CHAPTER 5

RESULTS

Floral Development and Morphology, Flowering Phenology and Anthesis Process

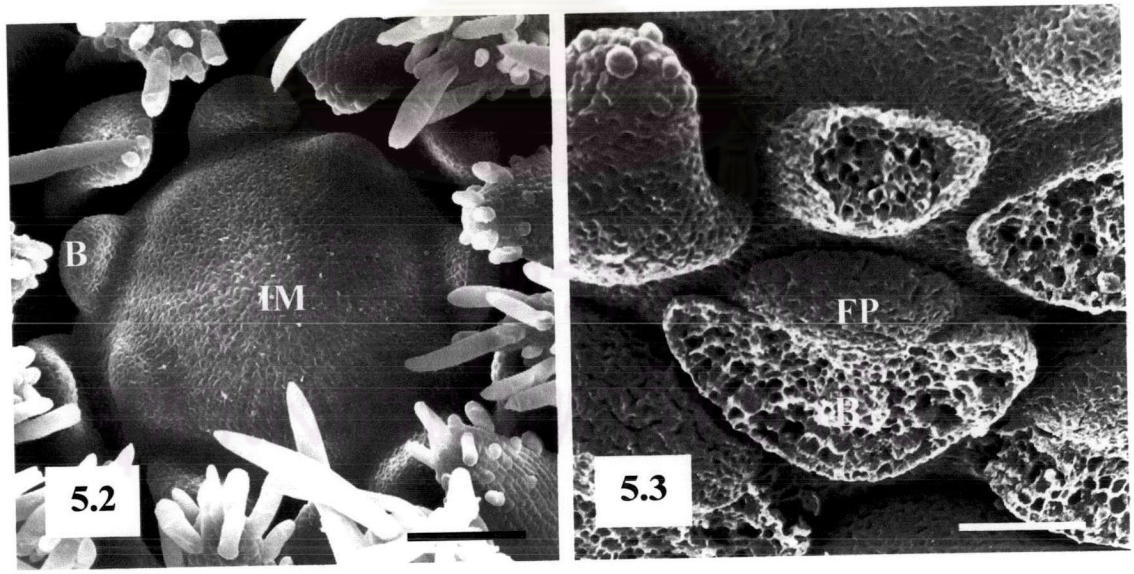
1. Floral Morphology

Afgekia sericea Craib usually has long raceme inflorescence, bearing a large number of flowers, up to about 300 florets. Like the other fabaceous species, the floral structure of *A. sericea* Craib is a papilionoid flower (Figure 5.31-5.38). The perianth (calyx and corolla) is covered by white, pubescent hairs. The calyx is purplish red in color. Standard is greenish yellow with dark purple nectary guides. Wings are deep purple and keel is white. The calyx composed of 5 sepals, which are basally fused along their margins, forming a calyx tube, and the free upper part, calyx lobes, which arranged in bilabiate form, i.e. the two upper lobes and three lower lobes. The corolla composed of 5 free petals in papilionaceous form, i.e. one upper most petal, the "standard" or the "vexillum", the two lateral petals, the "wings", and the two lower most petals. The last two petals always, more or less united, forming a robust, boat-shape like structure, the "keel" (Figure 5.21), which enclose androecium and gynoecium. The two lateral petals are usually in some way laterally connected with the keels (Figure 5.1). The androecium composed of 10 stamens, which is pseudo-monadelphy (Figure 5.22-5.25). The gynoecium composed of one simple pistil and is embraced by united stamen filaments. The placentation is marginal with usually 1-2, rarely 3 ovules.

2. Floral Development

The inflorescence meristem of the raceme of *A. sericea* Craib produces bracts in helical sequence (Figure 5.2), each of which subtends a floral primordium (Figure 5.3). During the development of the flower, the floral primordium is gradually changed in shape from ellipsoid to globose and the area of apical meristem gradually diminishes as the successive floral parts arise (Figures 5.4, 5.5). The initiation of the floral organ is acropetal from sepals, petals, stamen and carpel respectively (Figure 5.29).

Figure 5.1 Flower structure of young and mature flowers: **B**, bracts; **S**, sepal; **C**, calyx tube; **V**, vexillum petal; **W**, wing petals; **K**, keel; **NG**, nectary guide; bar = 1 cm.

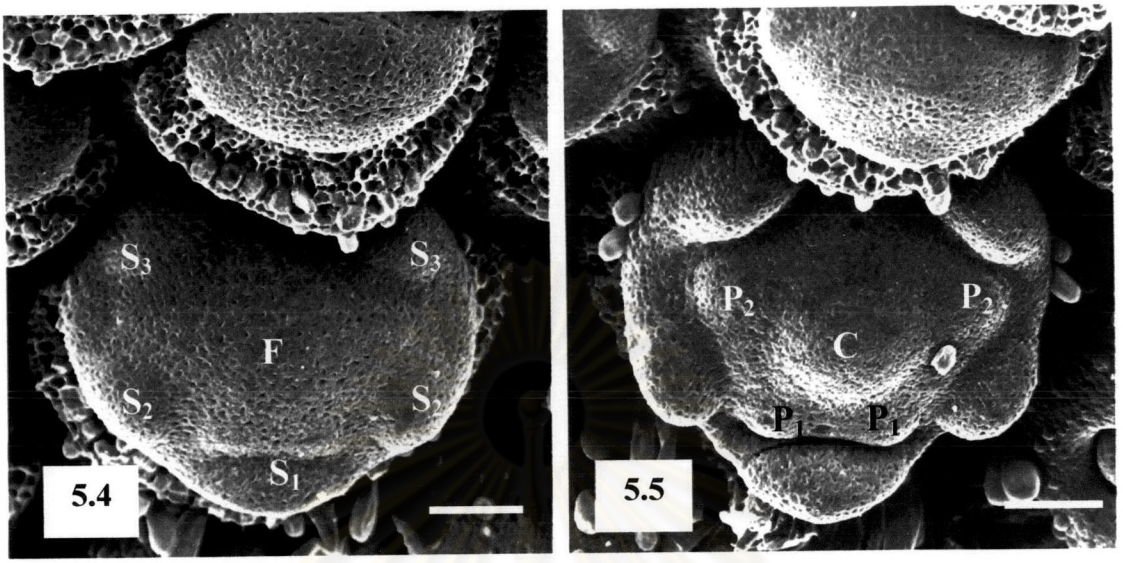


Figures 5.2–5.3 Floral organogenesis (SEM micrographs): **5.2** Inflorescence meristem (**IM**) after bracts initiation (**B**), bar = 100 μm; **5.3** Each of bracts (**B**) subtends an ellipsoid floral primordial (**FP**). Abaxial side is at base in figure **5.3**, bar = 25 μm.

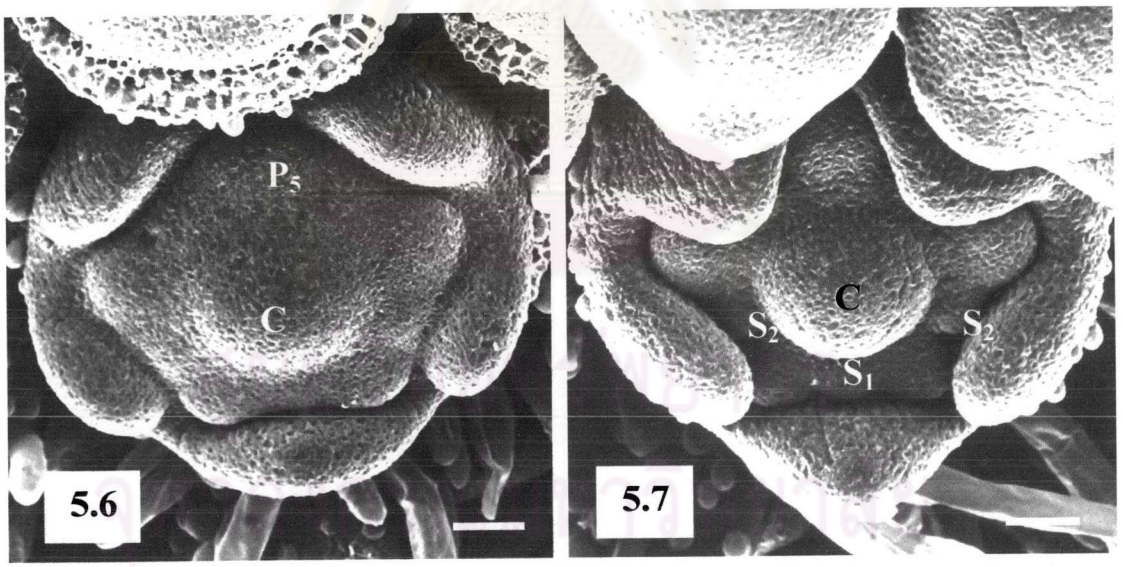
In the calyx, the lower most sepal is primarily initiated, follow immediately by the two lower lateral sepal primordia and the two upper sepal primordia, either simultaneous or very close successive stage (Figure 5.4).

After the establishment of all sepals, the petal primordial is then formed and alternate with sepal primordia. The lowermost petals (keels) primordia earlier appear and are alternate with the three lower sepals, followed by the arising of two primordia of lateral petal (wings) (Figure 5.5). The vexillum primordium seems to be the latest one to initiate but it is the first one to begin form differentiation (Figures. 5.6, 5.10, 5.11). Lamina formation begins at a height of 50 microns, and the vexillum continues to be the widest petal throughout the development (Figure 5.16). As in other papilionoid flower, the vexillum encloses the adjacent petal margins, in an imbricated arrangement. All five petals primordial grow marginally as flattened structures arching over the other organs (Figure 5.19). At anthesis the vexillum has a short claw with an oblong-elliptic blade, which is larger than the other four narrow petals (Figure 5.30).

After the establishment of all petal primordia, the stamen development is clearly divided into 2 series (Figures 5.7, 5.8, 5.9). Stamen primordia which are opposite to the sepals (antesepalous stamens) (Figure 5.7) are initiated before the ones that are opposite to the petal (antesepalous stamen) (Figures 5.8, 5.9). The sequence of stamen primordia development is start from the one which stand directly to the lower most sepal and the next are the two which opposite to the lower lateral sepals and then the last two primordia that opposite to the two upper sepals (Figures 5.7, 5.9). After that the series of stamen which is opposite to the petals (antesepalous stamen) start to initiate and alternate with the antesepalous ones (Figures 5.8, 5.9). The direction of the development of antepetalous stamens is the same as the antesepalous stamens, i.e. the two stamen primordia, which are opposite to the "keel", then the two primordia, which are opposite to the "wing", and then the vexillary stamen primordium.

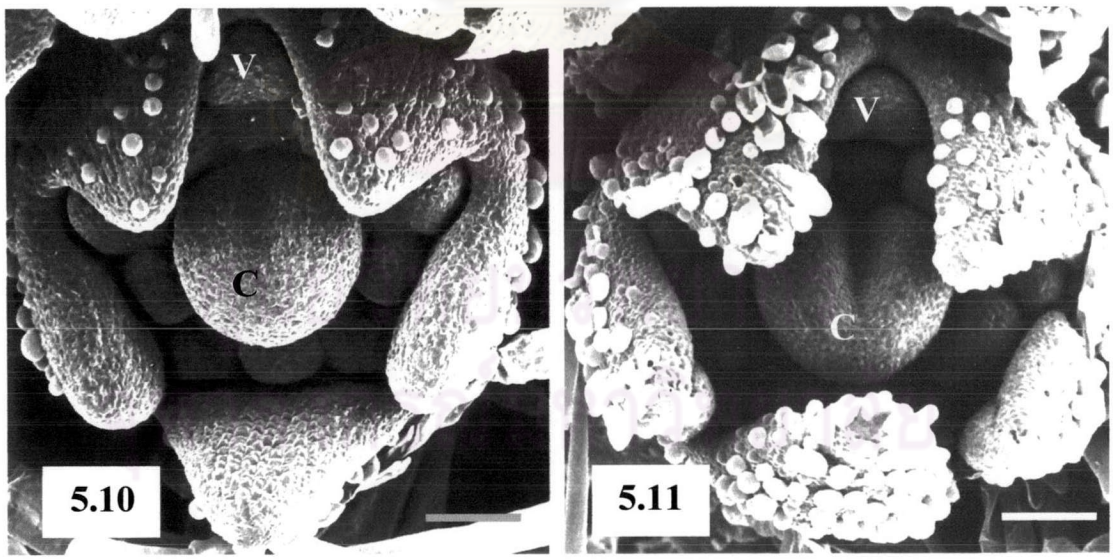


Figures 5.4-5.5 Floral apex: **5.4** Fives sepals initiated on floral apex (**F**), the first-produced sepal (S_1), two others (S_2), and S_3 the two adaxial sepals, bar = 50 μm; **5.5** Petal initiation (**P**) around initiated carpel (**C**), bar = 50 μm.



Figures 5.6-5.7 Floral apex: **5.6** Petal primordium at top will become vexillum, bar = 50 μm; **5.7** Initiation of first antesepalous stamen (S_1) and the other twos on abaxial side of initiated carpel (**C**), bar = 50 μm.

Figures 5.8-5.9 The carpel primordium becomes dorsiventral, two of the antepetalous stamen are at arrowheads (**5.8**), bar = 50 μm; **5.9** Antepetalous stamens (s_1-s_3) have been initiated, vexillum petal primordium (**V**) becomes larger, bar = 50 μm.

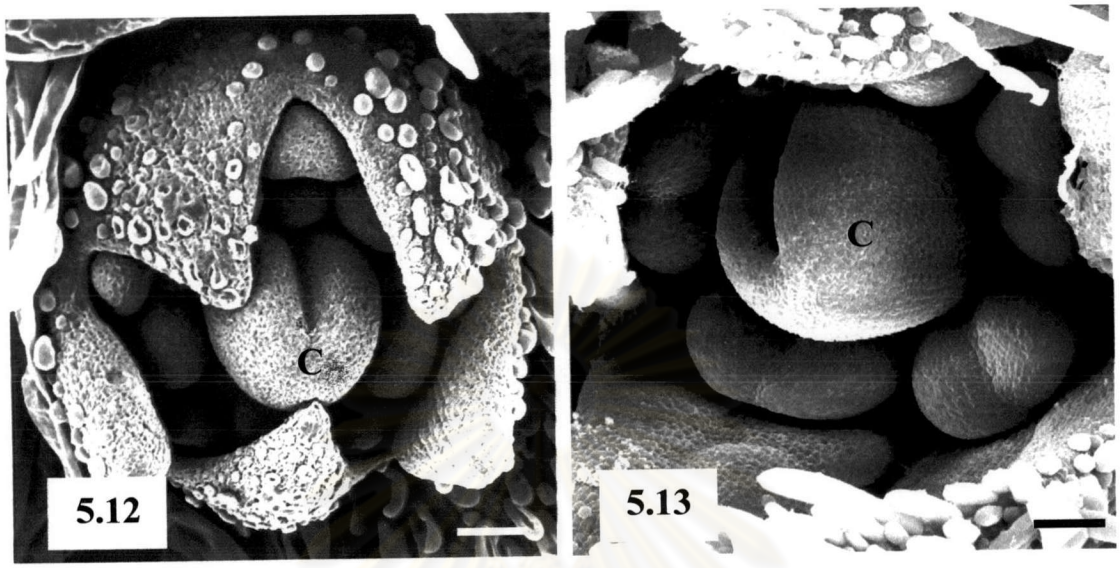


Figures 5.10-5.11 Carpellary cleft formed adaxially, vexillum primordium (**V**), is the latest one to initiate but it is the first one to begin form differentiation, bar = 50 μm.

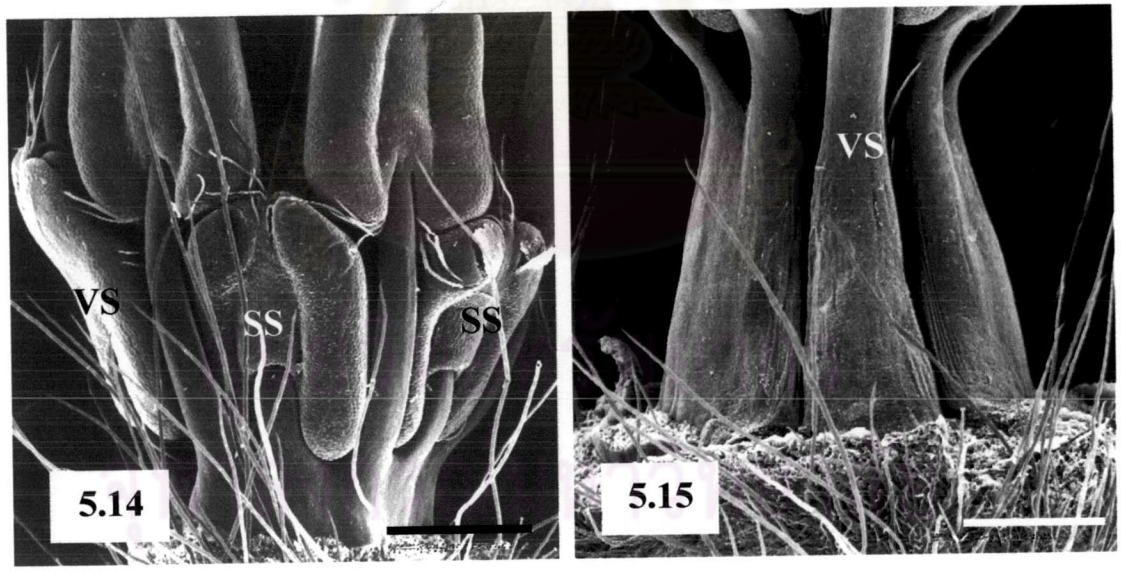
Stamen primordia of both whorls remain cylindrical (Figure 5.13) until gaining a height of 100 microns. At that stage the antesepalous stamen primordial slightly arched inward and appressed against the carpel (Figure 5.13) the stamens, thereafter develop a distal connective, elongate, introse anthers with thick filaments (Figure 5.14).

The antepetalous stamens have distinctly shorter filaments since the beginning of the development until a few days before anthesis (Figure 5.14), than these short filaments are very rapidly elongate before the day of anthesis, until they are equal in length to the antesepalous ones. After the filament and anther regions appear, the fusion of filaments has developed by zonal growth, forming a short incomplete stamen tube, including 9 stamens (Figures 5.14, 5.15) while the tenth (vexillary stamen) is free. This is resulted in diadelphous stage. Later on the nine-stamen tube has fused with the vexillary stamen to form a continuous tube, which is then turn into the pseudo-monadelphous stage.

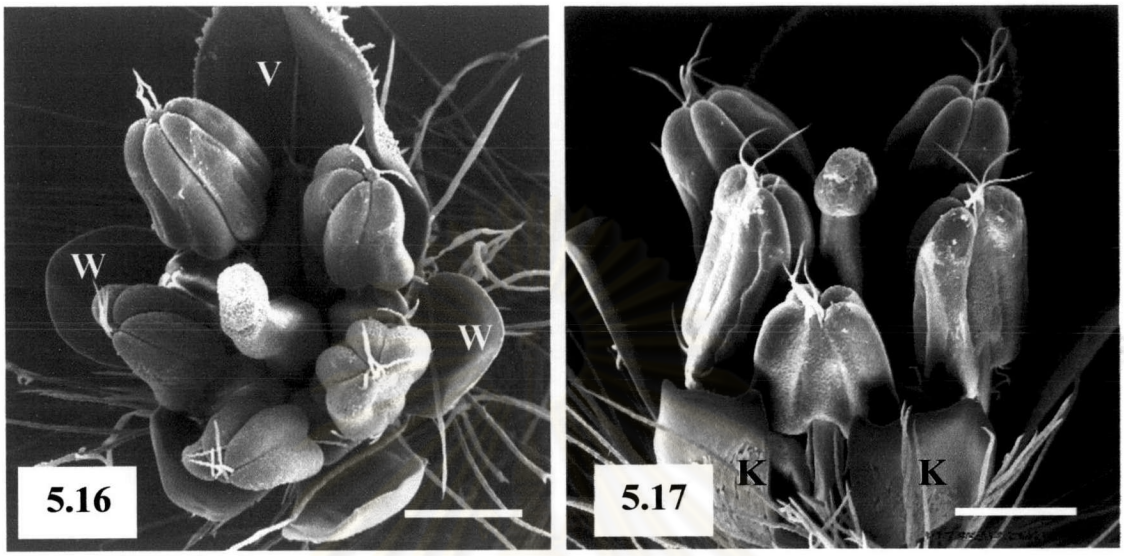
The inception of carpel is likely established soon after the initiation of stamen primordium (Figures 5.5, 5.6, 5.7). It looks like a mound at the centre of developing flower and later on becomes more radial hemispherical primordium (Figure 5.7). When the carpel primordium is at a height of about 60 microns, it then gradually changes to dorsiventral with flatten ventral side and round dorsal side (Figures 5.8, 5.9). The margin of ventral side is likely folded, forming a shallow depression on the median ventral side of carpel primordium (Figure 5.10). This depression gradually deepens and more discernible as a cleft, when the carpel reaches its height of about 100 microns, with the development and growth of the flower organs (Figures 5.11, 5.12). At this stage, it looks more or less like a horseshoe–shape (Figure 5.13) and then begins to show form differentiation by laterally compressed of carpel and large stigmatic area formation (Figures 5.16-5.20). The style and ovary are later elongate (Figures 5.17, 5.19, 5.26, 5.27). Stigma before-, on-, and after anthesis day were observed. In immature stigma, papillae were turgid (Figure 5.28a), although some secretions were already visible between papillae and the other cell of receptive surface. In mature stigma, papillae had a shrunken appearance (Figure 5.28b). While degenerated stigma papillae have collapsed (Figure 5.28c).



