The Effects of Dryness and Cellulase activity on Pod Dehiscence of Bauhinia variegata

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Abstract

Bauhinia variegata (宮粉羊蹄甲) is a commonly found tree which has pink flowers with dehiscent pods. Its pods dehisce when they turn black and mature in dry season. In this investigation, a series of observations and experiments were carried out to study the effect of dryness and activity of cellulase on the dehiscence of its pods. We observed that the pods dehisced when the dryness was higher and thus hydronasty was exhibited (*Wikipedia*). It was also found that the cellulase content was higher when the pods splited open. This shows that dehiscence of the pods relates to enzyme activity.

Besides, the effect of pressure on dehiscence was also studied. We found that the internal pressure was not necessary to be built up for dehiscence. Moreover, relationship of the height from which the seeds were dropped and the distance the seeds scattered was also investigated. It was found that the higher the pods grow, the further their seeds can be dispersed. Therefore, the seeds can be scattered further away for its reproductive purpose.

Introduction

In the playground beside the school hall, there are three Bauhinia variegata with many long pods hanging on it. One day, a pod suddenly exploded when we were passing by there. It surprised us and gave rise to our interest. As a result, we kept having observation on the growth of the trees and their pods. Then we found that the pods grew gradually from green to black and finally dehisced to scatter numerous seeds onto the ground. Moreover, it was noticed that they only dehisced in sunny days and some of them got decayed after a long period of rainy time.

Being a member of the family *Caesalpiniaceae* (*the Chinese University of Hong Kong, Hong Kong Flora & Vegetation website*), Bauhinia variegata, which has a common name called Camel's Foot Tree, is very popular in the tropical and subtropical climate. This species of tree can be found elsewhere in Hong Kong and is mainly regarded as an economically important plant for its uses as medicine and food. It is a medium-sized tree growing to 10–12 m tall, deciduous in dry season. Its leaves are broad and rounded; its flowers are bright pink or white, with five petals. The pods are its fruits, which are 15–30 cm long, containing several seeds. (Wikipedia)

(For simplicity,' B. variegata' represents' Bauhinia variegata ' in the remaining report)

Methods of investigation

It was observed that the B. variegata pods dehisced only when it was black and dry. And the black pod decayed if the weather had been wet for a long time, like something was breaking down it inside. Therefore, we focus on the effect of dryness and cellulase activity on the dehiscence of B. variegata pods.

In order to achieve the objectives mentioned above, the following methods were used:

1. **Observation of the B. variegata pods in the playground beside our school hall:**
   We recorded the conditions and length of the pods over 2 weeks. At times, photographs of the pods were taken by digital cameras. Meanwhile, temperature and humidity sensors were used to record the change in temperature and humidity.

2. **Experiments to study the effect of dryness and cellulase activity on the dehiscence of B. variegata pods:**

We picked several pods from the B. variegata grew near the playground to the Biology laboratory. In order to ensure the pods were fresh and in natural conditions, we only picked the pods just before the experiments began. Oven was used to speed up the removal of water in the B. variegata pods. A video recorder was set up to record the moment of dehiscence of the pods. Photographs were taken at intervals of time. A series of experiments were conducted to achieve our goals.

Summary of fieldwork and laboratory work:

Aim	Questions brought from observation and experimental results
A. Factor being studied: Dryness	
Field work:	
Observing how dryness affects the dehiscence of B. variegata pods	Is dryness a factor for the dehiscence of B. variegata pods?
Laboratory work:	
Experiment 1: To study the effect of dryness on the dehiscence of B. variegata pods	Which kind of pods is drier, black one or green one?
Experiment 2: To compare the dryness and seed glucose content of the green (immature) and black (mature) B. variegata pods	Will dryness affect the shape of B. variegata pod skin?
Experiment 3: To study whether dryness affects the degree of twisting of B. variegata pod skin	/
Experiment 4: To investigate effect of dryness on the growth of B. variegata pods	/
B. Factor being studied: Cellulase activity	
Field work:	
Observing the effect of cellulase on the dehiscence of B. variegata pods	Why does the pod decay? Is it due to the action of enzyme?
Laboratory work:	
Experiment 5: To study the effect of cellulase activity on the dehiscence of B. variegata pods	/
C. Other investigations	
Field work:	
Observing the effect of internal pressure on dehiscence of B. variegata pods and the scattered distance of seeds	What are the relationships between the height of pods and the distance of their seeds scattered? How does pressure affect the dehiscence?

Laboratory work	
Experiment 6: To study whether the **pressure** inside B. variegata pod affect its dehiscence	/
Experiment 7: To investigate the relationships between **the height of pods** and the distance of their seeds scattered	/

A. How does dryness affect the pod dehiscence?

Field work

In our field work, we have carried out observation on the B. variegata in our school; their height, canopy height, and canopy size were measured respectively with a measuring tape.

