography

1. 羅幹成，2003。吹棉介殼蟲，植物保護圖鑑系列 9- 柑橘保護，防檢局，台北。第 20-24 頁。
2. 王文哲，2000。台中區農情月刊第 6 期-常見介殼蟲介紹。2015 年 3 月 24 日下載自  
[http://tdares.coa.gov.tw/show\\_monthly.php?id=tdais\\_tdais\\_edit\\_20080312011343](http://tdares.coa.gov.tw/show_monthly.php?id=tdais_tdais_edit_20080312011343)