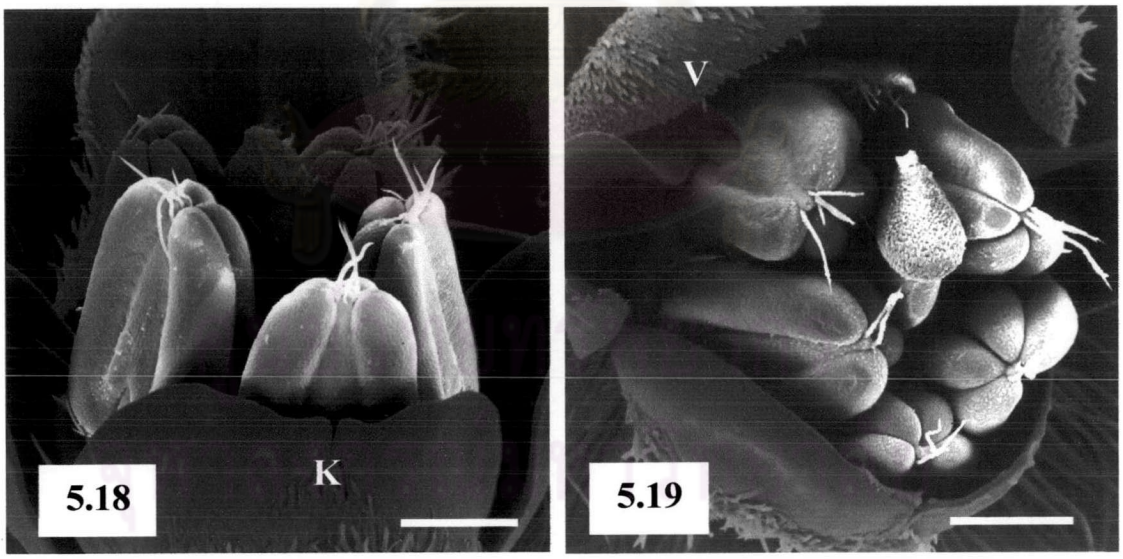
Figures 5.12-5.13 The margin of ventral side is folded, forming a depression on the carpel primordium, gradually deepens and more discernible as a cleft (**5.12**). This stage looks like a horseshoe–shape (**5.13**), bar = 50 μm.



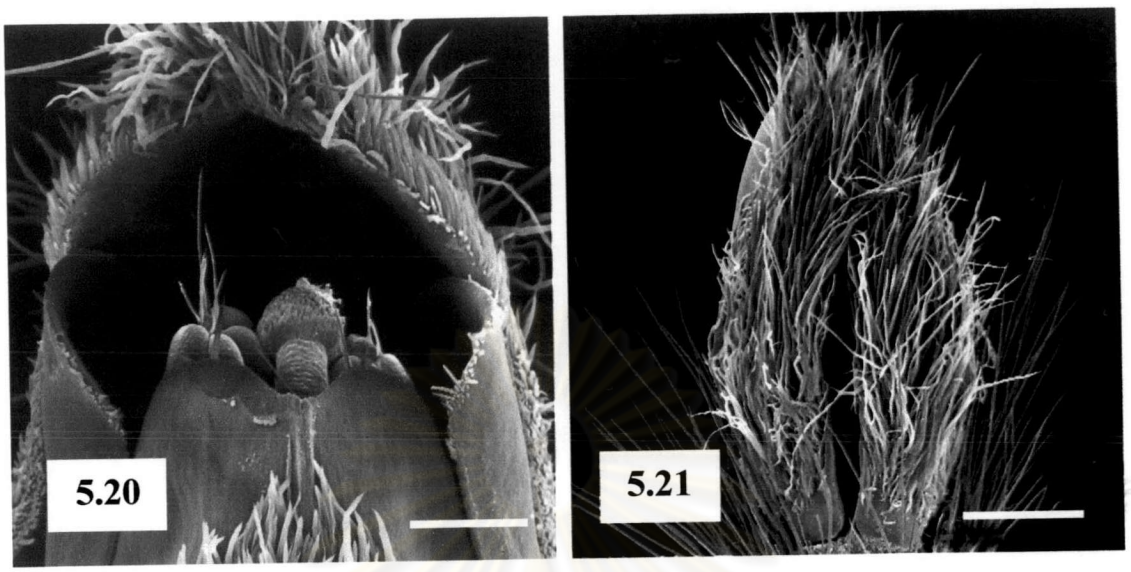
Figures 5.14-5.15 The antepetalous stamens (**SS**) have shorter filaments (**5.14**). The fusion of filaments has developed, forming a short incomplete stamen tube, including 9 stamens (**5.15**) while the tenth (vexillary stamen, **VS**) is free, bar = 500 μm.



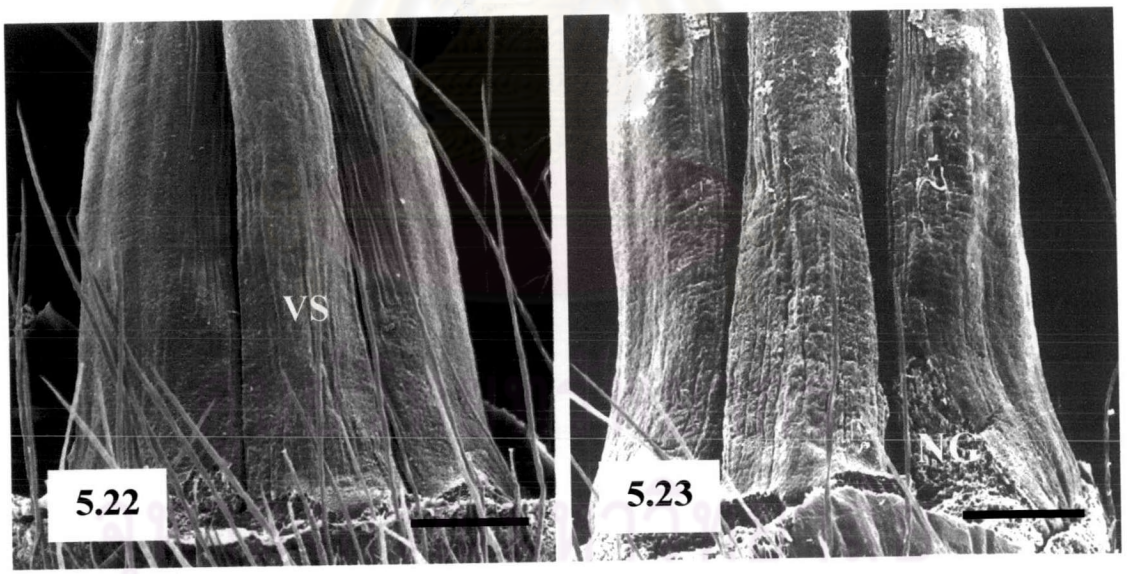
Figures 5.16-5.17 The vexillum, the widest petal throughout development (**5.16**). The lowermost petals (keels) and the two of lateral petal (wings) (**5.16-5.17**), bar = 500 μm.



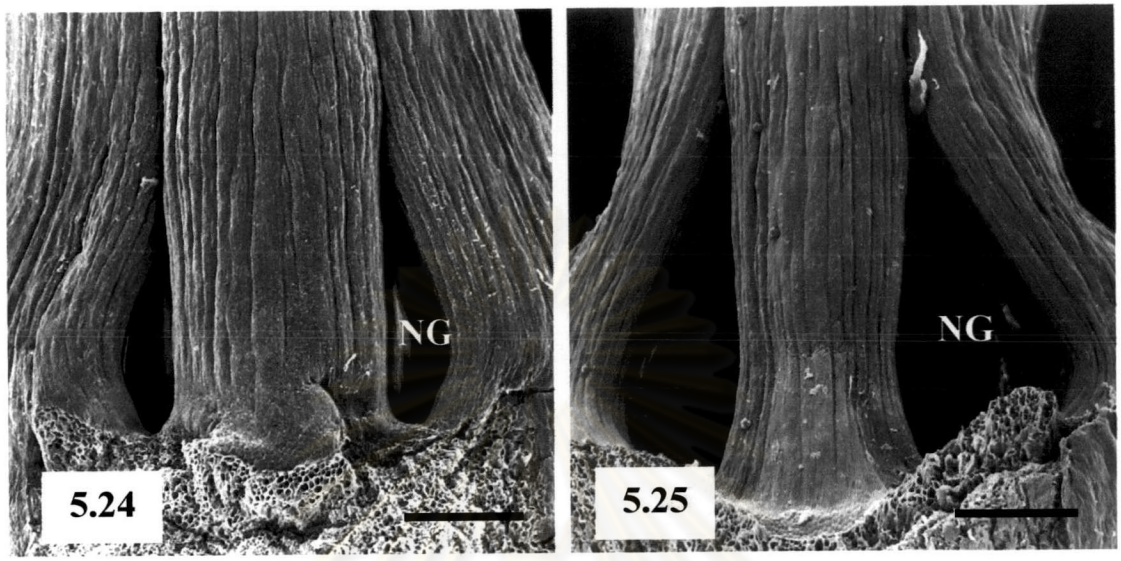
Figures 5.18-5.19 Keels are fused (**5.18**) and vexillum encloses the adjacent petal margins, in an imbricate arrangement. All five petals grow marginally as flatten structures arching over the other organs (**5.19**), bar = 500 μm.



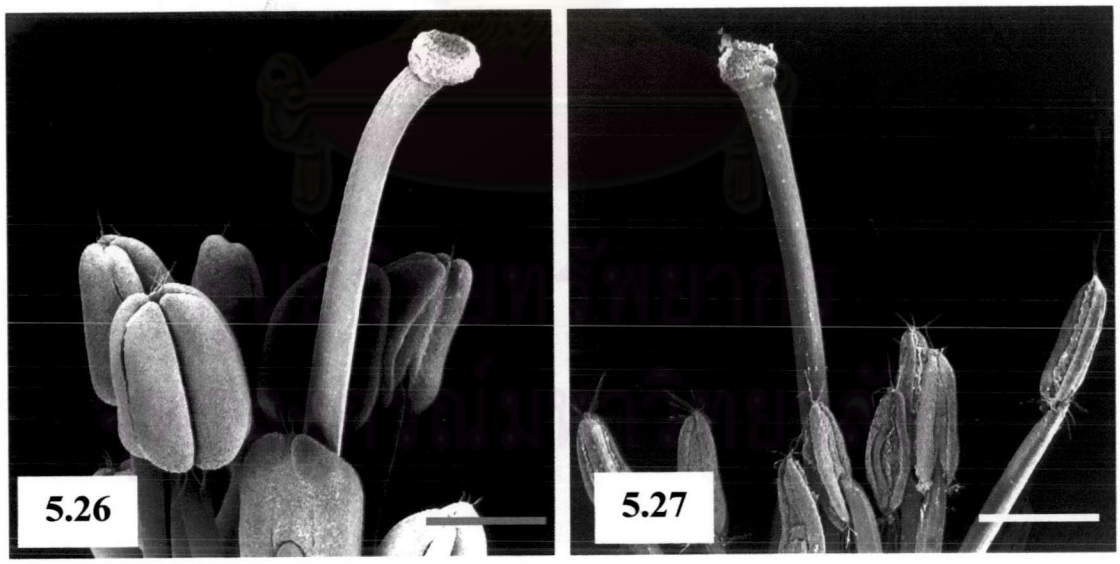
Figures 5.20-5.21 Keel start to fuse at the distal end (**5.20, 5.21**). The vexillum start to encloses the adjacent petal margins, in an imbricated arrangement, bar = 500 μm in **5.20** and 1 mm in **5.21**.



Figures 5.22-5.23 The fusion of filaments has developed, forming a short incomplete stamen tube, while the tenth (vexillary stamen, **VS**) is free (**5.22**), bar = 500 μm. Later on the nine-stamen tube has fused with the vexillary stamen to form a continuous tube and the nectar gates (**NG**) (**5.23**), bar = 500 μm.



Figures 5.24-5.25 The nine-stamen tube has fused with the vexillary stamen to form a continuous tube at pseudo–monadelphous stage; **5.24**, one day before anthesis, and **5.25**, on the anthesis day, bar = 500 μm.



Figures 5.26-5.27 The antepetalous stamens have shorter filaments until one day before anthesis (**5.26**), they are equal in length to the antesepalous ones on the anthesis day (**5.27**), bar = 500 μm.

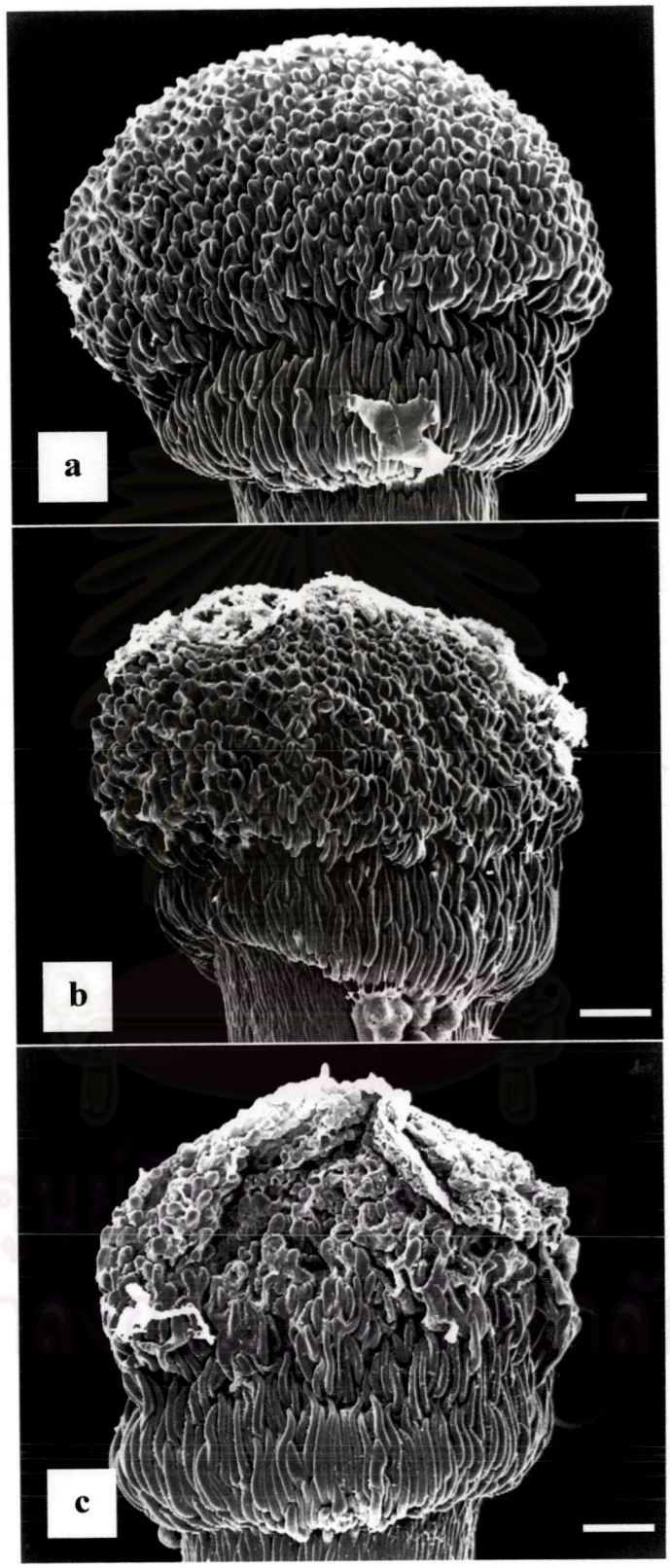


Figure 5.28 Stigma maturation: a, immature stigma; b, mature stigma; c, degenerated stigma, bar = bar = 100 μm.

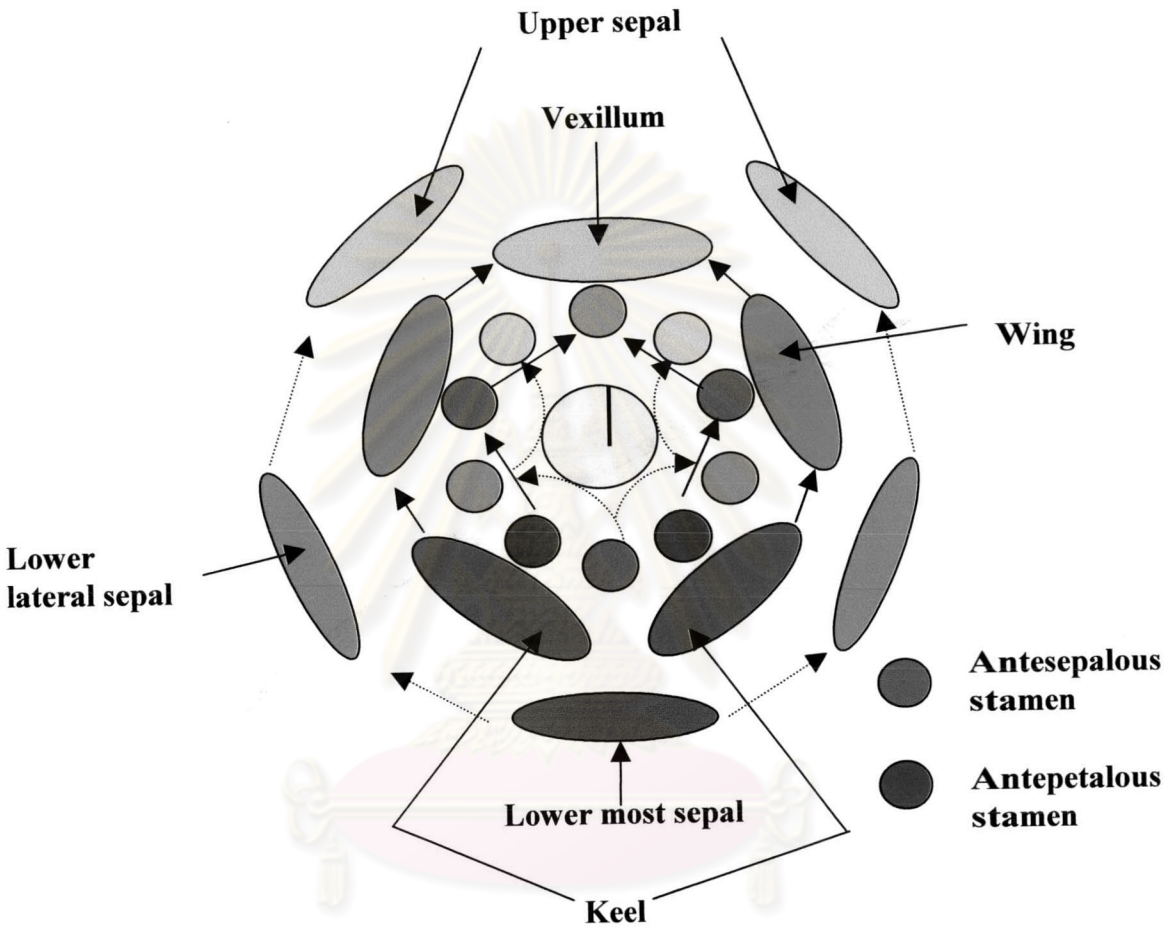


Figure 5.29 The initiation of the floral primordium of *A. sericea* is acropetal, starting from sepals, petals, stamen and carpel, respectively; dark color shade represented starting point of initiation.