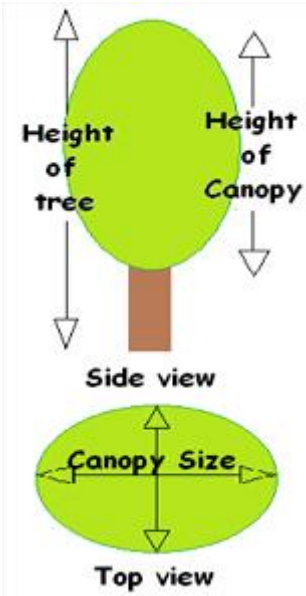
Tree	A	B	C
Height(m)	6.63	8.28	5.07
Canopy Height(m)	4.51	6.11	2.87
Canopy size(m^2)	6.01 x 5.05	5.2 x 4.86	6.21 x5.87
Keys  			

Fig.1 A straight dehisced pod is found after a rainy night

Fig.2 A twisted dehisced pod is found on sunny day.

In addition, the length of some selected B. variegata pods was measured with a string and ruler along their concave side. Also, some B. variegata pods were sealed in plastic bags as field experiment.

Day	0	3	5	6	9
Temperate (^{o}C)	22.7	22.6	21.9	20.9	17.4
Humidity (%)	81.3	100	80	62	100

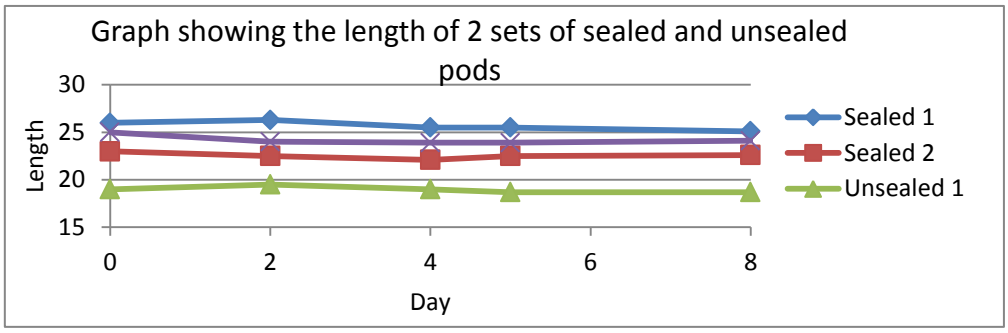


Fig.3 All the pods are green in color at first.

Fig.4 The sealed pods seem to be more immature after 9 days.

Fig.5 Conditions of pods after 9 days: The sealed pod (left) turned moldy but remained partly green in color; the unsealed pod (right) has turned totally black.

Fig.6 Seeds inside the green sealed pod in Fig. 5 have turned brown like those in unsealed pods.

Observation:

From our fieldwork, we found that sealing the B. variegata pods with plastic bags only affect their color, but not their length. The pods sealed in the plastic bags were found to turn black slower than those unsealed. (Fig.3, Fig.4)

With reference to the graph in p.4, we found that there was no significant change in length of both the sealed and unsealed pods. However, at the end of our field work, we found that nearly all the unsealed pods have turned black already, but some of the sealed pods were still partly green (Fig.5).

Nonetheless, even though the skin of the pod have not turned black (mature), the seeds inside are as same as those black mature pods. (Fig.6)

Therefore, we supposed that the humidity and the temperature would only affect the color of the pod skins but not the seeds, further laboratory work should be carried out.

Besides, some dehisced pods were straight while some were twisted. (Fig.1, Fig.2) Since they were discovered under different weather conditions, we suspected that their shapes were affected by dryness. **As a result, we decided to further study the effect of dryness on the pods in the laboratory work.**

Laboratory work

Experiment 1:

To study the effect of dryness on the dehiscence of B. variegata pods

Test 1: Study whether the pods dehisce under dryness

Principle of investigation:

We can study whether dehiscence occurs under dryness by providing a dry environment.

Identification of variables		
Independent variable	**Dependent variable**	**Controlled variable**
-Dryness of pods	-Dehiscence of B. variegata pods	-Temperature
Apparatus		
-Electrical dry cabinet X 1	-Camera X 1	
Materials		
-Black pod of B. variegata X 2	-Green pod of B. variegata X 2	

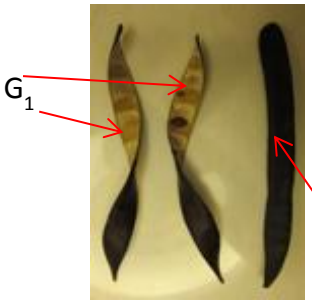
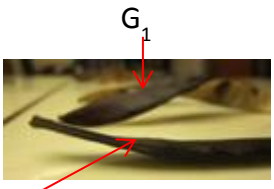
Procedures:

1. Label the 4 pods B_1, B_2, G_1 and G_2 respectively.
2. Place them in an electrical dry cabinet
3. Measure the time for dehiscence for each B. variegata pod.
4. Observe and record the result obtained.

Fig.7 Electrical dry cabinet was used to draw water out of the pods.

Result:

Table showing the dehiscence of the B. variegata pods

Set	B_1	B_2	G_1	G_2
Time of dehiscence	6 days	7 days	8 days	Didn't dehisce after 9 days
Photos				 (a)
Remark	In general, the pods turned black, and then dehisced and twisted with the seeds scattered. Although G_2 didn't dehisce, it has twisted a bit already. (a)			

Interpretation of result:

In this experiment, the black pods were found to dehisce more quickly than the green ones.

The humidity reading of the electrical dry cabinet dropped from 50% to 39% in the experiment, which indicates that water is drawn out from the pods and they become drier and drier. Therefore, from the result, we can deduce that the B. variegata pods are able to dehisce when they are dry.

Sources of error:

The two black pods are not identical so that their original water content could be different.

Conclusion:

B. variegata pods can dehisce when they are dry.

Follow up:

We have found that dry B. variegata pods can dehisce in this experiment. For further investigation, we want to find out whether dryness is necessary for the dehiscence of B. variegata pods

Test 2: Study if dryness is necessary for dehiscence

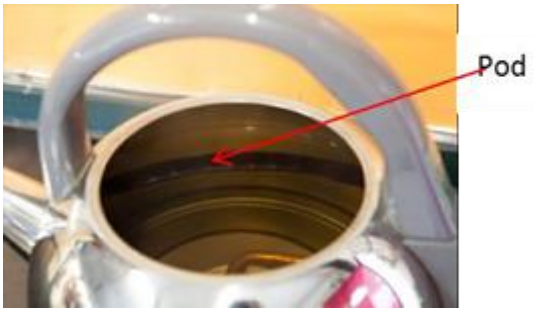
Principle of investigation:

In order to study the necessity of dryness for dehiscence, we carry out experiment by providing a wet environment to the pods, and check if dehiscence occurs.

Identification of variables					
Independent variable		**Dependent variable**		**Controlled variable**	
-Dryness of pods		-Dehiscence of B. variegata pods		-Temperature	
Apparatus					
-Electrical kettle	X 1	-Oven			X 1
-Electronic balance	X 1	-Timer			X 1
Materials					
-Black pod of B. variegata	X 2	-Distilled water			

Procedures:

1. Label the 2 pods A and B respectively.
2. Prepare the oven to 100°C and boil a kettle of water
3. Place A in the boiling water and B in the oven.
4. Measure the time for dehiscence for each B. variegata pod.
5. Observe and record the result obtained.



Set A: Put into boiling water

Set B: in oven of 100 °C

Result:

Table showing the dehiscence of the B. variegata pods

Set	Condition	Time of dehiscence	Remarks/Photos
A	In boiling water	Didn't dehisce after 9 hours	The pod didn't dehisce and remained intact. It also gained weight after the experiment. 
B	In oven of 100 ^{o}C	1 hr 36 minutes	The pod coiled spirally and turned black, then it finally dehisced with the seeds scattered. 

Interpretation of result:

In this experiment, we found that only the pod in set B dehisced while that in set A did not. Since the temperature of set A and set B are equal, the factor of heat is ruled out. In set B, the pod is placed in an oven, which facilitates the evaporation of water inside the pod. In contrast, although the pod in set A is also under the same temperature (100°C), it is bathed with water and its water content remains very high throughout the experiment. As a result, the experiment proves that dryness is necessary for dehiscence of the B. variegata pods.

Sources of error:

The two black pods are not identical so that their original water content could be different.

Conclusion:

Dryness is necessary for the dehiscence of the B. variegata pods.

Follow up:

We believe that the dehiscence of B. variegata pods depends on either its dryness. This test shows that **dryness is necessary for dehiscence** to occur. We want to study whether the black pods are drier and more mature, so another experiment is carried out.

Experiment 2:
To compare the dryness and seed glucose content of the green (immature) and black (mature) B. variegata pods

Principle of investigation:

The glucose content of the seeds of B. variegata pods can be compared by using clinistix paper, which changes color in different amount of glucose present. We can compare their dryness by their weight difference after being heated.

Identification of variables		
Independent variable	**Dependent variable**	**Controlled variable**
-Using the green pod or the black pod	-Water content of B. variegata pods -Glucose content of seeds	-Temperature

Apparatus		Materials	
-Oven	X 1	-Green pod of B. variegata	X 1
-Video recorder	X 1	-Semi-green pod of B. variegata	X 1
-Electronic balance	X 1	-Black pod of B. Variegata	X 1
-Timer	X 1	-Clinistix paper	X 2

Procedures:

1. Label the 3 pods of B. variegata as Green, Semi and Black respectively.
2. Prepare the oven to 105°C
3. Place them into the oven for 8 hours.
4. Videotape the B. variegata pods and measure the time for dehiscence of each B. variegata pod.
5. Grind the seeds obtained for each pod into paste.
6. Filter the paste with a piece of muslin cloth to get the filtrate for clinistix test.
7. Observe and record the result obtained.

Fig.8 The dehiscence of pods was videotaped

Result:

Table showing the time required for dehiscence of the B. variegata pods

Set	Time for dehiscence	Remarks/Photos
Green	7hrs 16min	The pod dehisced and turned black.
Semi	5hrs 39min	
Black	2hrs 15 min	

Fig.9 Continuous shots of the dehiscence of one of the pods.