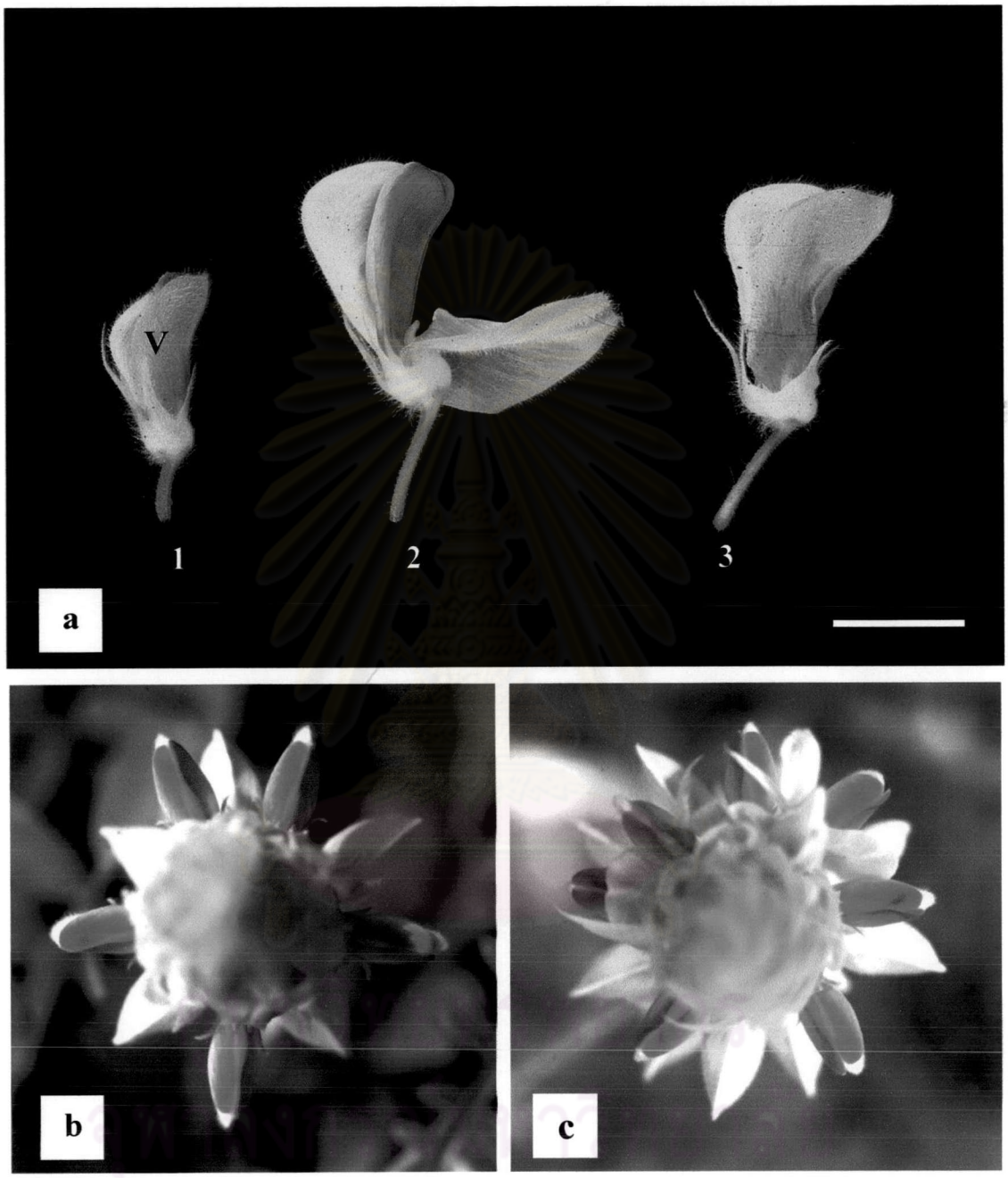
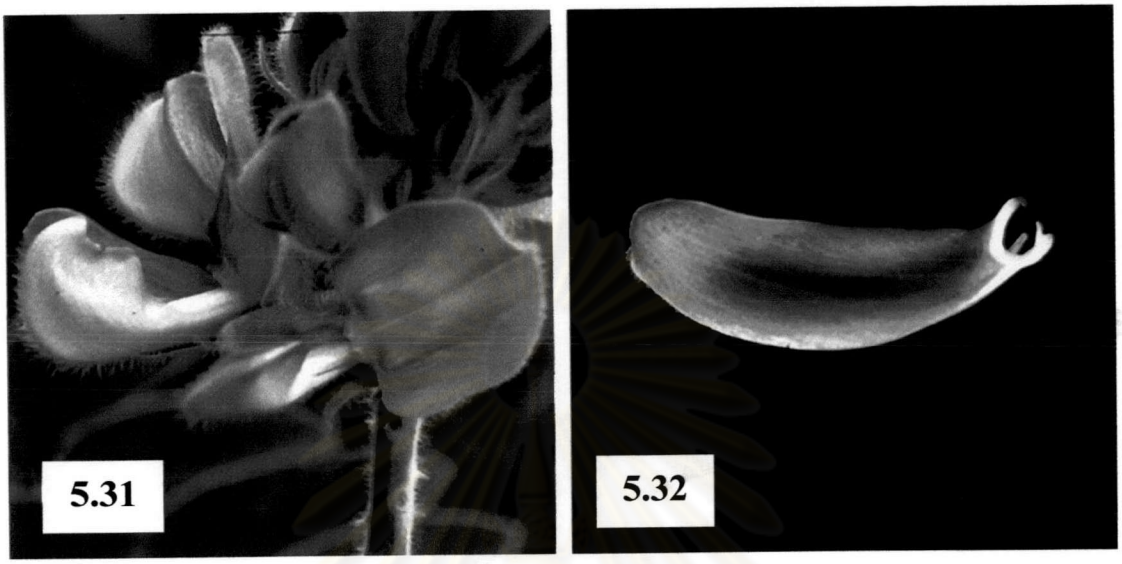
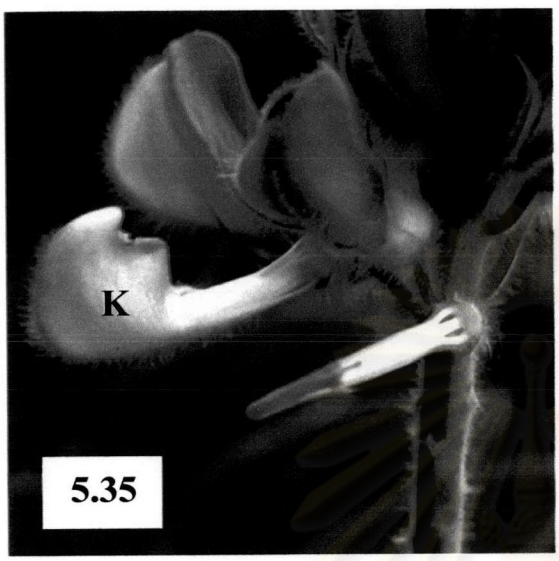


Figure 5.30 Before anthesis all five petals are arching over the other organs (**a1**). At anthesis the vexillum is shortly clawed with an oblong-elliptic blade (**a2**). All five petals of one-day flowers arching over the other organs again after blooming day (**a3**), bar = 2 cm.; **b** and **c** inflorescences top view.

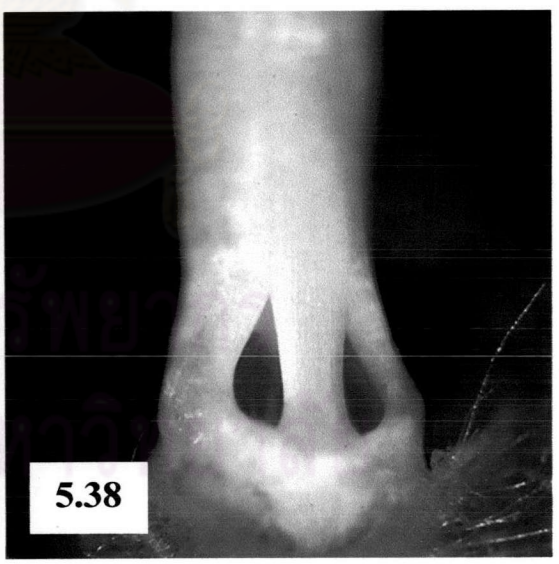


Figures 5.31-5.32 Wing removed flowers (**5.31**); wing (**5.32**), (**5.31** = 1 X; **5.32** = 2 X).

Figures 5.33-5.34 Vexillum and wings removed flowers (**5.33**); vexillum (**5.34**), (**5.33** = 1 X; **5.34** = 2 X).



Figures 5.35-5.36 Vexillum, wing, and keel (**K**) removed flowers; (**5.35** = 1X; **5.36** = 2 X).



Figures 5.37-5.38 Vexillum, wing, keel and staminal tube removed flower (**5.37**); **5.38** Staminal tube (**5.37** = 2 X; **5.38** = 20 X).

3. Flowering Phenology and Anthesis Process

3.1 Events in the single inflorescence studies

3.1.1 One full opening cycle from the first day to the last day of anthesis takes 68.60 ± 8.74 days (mean ± SD, n = 5) that is ca. 2 months.

3.1.2 Average inflorescence length of 15 inflorescences from 5 plants was 49.24 ± 9.46 cm, and the mean of the floret numbers were 228.93 ± 68.93.

3.1.3 In the flower anthesis studies, it was found that bract of all florets that going to anthesis fell before midnight, all anther dehiscence was between 01:00-02:00 hour. Timing of flower opening starting form 04:00 hour until full blooming of all florets at 09:00 hour, flower opened acropetally (Figure 5.39, 5.40).

3.2 Dynamics of flowering of the fifteen plants with 15 inflorescences each were investigated. It was found that flowering magnitude of 225 inflorescences from floral bud until the last floret blooming takes 13 weeks. On the 4^{th} week 36% of all inflorescences are in bloom, on the 7^{th} to 10^{th} week all inflorescence are in bloom, and on the 13^{th} week only 39.11% of all inflorescences are still in bloom (Figure 5.41).

3.3 Flowering course of the whole population

As compared with the weather data of 2000 and 2001 (Figure 5.42), the start of flowering season of *A. sericea* coincided with the beginning of the rainy season. Figure 5.43 shows the flowering phenology in study plot. In 2000, flowering commenced on the 8^{th} of April and ended with the wilting of the last flower on 25 November, ca 8 months later. Peak flowering, i.e., the time of maximum flower number per time unit, occurred on the 5^{th} of August. On that day, 421 inflorescences from 30 plants were in anthesis, and 26.67% (8/30) started fruit setting, fruit setting commenced on 29 July, 20% (6/30) was found. In 2001, flowering commenced on the 9^{th} of June and end on the 17^{th} of November, ca 6 months later. Peak flowering occurred on the 11^{th} of August. On that day, only 292 inflorescences from 30 plants were in anthesis, and 36.67% (11/30) started fruit setting, fruit setting commenced on the 7^{th} of July, 3.33% (1/30) was found.

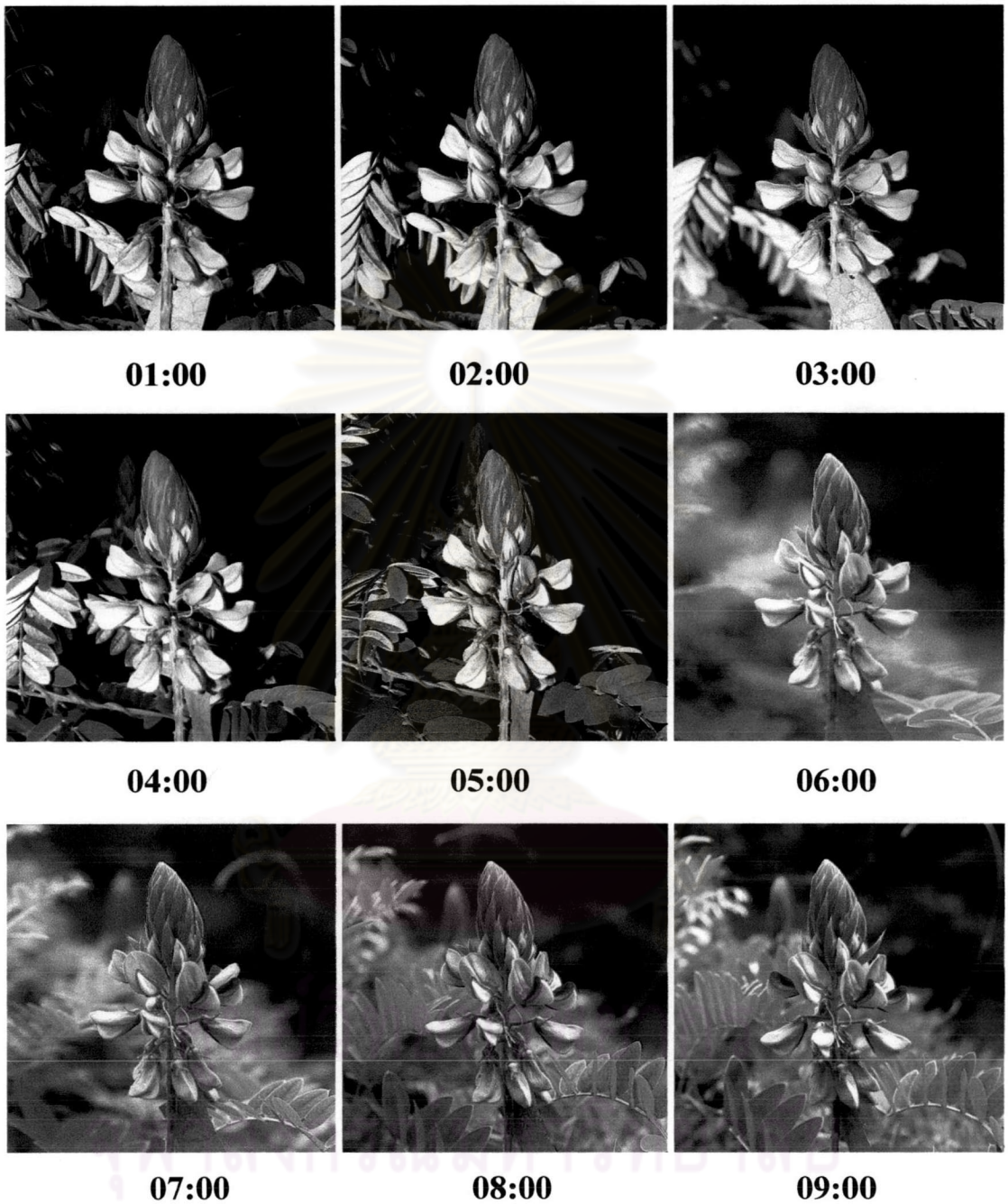


Figure 5.39 Timing of flower opening is starting form 04:00 hour until full blooming of all florets at 09:00 hour, flower opened acropetally.

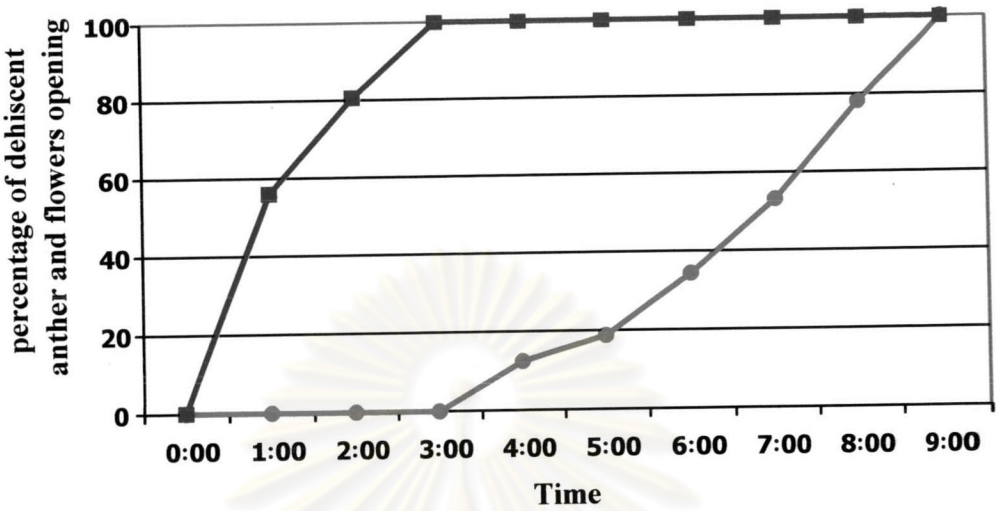


Figure 5.40 Percentage of dehiscent anthers (■) and opened flower (●) from mid night to 9:00 hour at Sakaerat Environmental Research Station on mid of August 1999.

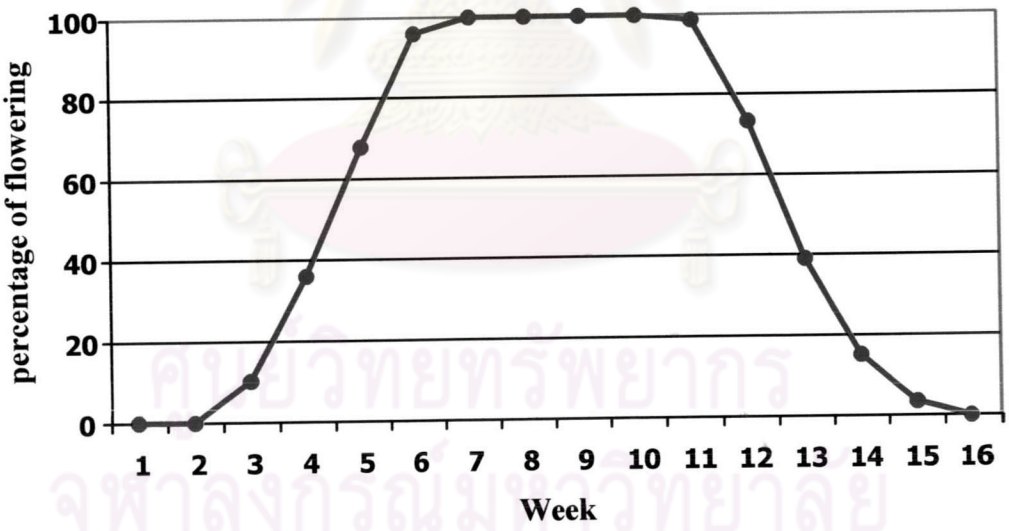


Figure 5.41 Dynamics of flowering of the fifteen plants with 15 inflorescences each from early June 2000 to late September 2000 at Sakaerat Environmental Research Station.