Table showing the color change of clinistix paper test

Pods	Results	Glucose content
Green	No change	No
Semi	Change from pink to blue	Higher
Black	Change from pink to dark blue	Highest

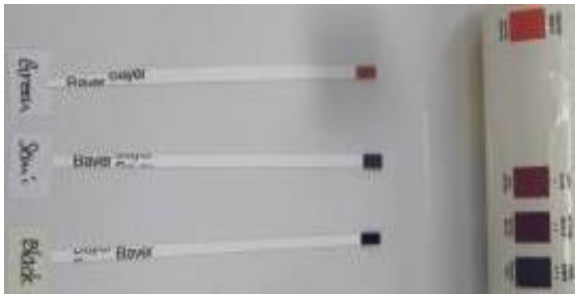


Fig. 10 Photo showing the result of clinistix test

Interpretation of result:

From this experiment, we can see that the dryness of the pod skin and the seed glucose content of the black B. variegata pod are the greatest.

From the result, we can deduce that the B. variegata pods gradually dry up during their growth due to evaporation. Moreover, the B. variegata tree may also stop supplying water to its pods for reproduction by dehiscence.

It is also found that the seed glucose content of B. variegata pod increases as it grows. Possible explanation is that when the B. variegata pod turns black and mature, more stored carbohydrate is broken down into glucose.

Sources of error:

Glucose content may not be a good indicator for the growth stage of seeds.

Conclusion:

The black B. variegata pod has the driest pod skin and highest seed glucose content.

Follow up:

In the experiment, we found that the black B. variegata pod is relatively drier and mature than the others and the time for dehiscence of the B. variegata pod increases with its water content. Moreover, the pods **become more twisted** after it dehisced. In order to study whether these changes were caused by dryness, further investigation was made to test the effect of dryness on the shape of B. variegata pods.

Experiment 3:
To study whether dryness affects the degree of twisting of B. variegata pod skin

Principle of investigation:

Since cell wall is freely permeable to water, B. variegata pods can soak in water. By comparing the shape and weight of the skin of the B.variegata pods, we can find out if dryness affects the shape of B. variegata pods.

Identification of variables		
Independent variable	**Dependent variable**	**Controlled variable**
-dryness of pods	-Degree of twisting of B.variegata pod skin	-Size of B.variegata pod skin
Apparatus		
-Beaker X 1	-Camera X 1	
-Electronic balance X 1	-Oven X 1	
Materials		
-Skin of black pod of B. variegata X 1	-Distilled water	

Procedures:

1. Weigh the skin of a twisted B. variegata pod.
2. Immerse half of it into a beaker of distilled water for 1 day.
3. Take photos of the skins. Then blot dry the water on the skin surface.
4. Dry it in an oven.
5. Record and observe the result.

Fig.11 Shape of pod skins before (left) and after (right) immersing in water

Fig.12 The immersed part of the pod skin turned straight.

Fig.13 Pod skin turned twist again after drying

Result:

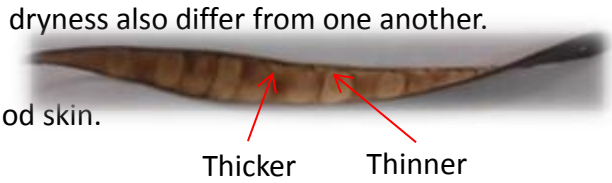
Table showing the change of weight and shape of pod skins in the experiment

Day	Condition	Weight of pod skin (g)	Shape of pod skin	
			Immersed part	Non-immersed part
0	Before immersing	3.583	*Twisted*	*Twisted*
1	After immersing	4.940	*Twisted*	Flattened
3	After drying	3.037	*Twisted*	*Twisted*
5	After immersing	4.955	*Twisted*	Flattened

Interpretation of result:

In this experiment, the originally twisted skin of pod turned flattened after being immersed into the water. By comparing its weight in different period of time, we can see that the skin was particularly heavier after being immersed into water as water molecules entered the plant cells freely, hence the skin gained water. However, after being dried in the oven, it turned twisted again due to the vaporization of water. This shows that dryness can cause the skin of B. variegata pods to twist and coil.

The possible explanation of its twisting is the uneven distribution of thickness. The B. variegata pods are unevenly shaped as seed compartments are developed at intervals. Since the thickness of different parts of the pod is different, their extents of shrink upon dryness also differ from one another.



Sources of error:

Temperature may also play a role on changing the shape of pod skin.

Conclusion:

The B. variegata pod skin will twist and coil on dryness. The degree of twisting of pod skin increases with dryness.

Experiment 4:
To investigate the effect of sealing the B. variegata pods with plastic bags on their growth

Principle of investigation:

In the field work, we sealed the pods with plastic bags, we want to compare the growth of the pods sealed and unsealed with plastic bags in this experiment. We will investigate the pods' growing stage by observing their skins and seeds. Moreover, since we have proved that the glucose content of the mature pods is higher (experiment 2), we will test the glucose content present in the seeds by carrying out clinistix test.

Identification of variables		
Independent variable	**Dependent variable**	**Controlled variable**
-The B. variegata pods sealed or not sealed with a plastic bag	-Growth of the seeds	- B. variegata pods from the same branch

Apparatus			
-Scissors	X 1	-Camera	X 1
-Electronic balance	X 1	-Mortar and pestle	X 1

Materials			
- B. variegata pods sealed with a plastic bag from the same branch	X 1	- B. variegata pods not sealed with a plastic bag from the same branch	X 1
-Clinistix paper	X 2		

Procedures:

1. Observe the color difference of the B. variegata pods.
2. Open the pods and observe the appearance of their seeds.
3. Weigh the seeds of the B. variegata pods respectively.
4. Grind the seeds to get extract for clinistix paper test.
5. Record and observe the result obtained.

Fig.14 The skin of sealed pod is still green in color; The skin of unsealed pod has already turned black.

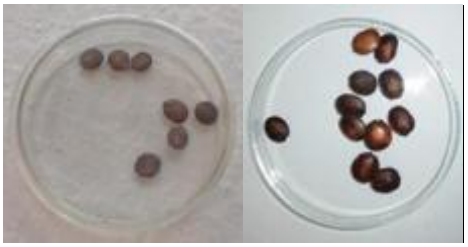


Fig.15 The seeds of both the sealed (left) and unsealed (right) pods are dark brown in color.

Fig.16 The seeds were grinded to get extract for clinistix test.

Result:

Table showing the observation

Pods	Color of pods	Appearance of seeds	Average weight of 6 seeds (g)
Sealed	Partly green	Swollen and dark brown	0.681
Unsealed	Black	Flat and dark brown	0.396

Table showing the color change of clinistix paper test

Labels	Remark	Glucose content
Sealed	Change from pink to blue	Same
Unsealed	Change from pink to blue	

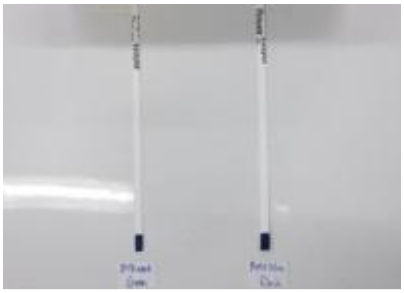


Fig.17 Photo showing the result of clinistix test

Interpretation of result:

In this experiment, it is found that the 2 pods and their seeds have different colors and weight. The difference of appearance of the 2 pods is mainly due to their different water content.

For the pod sealed with plastic bag, water vapour is trapped inside the plastic bag and hence provides a higher humidity. Therefore, its weight is larger which indicates its water content is higher. As we have shown that the pod skin will turn black on dryness (experiment 2), a pod with higher water content can remain green.

We also found that even though the colors of the 2 pods were different, their glucose contents were the same. This shows that the pods are in the similar growing stage. Since color reflects dryness of the pod skin, so we can conclude that dryness of B. variegata pods will not affect the growth of the seeds inside.

Sources of error:

Clinistix paper test is only semi-qualitative and it cannot show a small difference of the glucose content.

Conclusion:

The **dryness of B. variegata pods will not affect the growth of the seeds** inside.

B. How cellulase activity affects dehiscence of pods?

Field work

We discovered that some pods decayed from inside, like something was digesting it. (Fig.18 & Fig.19) Since it is suggested that breakdown of the cell wall by celluslase is a basic event in the process of dehiscence (*Regulation of fruit dehiscence in* Arabidopsis, *Cristina Ferrándiz*).

Therefore, we thought that it could be the result of cellulase activity. Since the environment was not suitable for the mature pods to disperse their seeds, the excess cellulase hence breaks down the skin of the pods. In order to study whether, we carried out experiments to find out the effects of cellulase to the pods.

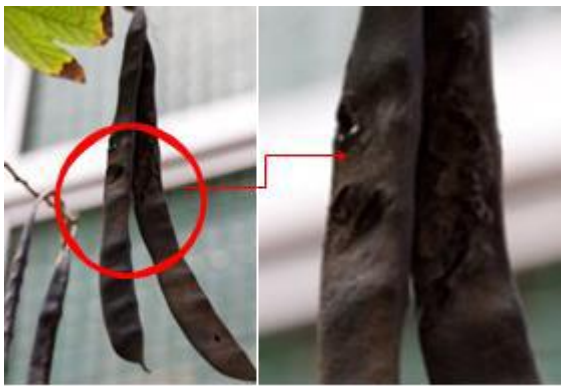


Fig.18 & Fig.19 Pods have decayed after a long period of rainny days and they don't dehsice.

Laboratory work

Experiment 5:

To compare cellulase content in the seeds of green and black B. variegata pods

Principle of investigation:

Cellulase is responsible for digesting insoluble cellulose and giving out soluble glucose as products. With the similar mass of substrate provided, the greater content of cellulase, the greater the rate of converting cellulose to glucose. The weight loss of cellulose is used to estimate the cellulase content.

Test 1:

We have carried out an experiment by using filter paper as the source of cellulose, but we found that the result was not significant. As the cellulase activity cannot be determined in this experiment, so we are going to modify our experiment for a more accurate result.