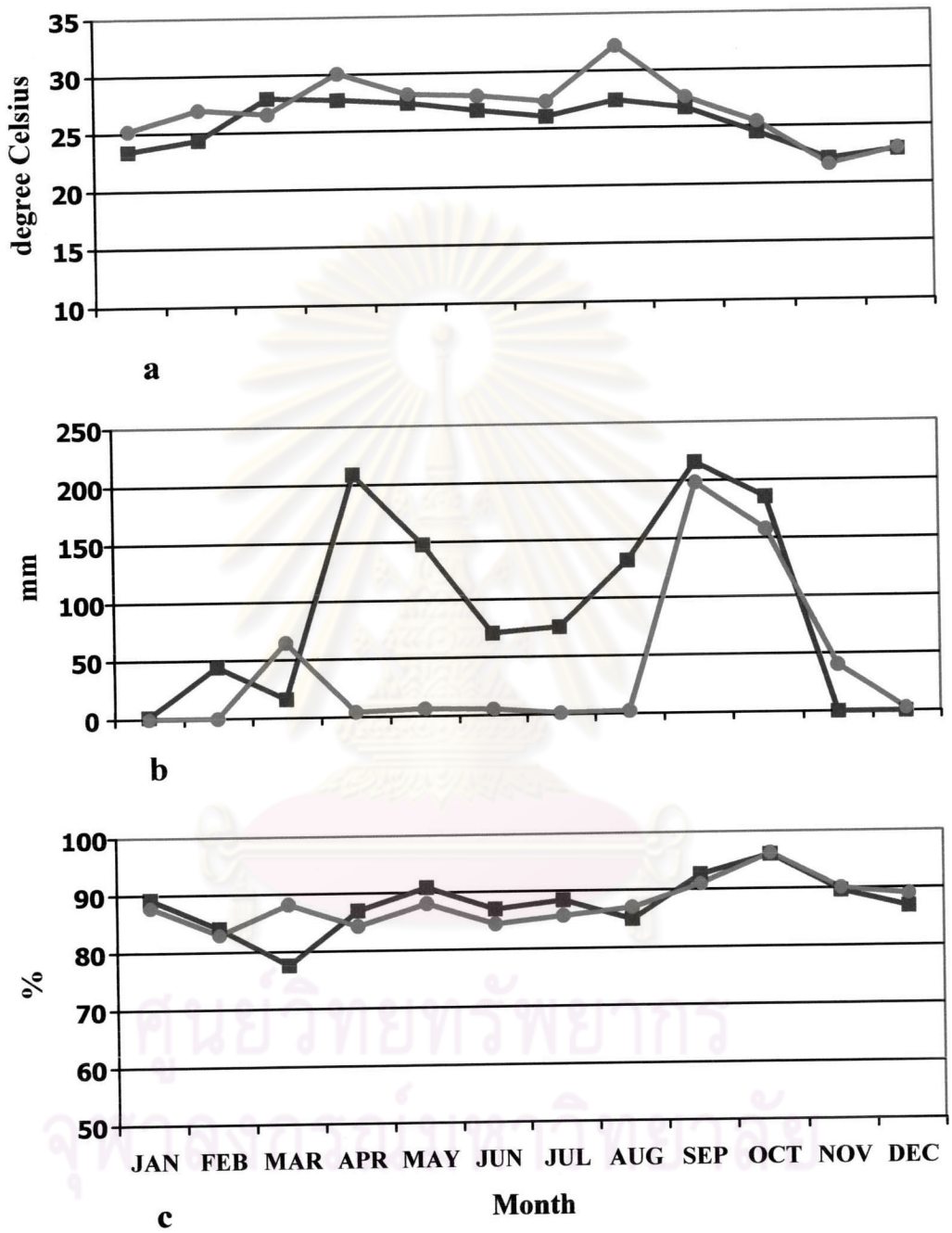


Figure 5.42 a, average monthly temperature; **b**, average monthly total rainfall and **c**, average monthly relative humidity of Sakaerat Environmental Research Station in the year 2000 (■) and 2001 (●).

The number of flowering inflorescences per day from 30 plants ranging from 0 to 421 in 2000 and 0 to 292 in 2001, depending largely on the overall phenological state of the plant and precipitation. In 2000 total annual precipitation was 1101.2 mm and the peak flowering is 421 inflorescences but in 2001, total annual precipitation was only 484.7 mm and the peak flowering was only 292 inflorescences (Figure 5.43).

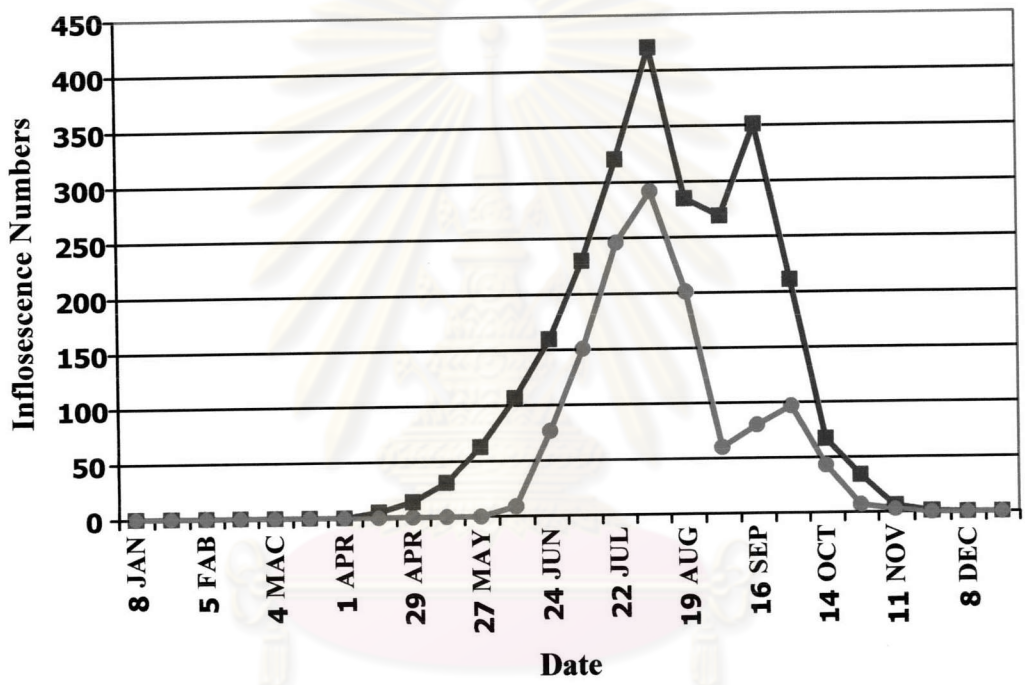


Fig 5.43 Phenology of *A. sericea* in 2000 (■) and 2001 (●) at Sakaerat Environmental Research Station. The total number of inflorescences is given censused per time unit.

4. Nectar Studies

4.1 Studies on the floral nectary structure

The flower produces nectar by a nectary gland at gynoecium base (Figure 5.44, 5.45). It is a concave ring between the stamens and the ovary base, about 2 mm wide and 3 mm height. The gland is composed of small isodiametrical cells with thin walls, relatively large nuclei, intensely stained, with dense granular cytoplasm and small vacuoles (Figures 5.46-5.49). The gland does not have special vascular supplies, so that the receptacle bundles must fulfill this function. The glands do not have stomata, and the nectar has to be exuded through the rugose cuticle (Figures. 5.50, 5.51).

4.2 Nectar amount and nectar concentration

Overall nectar production ranged from 1.35-3.47 μl, and the mean nectar volume ± SD is 2.16 ± 0.58 μl at Siri Ruckhachati Garden, Mahidol University, Nakhorn Pathom; and ranged from 0.93-3.53 μl with the mean nectar volume ± SD of 2.11 ± 0.70 μl at Sakaerat Environmental Research Station, Nakhorn Ratchasima.

Nectar sugar concentration was an average of 65.68 ± 2.80%, ranging from 60.6% to 69.6% at Siri Ruckhachati Garden, and 63.02 ± 2.44%, ranging from 58.2% to 67.2% at Sakaerat Environmental Research Station

4.3 Determination of nectar sugars

Nectar can be analyzed for sugar composition using thin-layer or high-performance liquid chromatography (HPLC) (Stuessy, 1990), in this investigation kinds of sugar were identified via HPLC, four samples were examined, and the only sugars identified for all samples were the usual three, i.e. sucrose, fructose, and glucose (Table 5.1). There was some variability in sugar ratios, however all samples were sucrose dominant. The hexose ratios showed that in all samples fructose pre dominates over glucose. There also was variability among the samples in the hexose proportions.

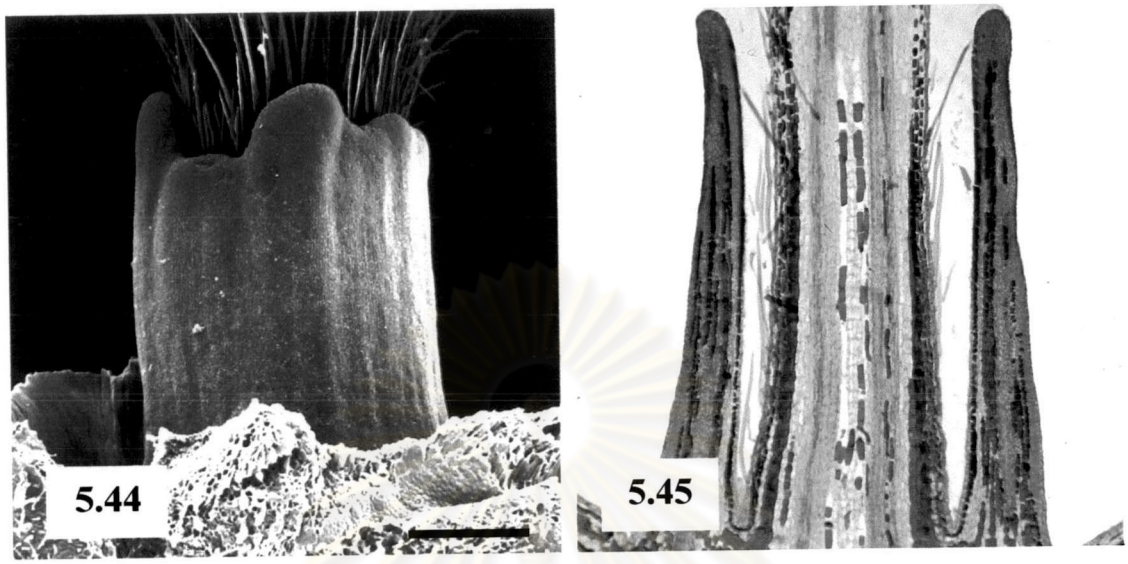


Figure 5.44-5.45 Nectary structure: **5.44** Nectary SEM micrographs, bar = 500 μm; **5.45** Nectary in longitudinal section (25 X). The floral nectary, a disk or collar around the gynoecium base.

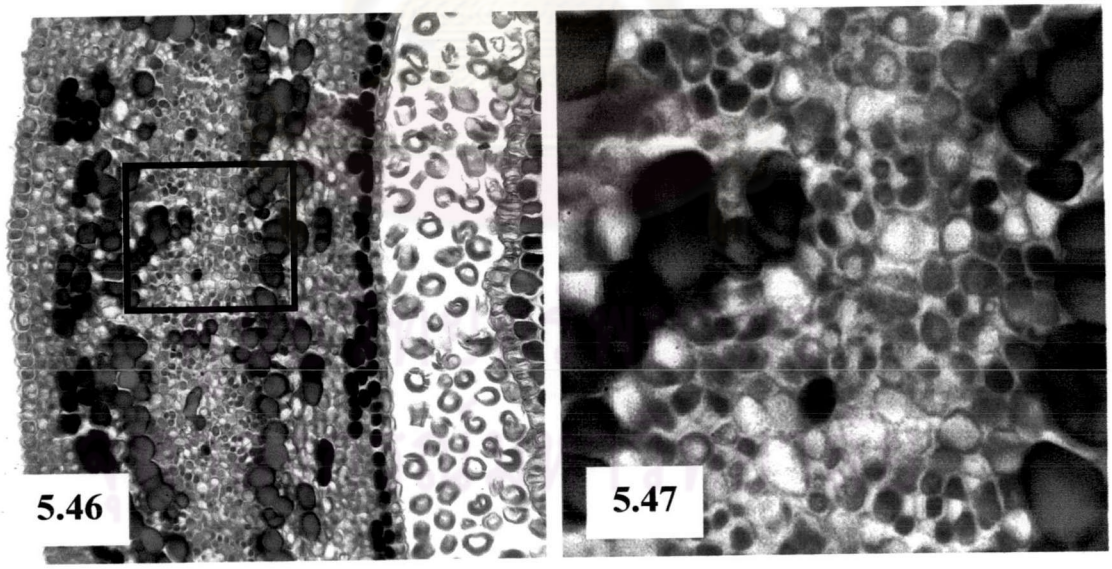


Figure 5.46-5.47 5.46 Nectary in cross-section (100 X). **5.47** The gland is composed of small isodiametrical cells with thin walls, relatively large nuclei, intensely stained, (300 X).

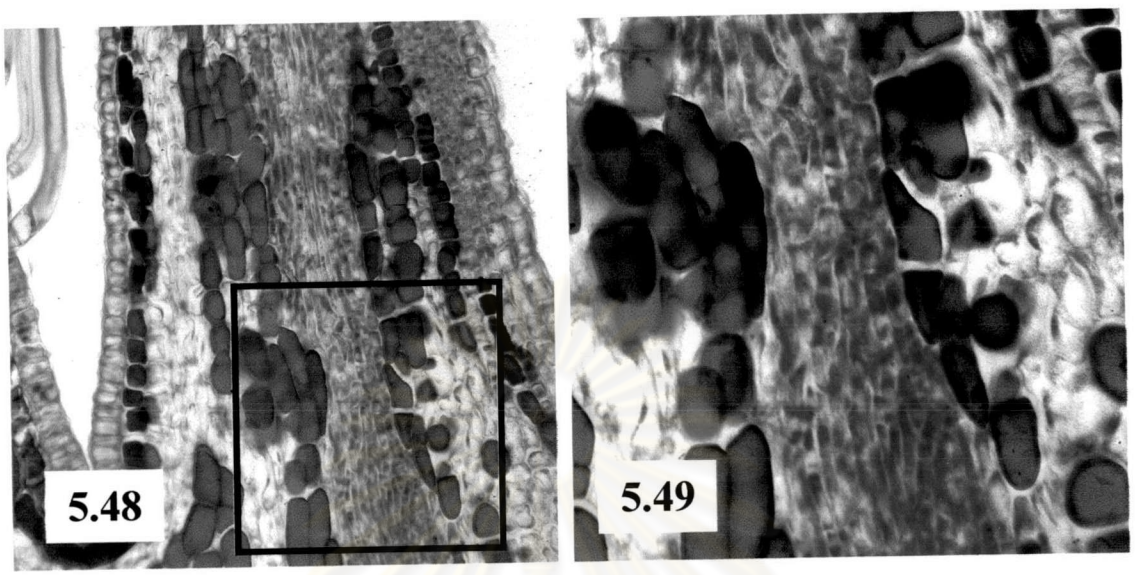


Figure 5.48-5.49 5.48 Partial nectary longitudinal section (100 X). **5.49** The gland in details, showing the nectariferous tissue, composed of small cells with thin walls, large nuclei, intensely stained, with dense cytoplasm and small vacuoles (300 X).

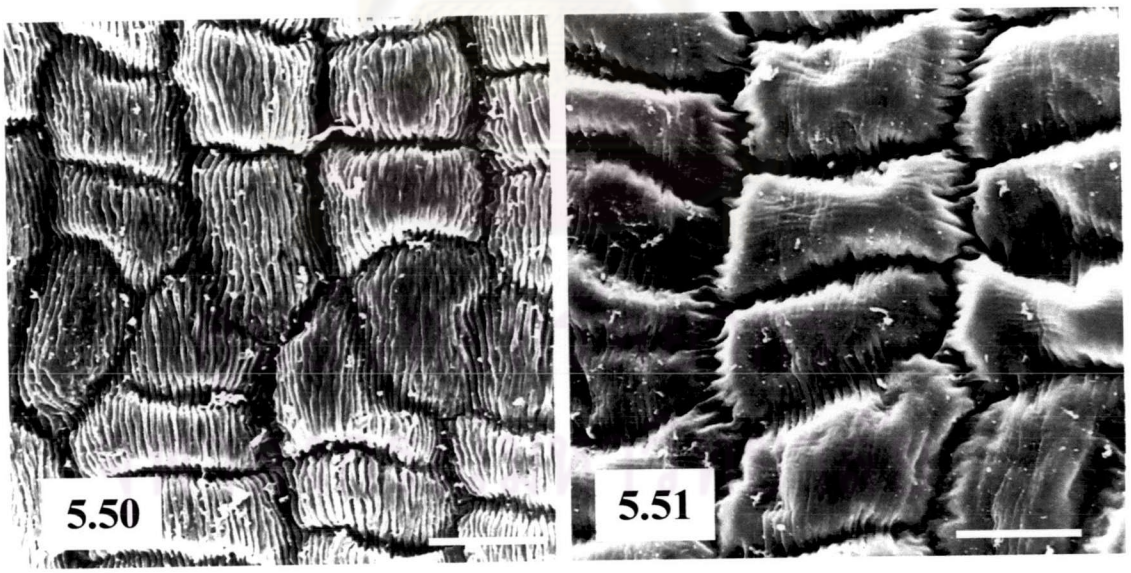


Figure 5.50-5.51 5.50 Outer epidermis of the nectary, the epidermis does not bear modified stomata. **5.51** Inner epidermis of the nectary, the epidermis also does not bear modified stomata, bar = 1,500 μm.

Table 5.1 Nectar sugar composition and concentration in selected samples from Siri Ruckhachati Garden, Mahidol University, Salaya campus Nakhon Pathom and Thai Commemorative Garden Kasetsart University, Bangkok,

Sample	Nectar Sugar Conc. (%)	Sucrose (%)	Fructose (%)	Glucose (%)
Salaya 1	48.96	69.32	18.26	12.42
Salaya 2	55.34	65.99	19.64	14.37
Kasetsart 1	45.16	65.37	19.44	15.19
Kasetsart 2	44.52	71.97	16.16	11.44

Pollen Viability and Germination, Pollen-Stigma Interaction, and Self-Incompatibility

1. Pollen Viability and Germination

1.1 Pollen viability, investigated during anthesis at 9.00 hour, Tetrazolium test was used. Quantitative data concerning the viability (%) were obtained, scoring of 200-300 pollen grains from 10 microscopic fields for each treatment.

Pollen staining with 2,3,5-Triphenyltetrazolium chloride (TTC) gave different results at different age of pollens. The highest percentage viable pollen was on anthesis day, it was 91.29% in pollen samples from Salaya and 89.45% in the sample from Sakaerat, and a steady reduction of pollen viability was observed when the time passed to approximately 30% in 2 days after anthesis. Almost no viable pollen, only less then 10%, was found at 72 hours after anthesis treatment (Figure 5.52, 5.53). Vital staining with TTC revealed that the pollen remains viable for approximately 2 days. Figure 5.53 shows that viability of pollen grains decreases gradually, dropping to 50% line at circa 21:00 hour of the second day of anthesis. In all samples, viability elapsed at the morning of the fourth day.

1.2 Study on pollen germination

Pollen germination tests were conducted in 7 media of different sugar concentration at 9:00 hour on anthesis day. The media which composed of 50% sucrose gave the highest percentage germination. In contrast, the formula, which composed of 10%, 20%, 30% and 70% sugar, showed the low percentage germination of pollen on the anthesis day in all samples of either from Sakaerat or from Salaya. The results of both samples are showed in Figures 5.54, 5.55.

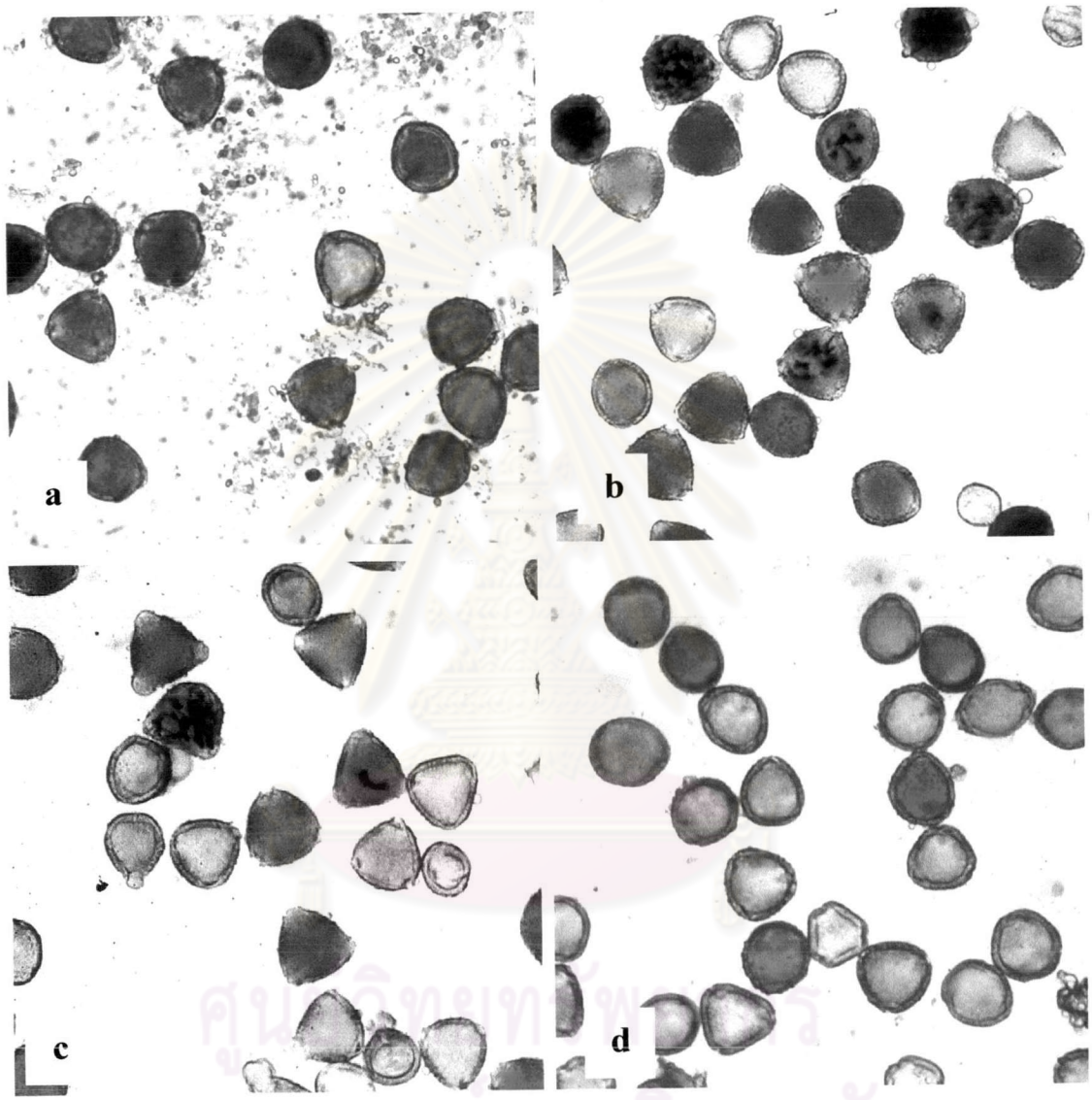


Figure 5.52 Pollen viability tetrazolium tests, **a**, on anthesis day, **b**, **c**, and **d** at 1, 2 and 3 days after anthesis, respectively. The differences in the tonal quality of the pollen grains indicated the gradation in formazan color development from deep red to light red.

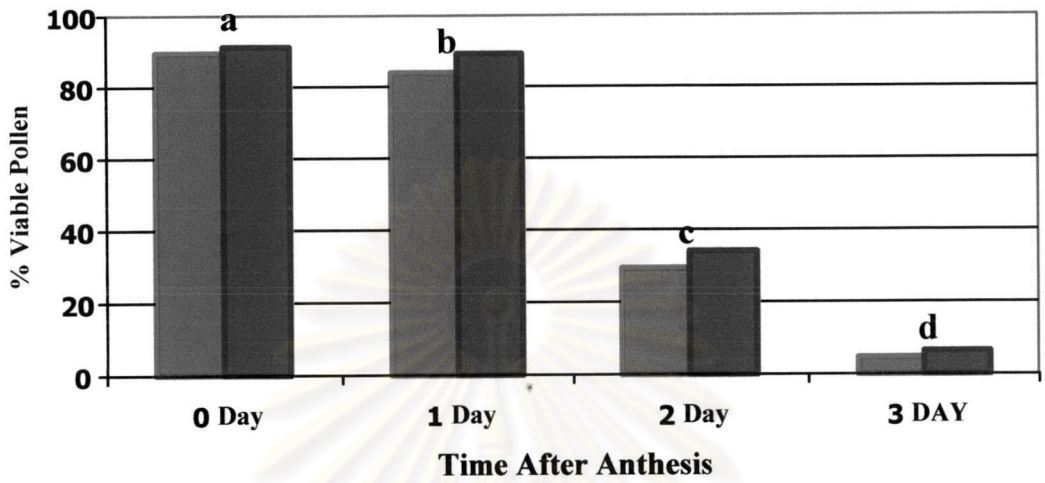


Figure 5.53 Comparison of percentage viable pollen indicated by TTC staining, at 0, 1, 2, and 3 days after anthesis (■ pollens sample from Sakaerat and ● pollens sample from Salaya).

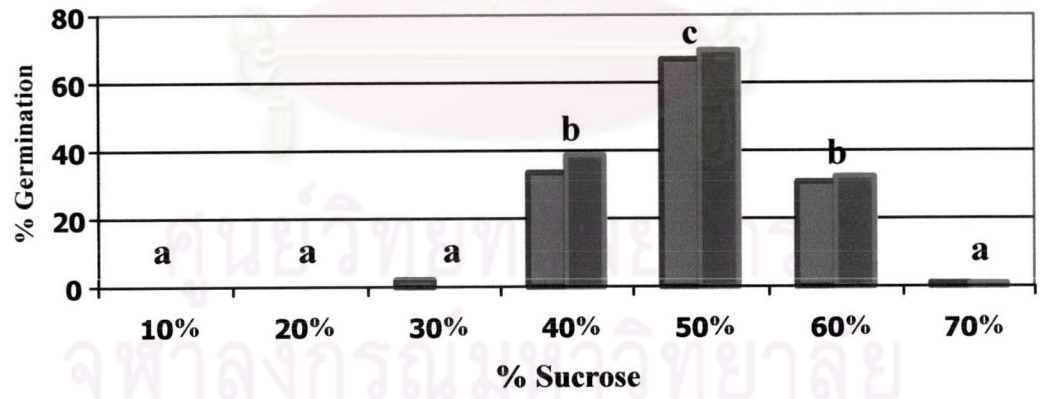


Figure 5.54 Comparison of percentage pollen germination indicated by *in vitro* germination in 7 types of sucrose concentration at 9:00 hour on anthesis day (■ pollens sample from Sakaerat and ● pollens sample from Salaya).

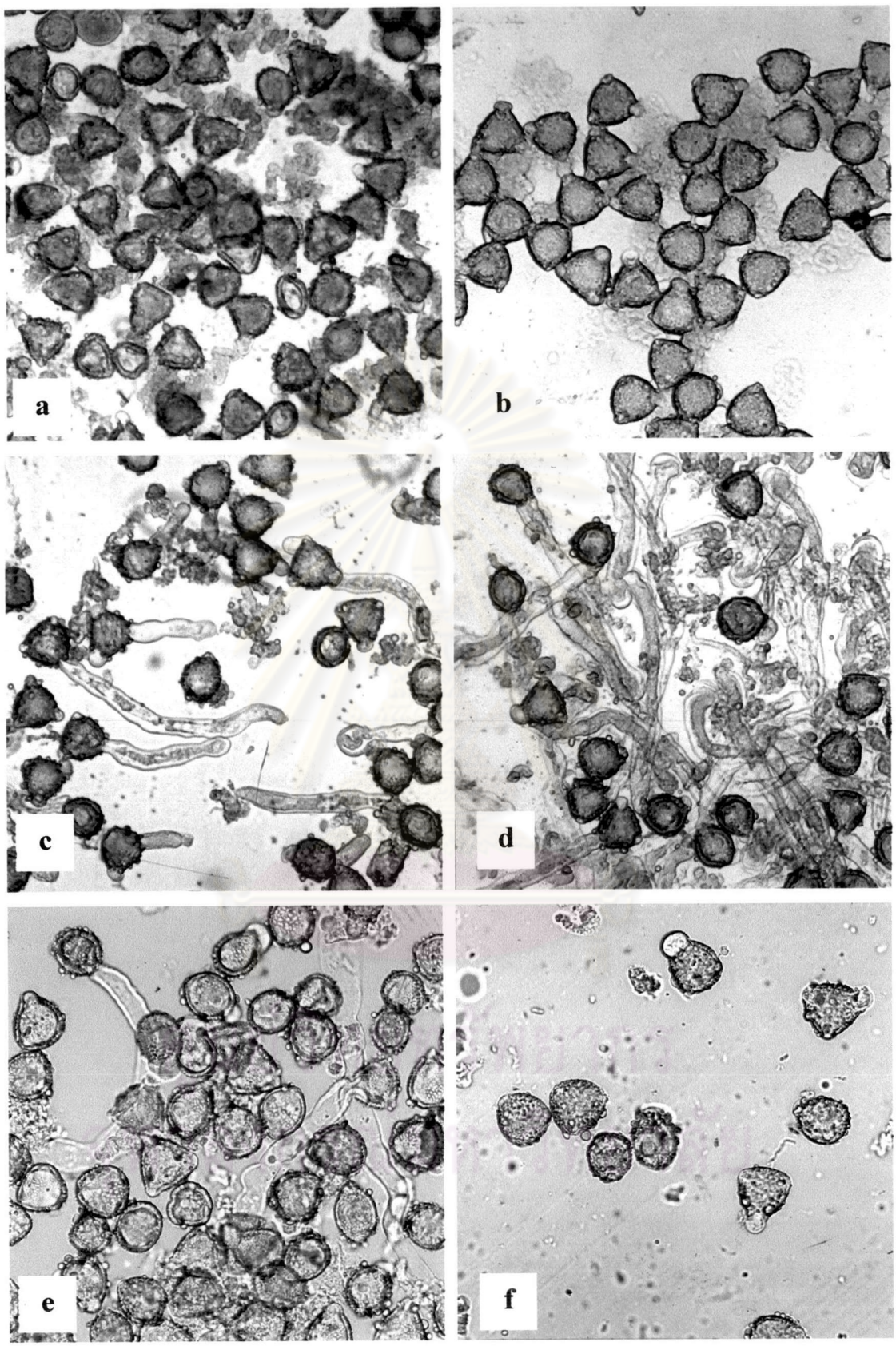


Figure 5.55 Pollen germination tests in 7 sucrose formulas, in **a-f** the result form sucrose concentration of 20%-70% are shown, respectively.

2. Pollen-Stigma Interaction

2.1 Sample of 5 flowers each was taken 16 time intervals on the day before anthesis to the day after anthesis. Cytochemical localization of esterases on stigma surface was used to determine stigma receptivity.

From the investigation it was revealed that, the white and smooth stigma becomes wet and receptive before the day of anthesis. The receptivity lasted until ca. 12:00 hour on anthesis day (Figure 5.56). From then onwards, receptivity diminished slowly, until the day after anthesis. During that time, most stigmas showed signs of wilting, viz. changing color from white to brown.

2.2 Pollen *in vitro* germination on cellophane membrane method was used to test pollen tube growth related to pollen age.

Pollen *in vitro* germination in media composed of 50% sucrose concentration, pollen samples was taken at 16 time intervals from 09:00 hour on the day before anthesis to 09:00 hour on the day after anthesis. It was found that, pollen at 9:00 hour on the anthesis day gave the highest percentage germination. In contrast, pollen on the day before and after anthesis day, showed the lowest percentage germination of pollen. It also found that pollen begin to germinate at 21:00 hour on the day before anthesis, and stop germination on the day after anthesis. The results are showed in Figure 5.57, 5.58.

2.3 Multiple staining for localizing pollen in the pistil was used to localize pollen tubes in the stigma and in the style.

In this investigation, pollen-pistil interaction method for study pollen tube growth *in vivo* was used. It showed the bagged flowers and selfing flowers with a large amount of ungerminated pollen grains on the globose stigma. Pollen counting on stigmas yielded germination, if occurred, with short pollen tube in artificial crossed. Figure 5.59 depicts stigma from an unbagged or open flower at Sakaerat, open to pollination by flower visitors. The germination pollen grains with the pollen tubes indicated successful pollination. Only a small amount of pollen grains are on the stigma surface.

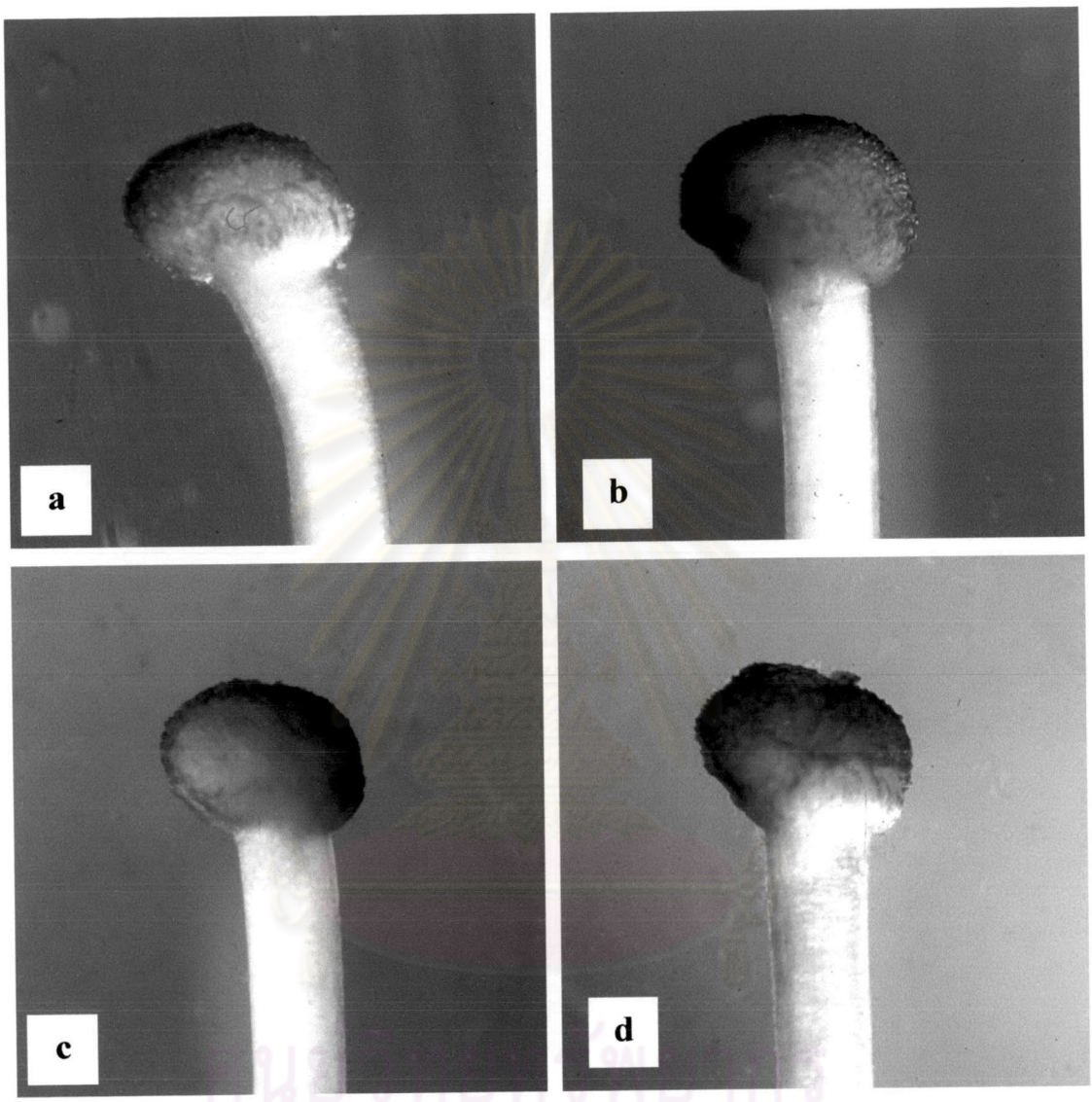


Figure 5.56 The results of cytochemical localization of surface esterases in stigmas after treat with α-nephthyl acetate. The esterase activity is obvious in stigma exudates, seen deep red as a result, at 03.00, 09:00, 15:00, 21:00 hours on anthesis day in **a**, **b**, **c** and **d** respectively.

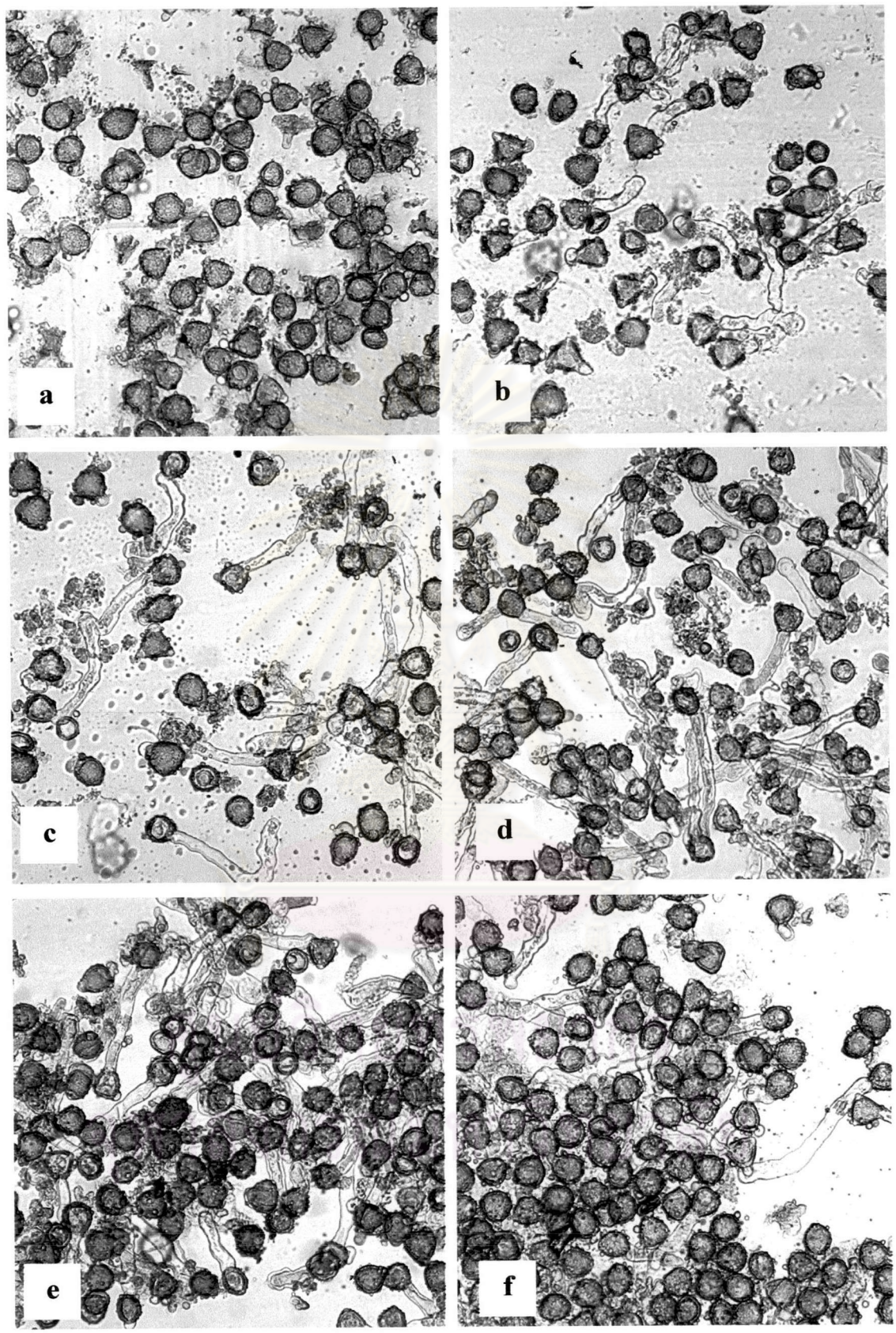


Figure 5.57 Pollen germination tests from mid night to 15:00 hour on anthesis day, three hours interval, the result are shown in pictures **a-f** respectively.