Test 2:

In test 2, we modified our experiment by using cellulose powder directly as the source of cellulose.

Identification of variables		
Independent variable	**Dependent variable**	**Controlled variable**
-Extract from the seeds of green or black pods	-Cellulase activity	-Temperature and pH -Time duration of experiment

Apparatus				**Materials**	
-Mortar and pestle	X 2	-Pipette filler	X 2	-Distilled water	
-Beaker	X 2	-Centrifuge tube	X 4	-Melting ice	
-Plastic vial	X 4	-Test tube rack		-Buffer solution of pH 4	
-Filter paper	X 4	-Scissors		-Cellulose powder	
-Filter funnel	X 2	-Electronic		-Cellulase	
-Label	X 4	balance		-Green pod of B. variegata	X 1
-Muslin cloth	X 1	-Oven		-Semi-green pod of B. variegata	X 1
-Pipette	X 2	-Centrifuge		-Black pod of B. Variegata	X 1

Procedures:

1. Open a green B. variegata pod and take out 8 seeds from it. Weigh the seeds.
2. Grind the seeds together with 20 cm^3 of pH 4 buffer solution into paste in an ice-water bath.
3. Filter the paste with a piece of muslin cloth to get the filtrate for test.
4. Centrifuge the filtrate and filter the mixture with a piece of filter paper.
5. Repeat step (5) to remove as much impurities as possible.
6. Test the extract with clinistix paper and record the color change.
7. Add 5 cm^3 of the extract to a plastic vial containing a weighed amount of dry cellulose powder.
8. Repeat steps (1) – (8) with a black pod and a semi green pod.
9. Label the extract obtained as $Green_2$, $Semi_2$ and $Black_2$ respectively.
10. Prepare a cellulase solution and a set of control with buffer. Label them Enzyme and Control respectively.
11. Incubate the mixtures at 45°C for 2 days.
12. Shake the powder-solution mixture well at intervals of time.
13. Filter each of the mixture with a piece of weighed filter paper.
14. Dry the residue-carrying filter paper in an oven.
15. Weigh the dried filter paper with residue on it.
16. Record and observe the results.

Fig.20 Cellulose powder

Fig.21 Cellulase

Fig.22 Three types of pods were used

Precaution:

-Filter paper should be kept in an the oven for removing moisture before be weighed

Fig.23 Seeds were grinded for getting extract.

Fig.24 Centrifuge was used in order to get a purer seed extract.

Fig.25 The seed extract with cellulose powder were placed in the oven

Fig.26 Insoluble cellulose powder is filtered with filter paper.

Result:

Table showing the weight difference of filter paper before and after the experiment

Sample	Weight of seeds (g)	Weight of cellulose powder (g)		Percentage weight loss of cellulose per gram of the seed (%)
		Initial	Final	
$Green_2$	2.744	0.105	0.093	-4.16
$Black_2$	2.250	0.102	0.091	-4.79
Control	----------	0.100	0.098	----------
Enzyme	----------	0.100	0.071	----------
Time of incubation		50 hrs		

Interpretation of result:

In this experiment, we found that the weight loss of cellulose powders in $Black_2$ is greater and that of $Green_2$ is the smaller.

The higher weight loss of cellulose in $Black_2$ indicates that there is a greater cellulase activity in black B. variegata pod. Black B. variegata pod has a greater cellulase activity because it needs to weaken its rigid skin for facilitating its dehiscence. In the skins of B. variegata pod, the long straight cellulose fibers form many cross linkage, and this keeps the pod in shape even when it is dry. Therefore, the black B. variegata pods ready to dehisce needs more cellulase to break down the cellulose fiber or pod skin for scattering the seeds.

From this experiment, we can deduce the B. variegata pods secrete cellulase gradually since it is green. Therefore, the content of cellulase builds up and gives the above result, i.e. a greater cellulase activity in black B. variegata pod

Sources of error:

There might be material loss in the experiment and weighing is delayed.

Improvement:

The incubation time should be longer for cellulase activity.

Conclusion:

Cellulase content of seeds in black pods is greater than that of green pods.

C. Other factors being studied

Field work

Some seeds were found on the ground, their distance from the closest trees was measured. The distances between tree A, tree B and tree C and the seeds are 8.15m, 7.20m and 10.41m respectively. Since the heights of different pods on the tree were different, we wanted to know whether the dropping

height of seeds will affect their scattered distance.

Moreover, the decayed pods (Fig.18 and Fig.19, p.12) didn't dehisce over a long period of time. We suspected that the holes formed due to decay have caused a release of the internal pressure, and hence the pods could not dehisce.

In order to further study our observation, a series of experiments were carried out.

Fig.27 A seed is dispersed on the ground.

Fig.28 A seed has fallen onto the soil, and begins to grow.

Laboratory work

Experiment 6:

To study whether the pressure inside B. variegata pod affect its dehiscence

Principle of investigation:

In order to study the effect of internal pressure on dehiscence of the B. variegata pods, we drilled holes on the pod's surface to release the pressure inside. If internal pressure is necessary to be built up, the pod will not dehisce even on dryness.