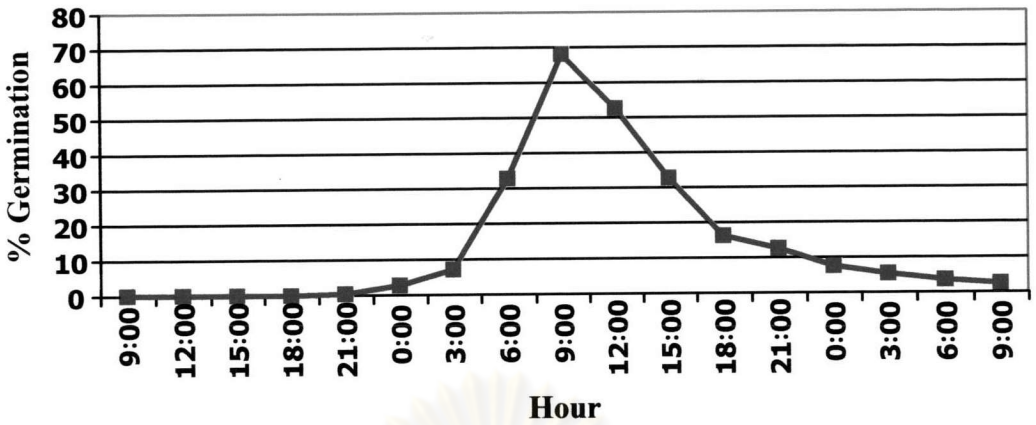


Figure 5.58 Comparison of percentage pollen germination, the results from 9:00 h on the day before anthesis to 9:00 h on the day after anthesis.

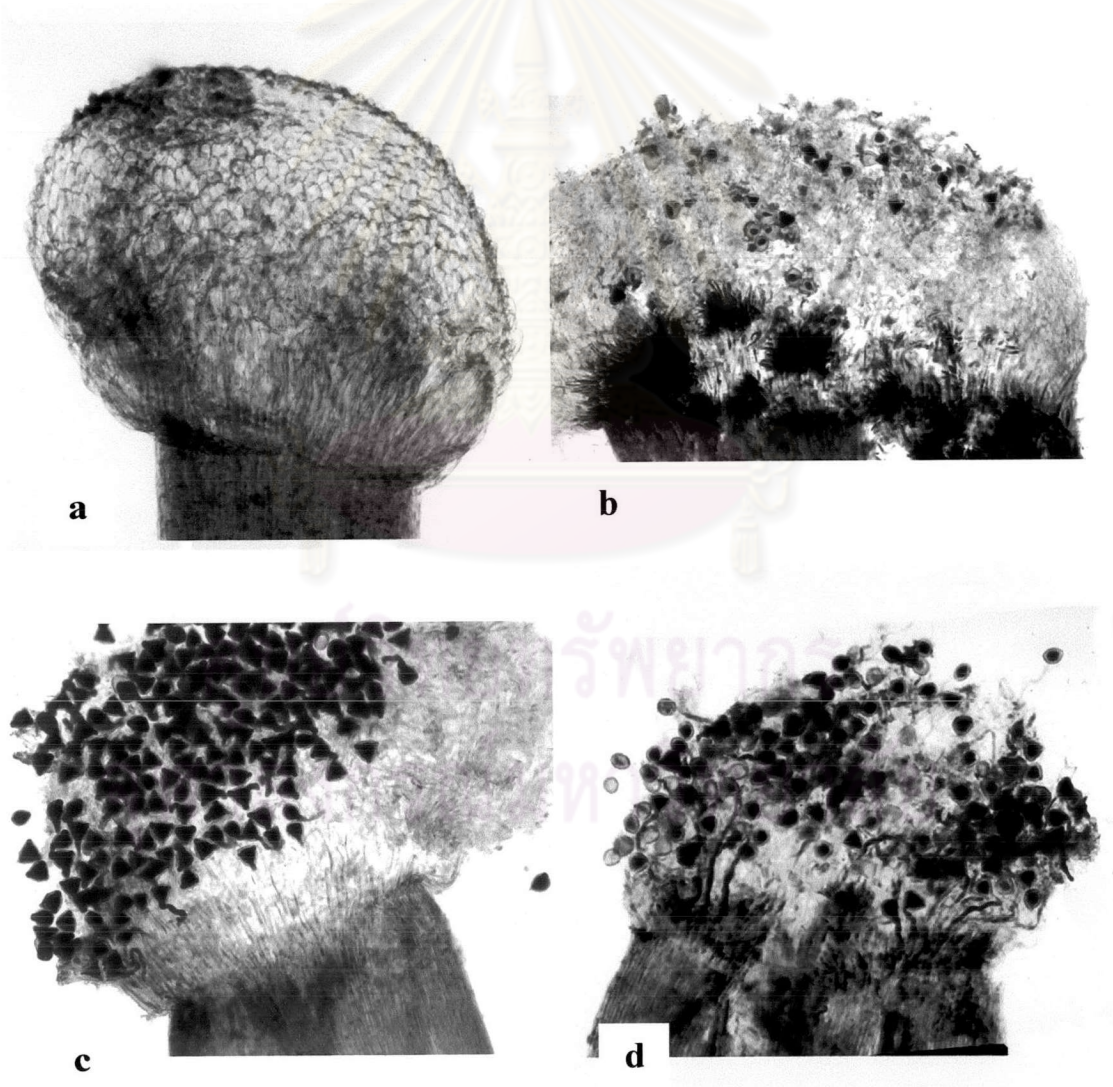


Figure 5.59 Pollen germinated on stigma in bagged (**a**), selfed (**b**), crossed (**c**) and open (**d**) flowers.

Table 5.2 The percentages of pollen germination on stigma in open and bagged flowers in September, October and November 2001.

Study Site	Month	Manipulations	With Out Pollens	With Pollens	With Germinating Pollens
Sakaerat	September	Open	36.24	11.59	52.17
		Bagging	66.67	33.33	0.00
Sakaerat	October	Open	37.59	51.13	11.28
		Bagging	86.67	13.33	0.00
Sakaerat	November	Open	34.83	64.05	1.12
		Bagging	61.62	38.38	0.00

Table 5.3 The results of pollen germination on stigma in open, bagged, selfed, and crossed flowers; the percentages from several manipulations in September and October 2001.

Study Site	Month	Manipulations	With Out Pollens	With Pollens	With Germinating Pollens
Salaya	September	Open	16.67	83.33	0.00
		Bagging	36.67	63.33	0.00
		Selfing	0.00	100.00	0.00
		MU X CU	0.00	20.00	80.00
		MU X KU	0.00	76.67	23.33
Salaya	October	Open	69.44	30.56	0.00
		MU XKU	4.76	76.19	19.05

3. Method for Self-Incompatibility Studies

3.1 Bagging experiment

In this study (Figure 5.60), it was found that none of 30-bagged inflorescences (Table 5.4) from 3 plants, 10 inflorescences each, did set pod. These results rule out the possibility of autogamy, because self-pollen, which is invariability deposited via automatically on the receptive area of the stigma, did not give fertilization to produce pods. This leaves xenogamy, i.e., the pollination and fertilization of flowers from different plant, the only mode of reproduction in this species. In this study fruit abortion in opened flower treatment also found (Table 5.4, Figure 5.61).

The bagging experiments with a single inflorescence from time to time in the earlier experiments support the results of these experiments: no flower did set pods, even though the pollen remain viable on the stigma for the entire duration of anthesis, i.e., 1-3 days. It was also observed that at all times the entire flowers was aborted in 1-2 day after anthesis finished.

3.2 In order to confirm the results of the bagging experiments, the bags with flower were examined for pollen tube growth under a fluorescence microscope compare with selfed, crossed and opened flowers.

In this investigation, pollen-pistil interaction method for study pollen tube growth *in vivo* was used again by fluorescence microscopy technique. The analysis of several hand cross-pollinated flowers showed high percentage of the stigmas with germinating pollen, with short pollen tube and ovary always abortion. This percentage was also high in open pollination. In open pollination analysis showed that high percentage of pistil had been penetrated by the pollen tubes.

Figure 5.60 Bagging experiment: **a**, Bagging at the study site in natural habitat; **b**, Inflorescences were isolated by covering before anthesis with fine mesh net.

Table 5.4 Bagging experiments in a period 7 weeks (W1-W7) at Sakaerat in the year 2001.

Month	Manipulations	W 1	W 2	W 3	W 4	W 5	W 6	W 7
August	Bagging	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Open	0.00	0.00	20.00	30.00	23.33	23.33	23.33
September	Bagging	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Open	0.00	0.00	30.00	30.00	36.67	53.33	46.67

The inefficiency of both bagged- and self-pollination treatments, indicates that, there is no self-fertilization in this species. It showed the bagged flowers and selfing flowers with a large amount of ungerminating pollen grains on the globose stigma. Fig. 5.62 and Table 5.5 depicts stigma from an unbagged or open pollinated flower at Sakaerat, open to pollination by flower visitors. The germination pollen grains with the pollen tubes indicated successful pollination. As no fruit was obtained from artificial crossed pollination, so pollen germination and pollen tube growth in pistil were used to Estimate self-incompatibility rate, the index to measure self-incompatibility (ISI) was used. Because there is no pollen tube occurred in artificial self-pollination (Table 5.5), so ISI = 0, it is indicated that this species is a completely self-incompatible.

3.3 Estimation of the out crossing level, the relation between the number of the pollen grains and ovule (P:O ratio) was observed to reflect the breeding system. The pollen-ovule ratio was determined by calculating the mean number of pollen grains produced per flower and dividing this by the mean number of ovules per flower.

The P:O ratios in this species were 1,5940, 16040 and 16130 in specimens from Chulalongkorn University, Kasetsart University and Mahidol University respectively, which points out to the obligate xenogamy.

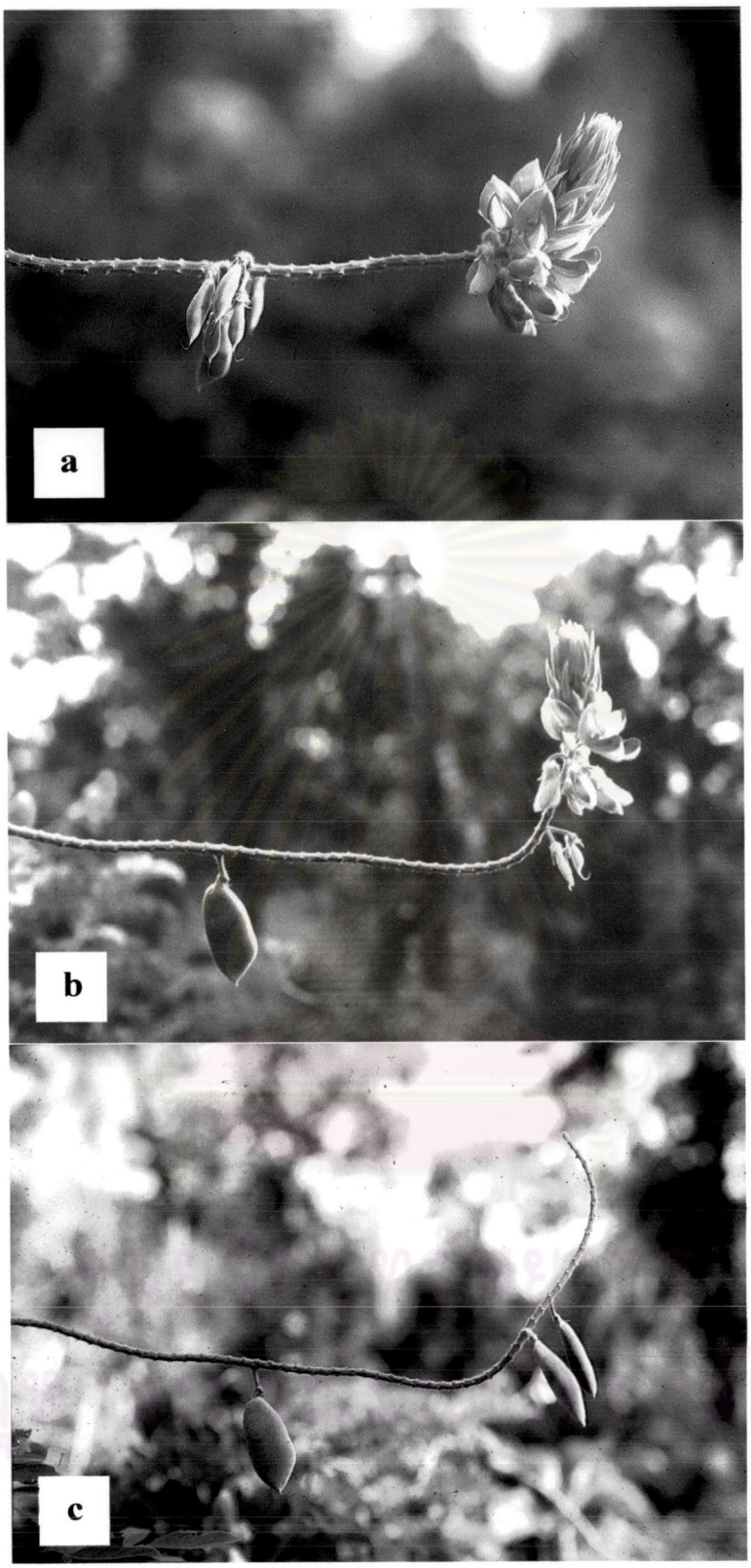


Figure 5.61 Fruit abortion; **A** on the first observation, **B** 2 weeks later, and **C** also 2 weeks after B.

Table 5.5 Pistil with germinating pollen from many manipulations in 3 study sites found that none of bagged and selfed inflorescences gave germination, pollen germinating only in open and crossed inflorescences.

Study sites	Month	Manipulations	With out Pollen	With Pollen	With Germinating Pollen
Sakaerat	July	Open	36	24	40
		Bagging	58	42	0
	August	Open	48	16	36
		Bagging	57	43	0
Salaya	July	Open	68	32	0
		MU X CU	0	43	57
Phanom Rung	August	Open	4	80	16
		PNR X PNR	0	82	18
		PNR X NBN	5	87	8
	September	Open	13	34	53
		Crossed	4	73	24
		Selfed	6	94	0

Note: MU = Siri Ruckhachati Garden, Mahidol University, Salaya, Nakhon Pathom
CU = Department of Botany, Faculty of Science, Chulalongkorn University
PNR = Phanom Rung Historical Park, Buri Ram Province.
NBN = Nong Bun Nak District, Nakhon Ratchasima Province

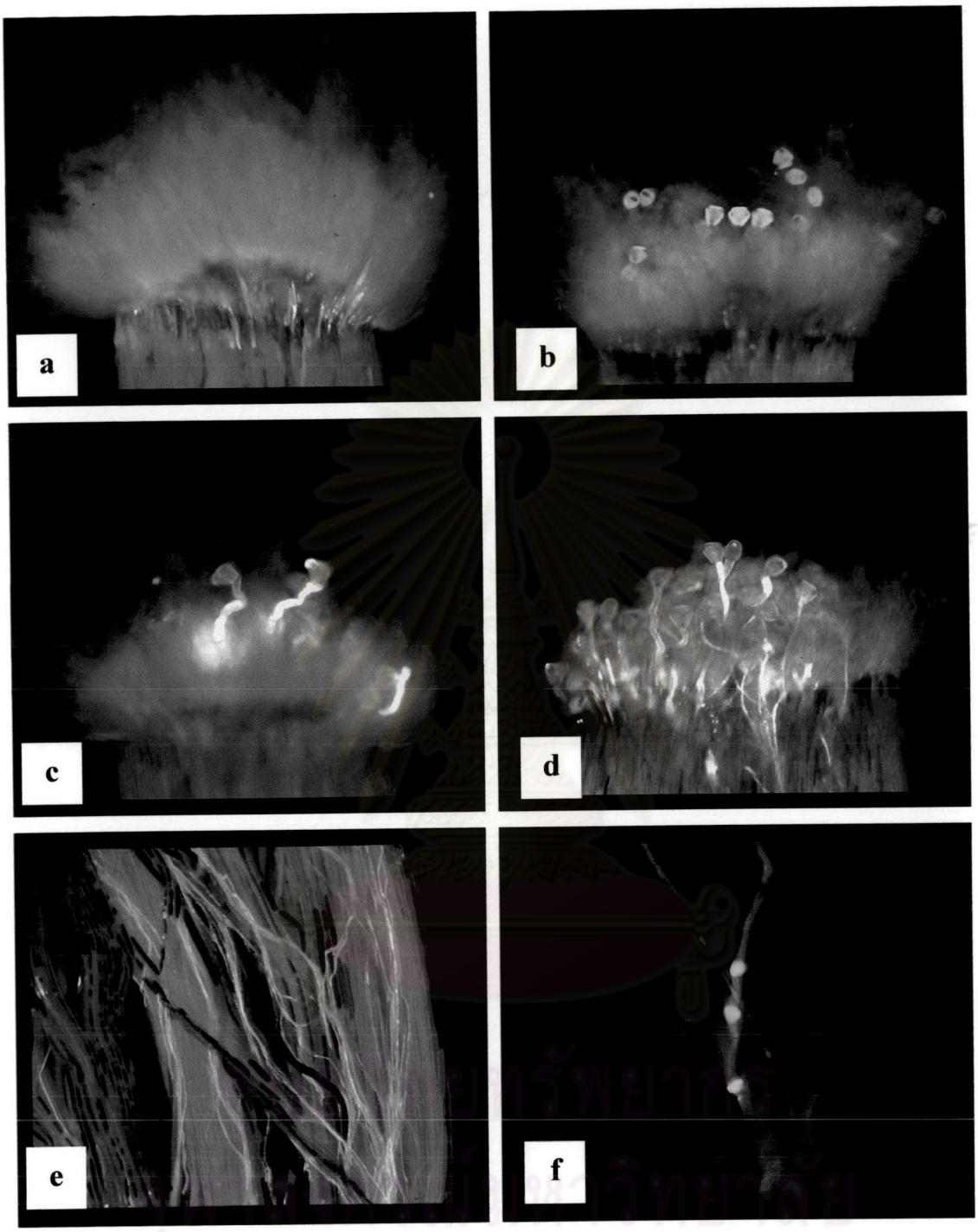


Figure 5.62 Microphotograph of stigma and uppermost part of the style. Note that no pollen tubes emerge form the pollen grains in **a**, bagged flower, and **b**, selfed flower. In hand crossed- **c**, and un-bagged flowers **d**, there are the numerous pollen tubes. In hand cross-pollinated flowers showed germinated pollen with short pollen tube. **e**, Pollen tubes in the style. **f**, Tube nucleus, the lowest one, and 2 sperm cells.

Behavioral Features of the Visitor and Pollinator-Plant Interaction

1. Visitor and Pollinator

Twenty animal species are found to be visitors of *Afgekia sericea* Craib in 5 study sites (Table 5.6) (Figure 5.63, 5.64). The majority of them are bees of the order Hymenoptera. The minority of these visitors are beetle of the genus *Mylabris* (*M. phalerata*), butterfly of the genus *Chilades* (*C. pandava*) and sunbird (*Nectarinia sperata*).

Among these Hymenoptera, the leaf-cutter bee (*Megachile* spp.) is the largest group, composes of 5 known species, i.e. *M. conjuncta*, *M. disjuncta*, *M. monticola*, *M. umbripennis* and *M. velutina*, and 3 unidentified species (*Megachile* spp. 1, 2 and 3). The others are two identified and one unidentified mining bees of the genus *Nomia* (*N. iridescens*, *N. elliotii* and *N.* sp.), two mining bees of the genus *Anthophora* (*A. crocea* and *A. zonata*), two carpenter bees (*Xylocopa aestuans* and *X. dissimilis*), one species of dwarf carpenter bee (*Pithitis smaragdula*) and one unidentified stingless bee species (*Trigona* sp).

These visitors can be categorized into two groups, pollinator and non-pollinator, according to their behavior that may take part in the pollination of *A. sericea* Craib or not. All species of *Megachile*, *Nomia* and *Pithitis* are found to be potential pollinators whereas the others are non-pollinator, as shown in Table 5.6.

***Megachile* spp.**

Leaf cutter bees get their name form the habit of some species that of cutting circles and ovals of leaves to line their burrows and most of them are solitary bees (McGavin, 2000). Many species have stout, dark brown to black bodies and may have yellow, white or pale marking. Pollen collecting species carry their loads in a brush of hair found underneath their abdomen (Deyrup, 2000).

Among the identified *Megachile*, *M. monticola* (Figure 5.66) is an only one large bee. It has wide-set and large mandibles, large and square head, and stout body. Its thorax and part of abdomen covered with dense orange hairs. *M velutina* (Figure 5.65) is a medium-sized bee with brown body. The other three species, *M.*

conjuncta (Figure 5.67), *M disjuncta* (Figure 5.68a) and *M. umbripennis* (Figure 5.68b) are small-sized bees. The first one has brown body whereas the latter two have black with white marking bodies.

Within unidentified *Megachile* (Figure 5.69-5.71), *Megachile* species-1 and species-3 are similar to *M. velutina* (Figure 5.69, 5.71) in its general morphology and color but differs in its smaller size and more dense, orange or yellow hairs covered on the thorax and part of abdomen. *Megachile* species-1 is then very closed to *M.* species-3, but having yellow color lower side abdomen in place of usually black other species.

M. velutina and *M. monticola* were abundantly found in the studied sites. However, the previous species seems to be more common as it has been found in all every studied sites but the latter not found at Salaya and Bangkhen. *M. conjuncta* and *M. disjuncta* were scarcely found at both Sakaerat Environmental Research Station and Salaya for eight and five times, respectively. *M. umbripennis* was even more rare. It has been found only once at Salaya during the study.

***Nomia* spp.**

Nomia elliotii and *N. iridescens* are more or less similar to each other but the latter is slightly larger and also has a longer proboscis (Figure 5.73-**a**, **d**). The unidentified *Nomia* differs from those two species in having yellow bands on the abdomen instead of green.

N. elliotii was found abundance at Sakaerat Environmental Research Station and also found at Phanom Rung Historical Park whereas *N. iridescens* was found only at Salaya and unidentified *Nomia* species was found only one time at Phanom Rung Historical Park.

***Pithitis* sp.**

Pithitis smaragdula (Figure 5. 74) is only one species of *Pithitis* found in this present investigation. It can be easily recognized by its small-sized and brilliant metallic green body. This potential pollinator was found in three studied sites, i.e. Sakaerat Environmental Research Station, Salaya and Phanom Rung Historical Park.

***Xylocopa* spp.**

Ten species of *Xylocopa* were recorded in Thailand (Hutacharern and Tubtim, 1995). Only two species, i.e. *Xylocopa aestuans* and *X. dissimilis* (Figure 5.76) were found to be visitors of *A. sericea* in this study. The first species was found in all five observed sites but the latter species was found only at Sakaerat Environmental Research Station. *Xylocopa aestuans* has medium-sized, black body with bright yellow hairs whereas *X. dissimilis* has larger size and black body without any mark.

***Trigona* sp.**

Stingless bee (*Trigona* sp.) was observed measures only 3-4 mm in size. In this species, pollen loads always found on corbiculae. The bee usually visit flower in groups (Figure 5.77).

***Anthophora* spp.**

Anthophora zonata is smaller than the last one. *A. crocea* (Figure 5.78) has deeper black body and wings than *A. zonata*. Both are similar in color band but *A. crocea* has more abundance black hair on thorax and legs. *A. zonata* is more commonly found than *A. crocea*. It has been found in Sakaerat Environmental Research Station, Phanom Rung Historical Park and Salaya but *A. crocea* has never been found in the last locality.

***Chilades* sp.**

At the present study, *C. pandava* (Figure 5.79) is only one butterfly species which is a visitor of *A. sericea* and found at Sakaerat Environmental Research Station only. It is a small butterfly measures only about 15 mm in size. *C. pandava* has brown wing, with black spots on hind wings.

***Mylabris* sp.**

M. phalerata or oil beetle (Figure 5.81) is a large beetle. It has a long and broad body (ca. 25 x 9 mm.), black wings cases with three broad, wavy, orange to yellow bands. It also has unique disagreeable odor. It has been found to be the pest of *A. sericea* in this present study and found just only at Sakaerat Environmental Research Station.

Table 5.6 Plant visitors at 5 study sites: 1. Sakaerat 2. Salaya 3. Bangkhen 4. Phanom Rung 5. Phatthalung. (Reward: P = pollen, N = Nectar)

Study Sites	1	2	3	4	5	Reward	Type of Visitor
Megachildae							
Megachile velutina Smith	*	*	*	*	*	P, N	Pollinator
Megachile monticola Smith	*			*	*	P, N	Pollinator
Megachile conjuncta Smith	*	*				P, N	Pollinator
Megachile disjuncta Fabricius	*	*				P, N, Leaf	Pollinator
Megachile umbripennis Smith		*				P, N	Pollinator
Megachile sp. 1	*					P, N	Pollinator
Megachile sp. 2	*					P, N	Pollinator
Megachile sp. 3				*		P, N	Pollinator
Halictidae							
Nomia elliotii Smith	*			*		P	Pollinator
Nomia iridescens Smith		*				P	Pollinator
Nomia sp. 1				*		P	Pollinator
Anthophoridae							
Pithitis smaragdula Fabricius	*	*		*		P, N	Pollinator
Xylocopidae							
Xylocopa aestuans Linn.	*	*	*	*	*	N	Non-Pollinator
Xylocopa dissimilis Lep.	*					N	Non-Pollinator
Apidea							
Trigona sp.	*	*	*			P	Non-Pollinator
Podaliriidae							
Anthophora zonata Linn.	*	*		*		N	Non-Pollinator
Anthophora crocea Bingh	*			*			Non-Pollinator
Lycaenidae							
Chilades pandava Horsfield	*					N	Non-Pollinator
Nectariniidae							
Nectarinia sperata	*					N	Non-Pollinator
Meloidae							
Mylabris phalerata Pallas	*					Petal	Flower Eating

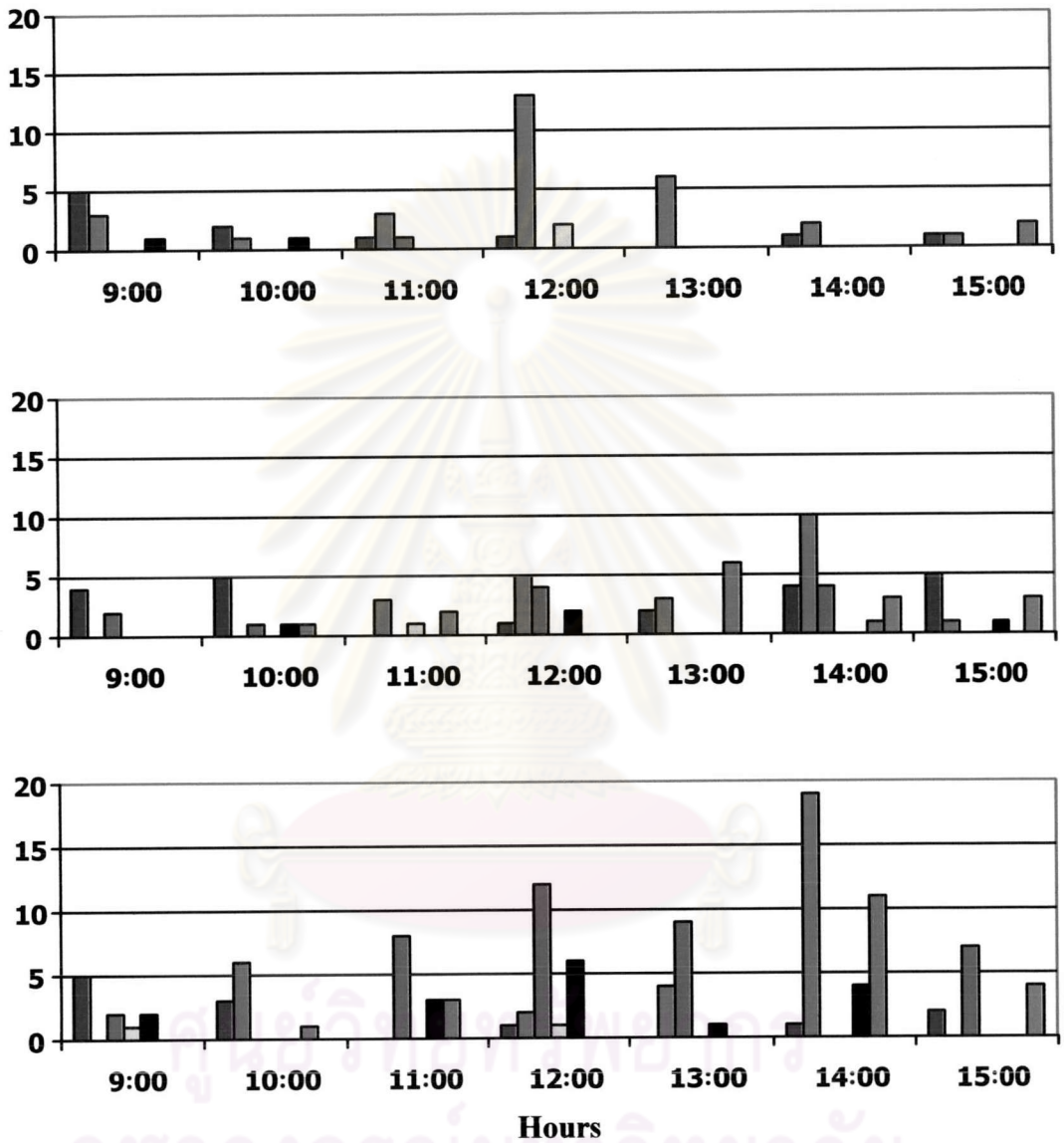


Figure 5.63 Frequency and diversity of plant visitors at Sakaerat Environmental Research Station on 3 rainy days; ■ *Mylabris phalerata*, ■ *Megachile velutina*, ■ *Megachile* spp., ■ *Nomia elliotii*, ■ *Xylocopa aestuans*, ■ *Pithitis smaragdula*, ■ *Nectarinia sperata*.

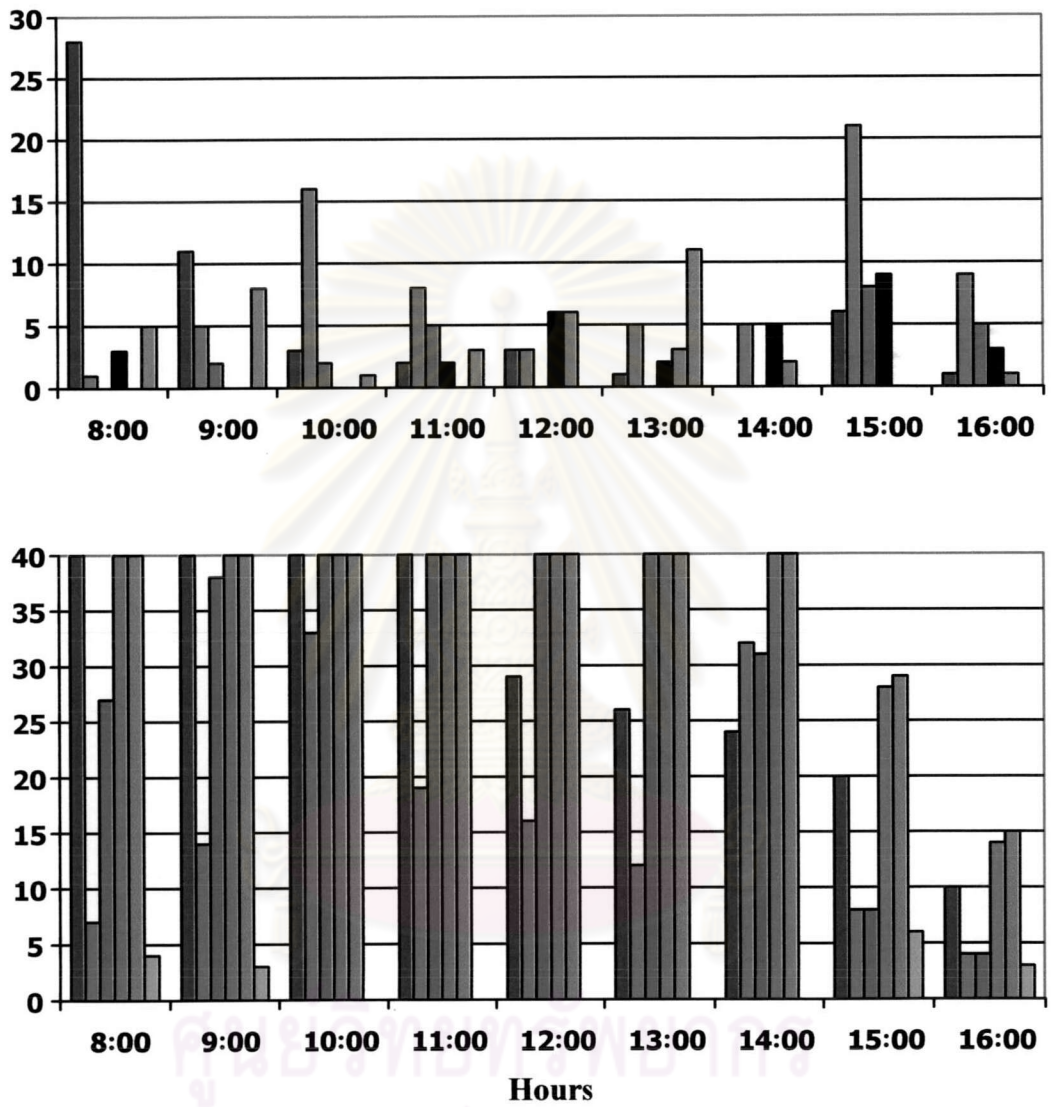


Figure 5.64 Frequency and diversity of plant visitors at Sakaerat Environmental Research Station on 2 sunny days, for convenient visiting frequency more than 40 times/h. all are recorded as only 40; ■ *Mylabris phalerata*, ■ *Megachile velutina*, ■ *Nomia elliotii*, ■ *Xylocopa aestuans*, ■ *Pithitis smaragdula*, ■ *Nectarinia sperata*. ■ *Trigona* sp. ■ *Chilades pandava*.

***Nectarinia* sp.**

N. sperata or purple-throated sunbird was found only at Sakaerat Environmental Research Station. Size is about 10 cm in length both sex are markedly different from each other (Lekagul and Round, 1999; Robson, 2000) (Figure 5.80).

2. Foraging Behavior of Pollinator and Non-pollinator

2.1 Pollinator behavior

***Megachile* spp.**

According to the investigation, *Megachile* spent approximately 10-15 seconds to cut and collect each leaf disc. As a pollinator, it visited flower of *A. sericea* for 60 to 120 seconds to collect nectar and pollen. The visiting bee usually lands and clings to the wings of the flower. It then inserts its proboscis into the path between the vexillum and the upper edges of the keel, sliding it down to reach the nectar at the base of the staminal tube. While it sucks the nectar, its lower body part presses against the wings and the keel. The keel is then moved downwards so that the pistil and stamens somehow exposed at the tip of the keel. The stigma and anthers then come into contact with the underside of the bee's body (Figure 5.72). The bee rakes pollen from the anther and stuffs it among dense rows of long stiff hairs (scopa) on the underside of abdomen until it is entirely packed with pollen. At the same time, the bee rubs its belly across anther and stigma. Finally the pollination is successful. As the bee left the flower, the wings and keel spring back to its initial position. The exposed stamens and stigma are then again covered.

Pollen grains deposited on *Megachile*'s body have the similar morphology as of *A. sericea*'s, in apertural characters, ornamentation pattern, shape and size (Figure 5.75).

Compared with other *Megachile* species, *M. velutina* seems to be the early bee to arrive on the flower, except for an occasional *Mylabris phalerata*. It is often found abundantly at approximately 10:00 hour and again at 14:00 hour, especially on sunny days. It is also restless and rather timid. It may nevertheless be the most

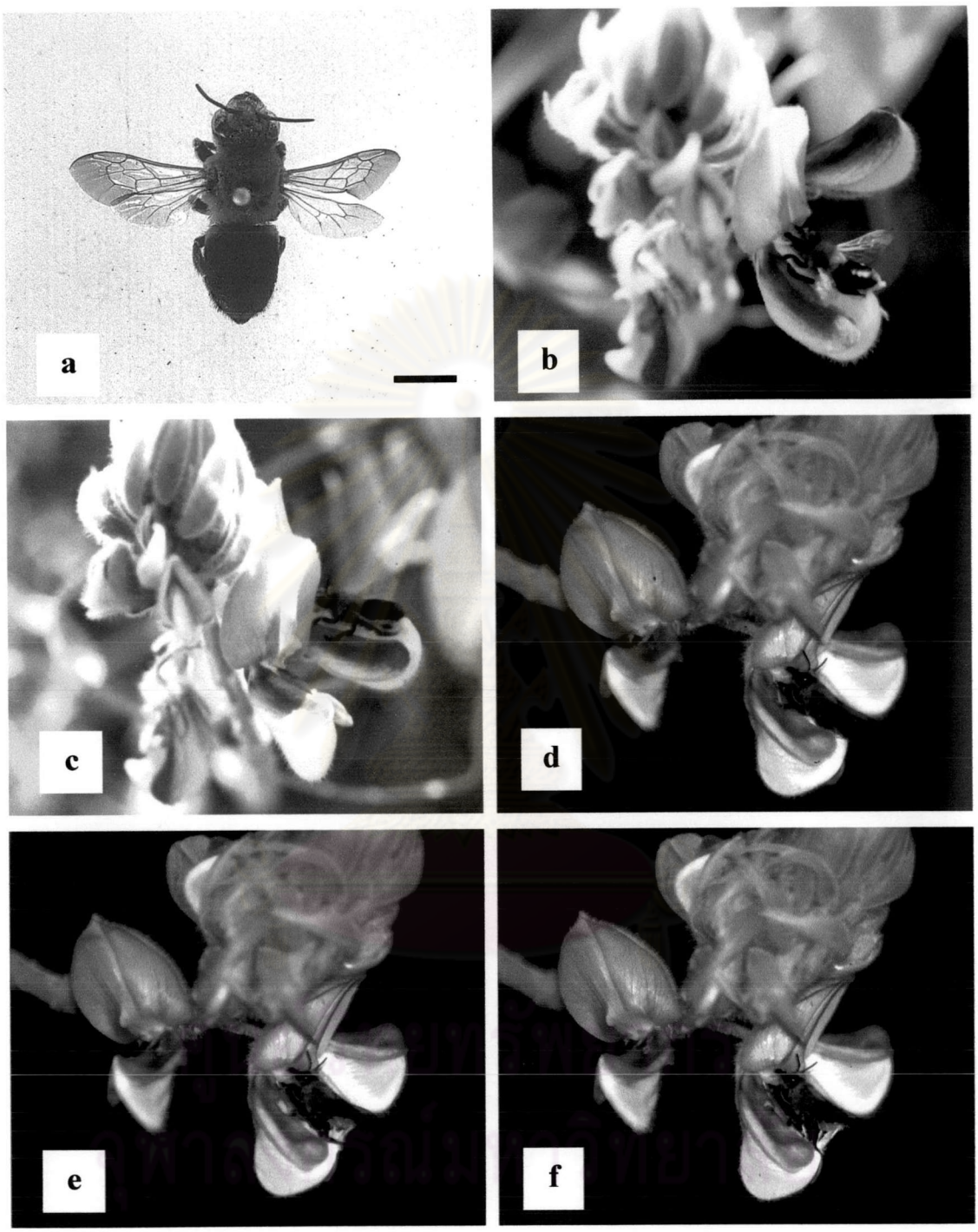


Figure 5.65 *Megachile velutina*, a main pollinator, foraging on *A. sericea*, the bee has approached the flower slightly from one side, and always moves from floret to floret in the same florescence (**b**, **c**). The visiting bee clings to the wings, forces its way into the flower, wings and keel are pressed down, uncovering the stamens and style which come into contact with the underside of the bee's abdomen (**b**, **c**, **e**, **f**), bar = 5 mm.