Identification of variables		
Independent variable	**Dependent variable**	**Controlled variable**
-Drilled or not drilled with holes on the B. variegata pods	-Dehiscence of B. variegata pods	-Temperature

Apparatus			
-Drill	X 1	-Camera	X 1
-Electronic balance	X 1	-Oven	X 1
-Timer	X 1		

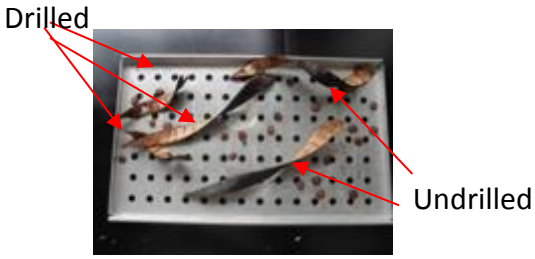
Materials	
-Black pod of B. variegata	X 2

Procedures:

1. Drill 3 holes on one of the B. variegata pods.
2. Prepare the oven to 105°C.
3. Place the two B. variegata pods prepared into the oven until both of them dehisce.
4. Videotape the two B. variegata pods and measure the time for dehiscence for each B. variegata pod.
5. Record and observe the result obtained.

Result:

Table showing the dehiscence of the B. variegata pods

Set	Time of dehiscence	Remarks/Photos	Photo
Drilled	1 hr 25 min	The pod turned black gradually and suddenly coiled and dehisced and the seeds were ejected.	
Undrilled	1 hr 36 min	The pod coiled and turned black, and then it dehisced and ejected the seeds.	

Interpretation of result:

In the above experiment, the B. variegata pods drilled with holes on it dehisced faster than the intact one. This shows that **pressure inside the B. variegata pods is not essential** for dehiscence

The possible explanation is that the pod drilled with holes gained a greater exposed surface area and this enhanced the rate of evaporation of water. Therefore, the dryness of pod increased more rapidly and dehisced within a shorter time.

Sources of error:

The original water content of the B. variegata pods may not be equal.

Conclusion:

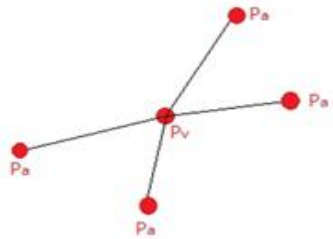
Pressure inside the B. variegata pods is not essential for dehiscence to occur.

Experiment 7:

To investigate the relationships between the height of pods and the distance of their seeds scattered

Principle of investigation:

To study this relationship, we dropped the seeds of black B. variegata pods from different heights. The distance P_v and P_a (refer to Keys)



P_v: landing position for a vertical drop

P_a: actual landing position for seed

Keys for measurement

Identification of variables		
Independent variable	**Dependent variable**	**Controlled variable**
-Height of releasing the seeds	-Distance of seeds of B. variegata pods scattered	-Force of releasing

Apparatus			
-Measuring tape	X 1	-Camera	X 1

Materials	
-seeds of black B. variegata pods	X 3

Procedures:

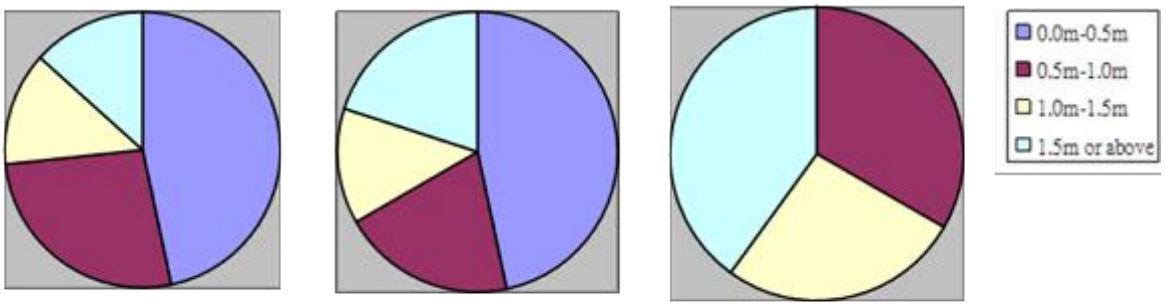
1. Release the seeds of black B. variegata pods from the height of 3 m.
2. Measure the distance of the seeds scattered from P_v

3. Repeat steps (1) and (2) for 5 times.
4. Repeat steps (1) to (3) from the height of 5 m and 8.5 m respectively.
5. Record and observe the result obtained.

Result:

Table showing the distance of the seeds scattered from the P_v

Height from the ground (m)	3	5	8.5
Mean of distance (P_v P_a) (m)	0.892	0.951	1.61
Maximum distance (P_v P_a) (m)	4.56	3.81	4.86



Piechart of distance distribution of height 3 m

Piechart of distance distribution of height 5 m

Piechart of distance distribution of height 8.5 m

Interpretation of result:

From the above experiment, we found that the average distance that the seeds could be dispersed was greater when the releasing height was greater, i.e. **the greater the height, the further the seeds can be dispersed** after the dehiscence of B. variegata pods.

The result can be explained by applying the acceleration due to gravity. Since a greater height is provided, the seeds gain a greater velocity towards the ground and they can rebound higher and further away. Moreover, the time for the pods to fall down from a higher position is also longer, there is a greater chance that the moving path of seeds is affected by wind. As a result, they can be dispersed for a further distance.

We also found that a few seeds were dispersed for a fairly long distance in the experiment. Possible explanation is that the seeds can roll along the ground surface due to their circular shape. The seeds of B. variegata pods are roughly circular in shape, so that they can roll for a distance after falling on the ground.

Sources of error:

The air movement cannot be determined and we cannot simulate the twisting action in dehiscence.

Conclusion:

The seeds of B. variegata pods which grow higher on the B. variegata trees can be dispersed further in dehiscence.

Overall Discussion

I. Effect of dryness on the dehiscence of B. variegata pods

In the field work, we observe that the B. variegata pods turn black as they grow and some of the dehisced pods twist. Moreover, some pods don't dehisce even though they are black and mature.

From the laboratory work, we found that dryness is essential for dehiscence of the B. variegata pods in experiment 1 and 2. Moreover, we also show that the pods will twist on dryness in experiment 3. By using these results, explanation of our observation can be made. Since the outside humidity remains very high during the days we conducted our fieldwork, so the pods were not dry enough to dehisce.

Therefore, we can conclude that dryness play an important role in the dehiscence of B. variegata pods. The benefits of being dry for dehiscence are worth further investigations in future.

II. Effect of cellulase activity on the dehiscence of B. variegata pods

In the experiment 5, we discovered that the cellulase activity in black B. variegata pod is greater since it needs to weaken its rigid skin for facilitating the dehiscence. This can explain our observation in the field work that some black pods decayed after a long period of rainy days. Since the B. variegata pods are not dry enough for dehiscence, its high cellulase content begins to digest its own skin and the pod hence decays. This shows that even though the cellulase content is high, dehiscence still cannot occur without dryness.

III. Roles of other factors on the dehiscence of B. variegata pods

From experiment 1 and 6, we can deduce that **internal pressure plays no role in dehiscence**. Even if the pressure isn't built up, the B. variegata pods can dehisce if they are dry enough.

In fieldwork, we found that some of the seeds were scattered on the cement ground, where the seeds could not grow, while some of the seeds were dispersed on the soil, which provided a favorable condition for them to germinate.

The result of experiment 7 shows that the higher the pods grow, the further their seeds can be dispersed. Combined with the observation we made in fieldwork, we can conclude that **the higher the B. variegata pods grow, the further its seeds can be dispersed**. This is because there is less competition between the same species in a particular place when the seeds are further apart. This profits the propagation of the B.variegata.

IV. Other observations - Relationship with other organisms

A larva was found inside a B. variegata pod during our experiment. It fed on the seeds and spun inside the pod. Since the some of the seeds are eaten, the presence of larva will affect the reproductive purpose of the pods. From this observation, we can see that other organisms are also using the B. variegata pods for their own survival.

Fig.29 The larva is being observed under microscope

V. Pros and cons of applying dehiscence for reproduction and methods of conservation in Hong Kong

By adopting this self-explosive mechanism, B. variegata can disperse its seeds without the help of external agents such as wind and aninals. However, the pod dehiscence can only occur under restricted weather conditions, i.e. those favourable for drying. Also, the dispersal distance by dehiscence will be shorter than that by animals.

According to the data of Hong Kong Observatory, the average rainfall of Hong Kong has been increasing since the past few decades. Therefore, the weather condition is getting unfavourable for B. variegata to dehisce. In our study, we have found some of the characteristic of mature pods, such as turning black and dry. We can identify the non-split pod with mature seeds; hence we can collect the mature seeds for conservation and propagation.

Limitation of investigation

We cannot simulate the dehiscence of pods in order to study how far the seeds can be scattered from trees. We cannot study if cellulase is necessary for dehiscence of the B. variegata pods. Also, the cellulase content in different parts of a B. variegata pod cannot be determined as well. Moreover, other factors affecting dehiscence cannot be studied due to limited time.

Suggestions of further investigation

-To study the mechanism of dehiscence
-To study cellulase content in different parts of a B. variegata pod
-To check if other factors in the environment affect the dehiscence
-To carry out germination experiment to assess the viability and maturity of seeds

Conclusion

In our study, we found that dehiscence of B. variegata pods depends mainly on the dryness. Dryness is necessary for the pods for dehiscence to occur. When the pod is moist, it cannot dehisce. The effect of cellulase activity on dehiscence is uncertain. In the laboratory work, we found that the cellulase content in black (mature) pods is higher, but we cannot show the necessity of cellulase in dehiscence.

However, we show that pressure is not necessarily to be built up in the pods for dehiscence; the pod can dehisce even if there are holes on it if it is dry enough. Also, we found that the scattered distance of seeds increases with their dropping height.

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