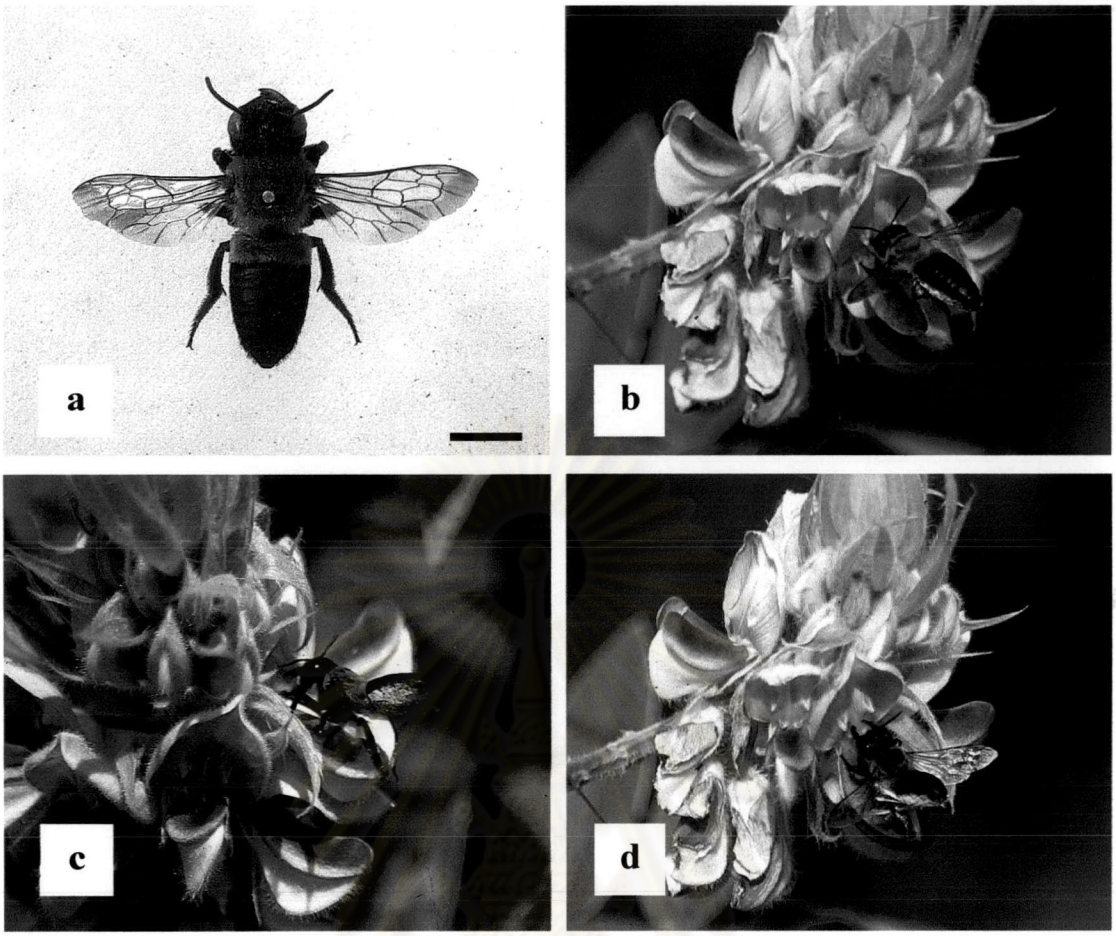


Figure 5.66 *Megachile monticola*, the largest species found from this study (**a**), bee clings to the wings (**b**), forces its and pressed down, uncovering the stamens and style. **c**, **d**), bar = 9 mm.

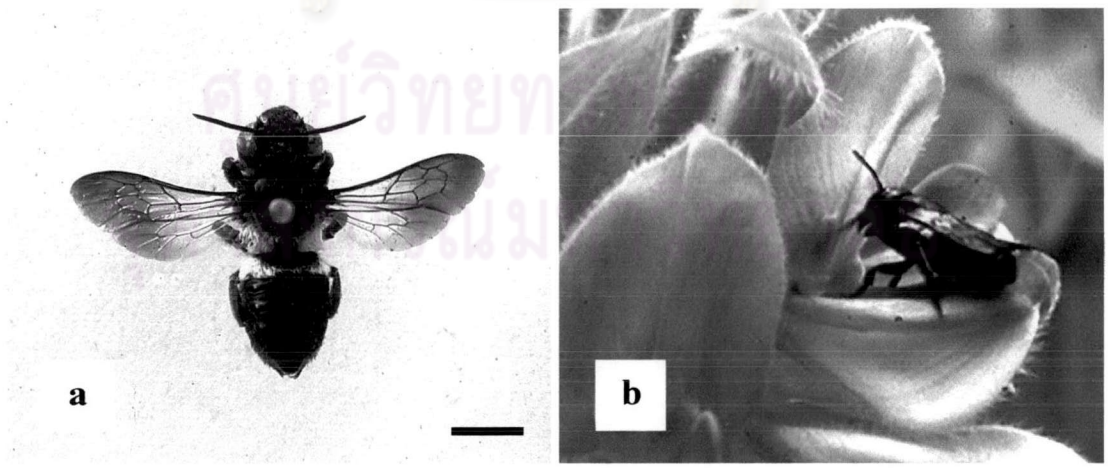


Figure 5.67 *Megachile conjuncta* General behavior was similar to that of *M. velutina*, but less daring in pollen collecting. Most specimens in this species found sucking nectar (**B**) rather than collected pollen, bar = 5 mm.

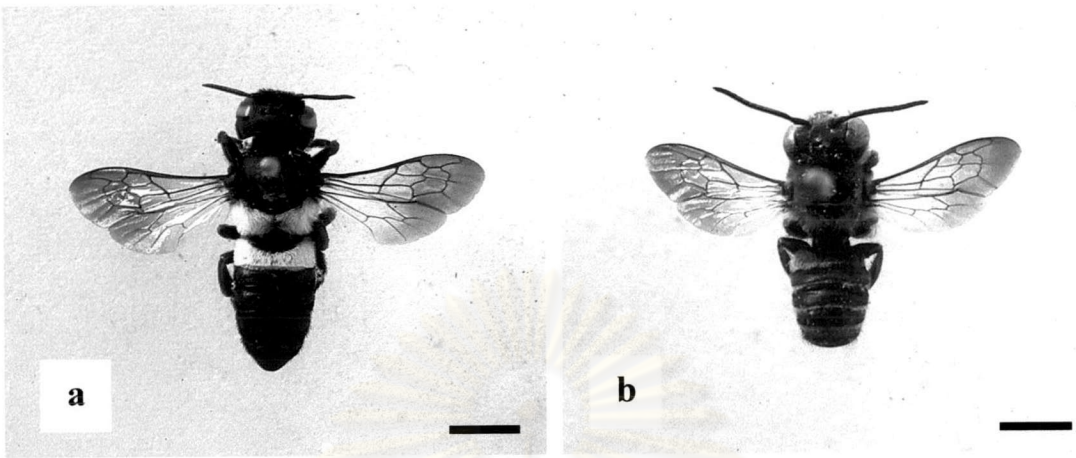


Figure 5.68 *Megachile disjuncta* (**a**) General behavior is similar to that of *M. conjuncta*, bar = 5 mm. *M. umbripennis* (**b**), a smallest of *Megachile* species, an active bee which quickly moving, only 1 specimen was found. Probably it is an efficient pollinator but too scarce when compared to others, bar = 3 mm.

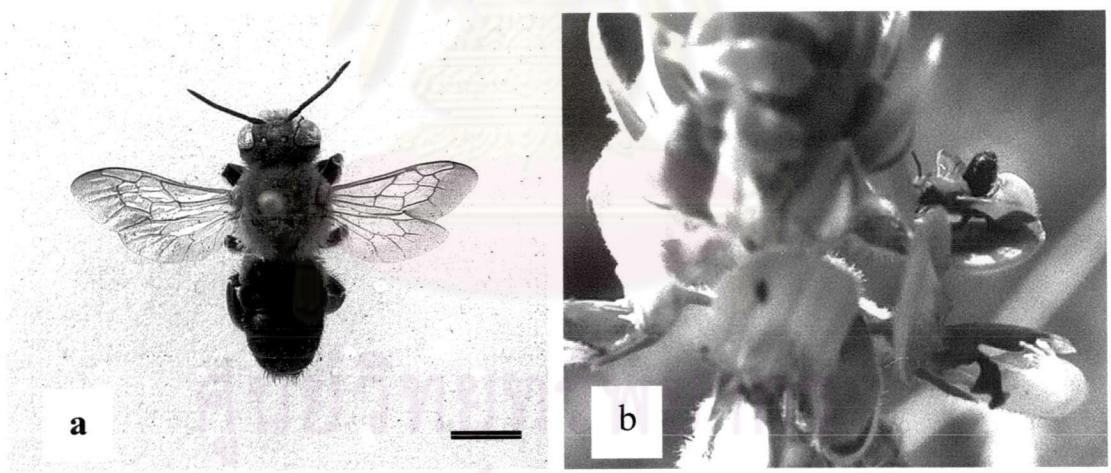


Figure 5.69 *Megachile* sp. 1, this species is similar to *M. velutina* in color (**a**), foraging behavior (**b**) but slightly smaller in size, having thorax and part of abdomen covered with more dense orange hair (**a**), bar = 5 mm.

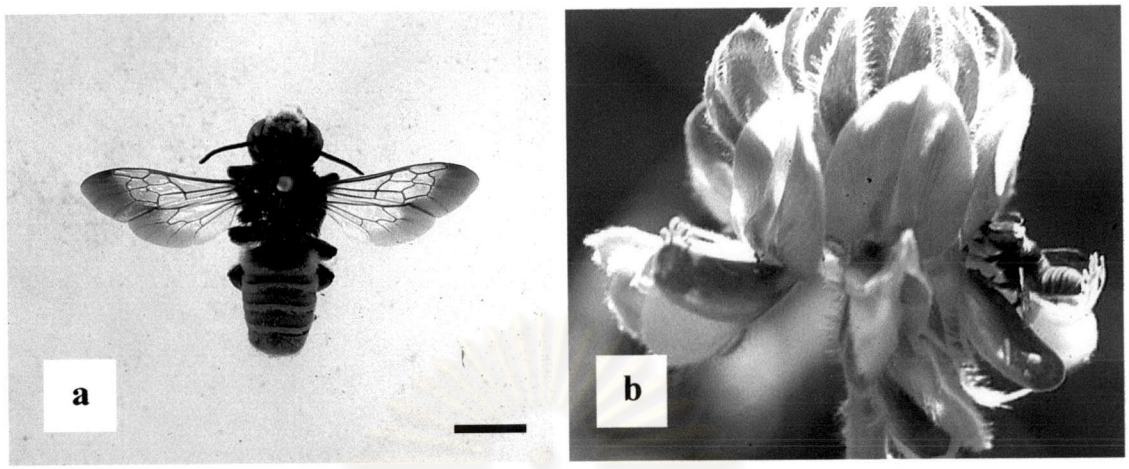


Figure 5.70 *Megachile* sp. 2 is slightly larger in size than *M. umbripennis*, but smaller than *M. velutina*, similar to *M. umbripennis* in general morphology (**a**), and to *M. velutina* in foraging behavior (**b**), has deep black thorax and upper side of abdomen covered with dense yellow hair. It is found only at Sakaerat Environmental research station, bar = 5 mm.

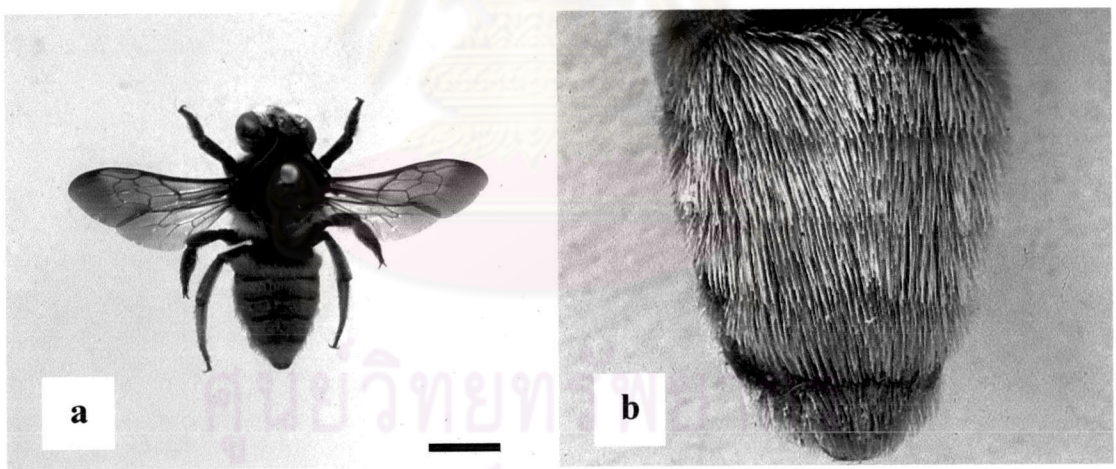


Figure 5.71 *Megachile* sp. 3, one specimen was found at Phanom Rung Historical Park, similar to *M. velutina* in general morphology (**a**) to *M.* sp.2 in deep black thorax and upper side of abdomen covered with dense yellow hair (**A**), slightly differ in yellow color of lower side of abdomen (**b**) in place of usually black in other species, bar = 5 mm.

efficient pollinator because of its relatively high rate of re-visiting and consistency to the flower of *A. sericea*.

M. monticola is the second most common pollinator. However, it is less restless and may less daring in collecting pollen of *A. sericea* than *M. velutina* since it has lower frequency of returness. Eventhough *M. conjuncta*, *M. disjuncta* and *M. umbripennis* had been found to carry pollen of *A. sericea* and have similar pattern of foraging behavior like *M. velutina* and *M. monticola*, but their appearance were so scarce. According to the present observation, they had been found only 8, 5 and 1 times respectively, during 2 years.

***Nomia* spp.**

According to the foraging behavior, *Nomia* seems to be interested only in collecting pollen as it has never touched or gone towards the base of the vexillum, where the nectar can be reached. It lands directly on the wing and presses its head towards the tip of the wings or crawls underneath the hood of the wings to open them up and then forcibly presses its head down into the keel to seek and collect pollen. It collects pollen in the pollen baskets or corbicular on hind legs like honey bee. *Nomia* has its daily emergence peak between 11:00 and 13:00 hours.

***Pithitis* sp.**

Like *Megachile*, *P. smaragdula* collects both nectar and pollen but in different pattern of visitation. When this bee finds for the nectar, it lands on the wings and move directly to the base of the vexillum. Contrary to *Megachile*, when it sucks the nectar, its head points outwards to the tip of the wings and keel, then its lower body part presses against the vexillum, not the wings or keels. On the other hands, it opens the wings and forcibly enters into the keel to collect pollen grains. It collects pollen in the pollen baskets or corbicular on hind legs like honeybee.

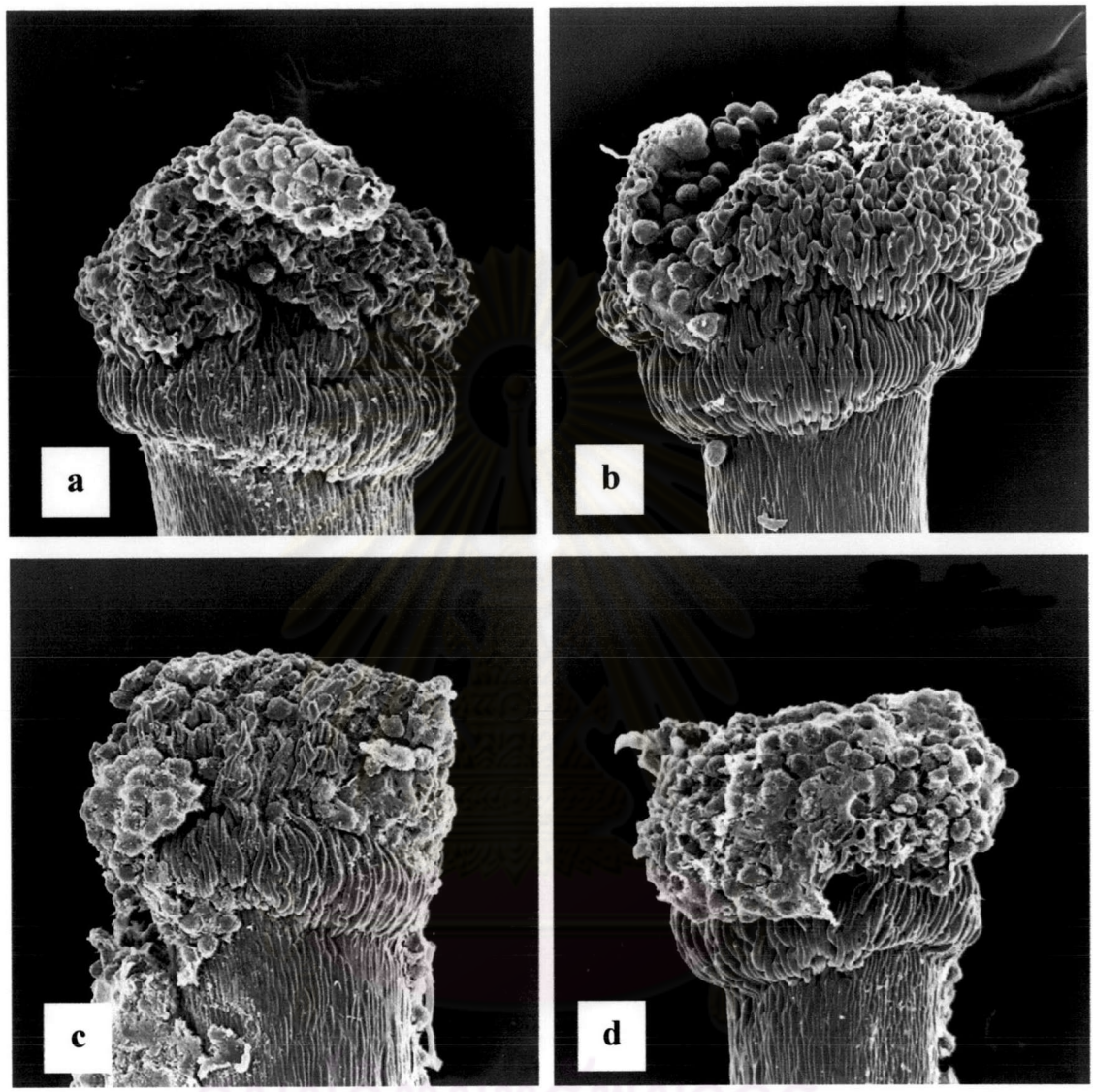


Figure 5.72 Stigma of *Afgekia sericea*; **a** artificial pollination, **b** and **c** pollination by *Megachile* sp., **d** pollination by *Nomia elliotii*, specimens were from Phanom Rung Historical Park. All are difference in abundance of pollen grain and also in stigma morphology.

2.2 Non-pollinator behavior

***Xylocopa* spp.**

The carpenter bees find only for the nectar from *A. sericea*. They show the same foraging behavior in visiting the flowers of this species. In stead of landing and facing directly on and the wings, the carpenter bees always cling on the side of the wings and keel and penetrate their proboscis down to reach the nectar which deposit underneath the base of the vexillum. In some case, they fly and land on the abaxial side of the vexillum of the flower, which situated in the lower position in the raceme (or the flower that open in the previous day) and sucks the nectar from the newly opened flower. They never touch directly on the tips of the keel and wings. These bees can found all daytime.

***Trigona* sp.**

Stingless bees visit flower of *A. sericea* in group all daytime. They spent 1-6 minutes per each flower. According to their behavior, they cling on the wings and keel and just move around but could not open them. This may be because these bees are rather clumsy, often loosing their grip and falling off from flower. They collect only pollen grains on parts of the flower, which left over by other visitors. They may also collect pollen directly from anthers but only from flower that keel is biting opened by other insects. They collect pollen grains by combined working of the forelegs and mouthparts. Pollen grains are then passed from the forelegs to the mid legs and then to the hind legs where they are gathered inside the pollen basket. This bee almost never contact stigma of the flower so should not be a pollinator in *A. sericea.*

***Anthophora* spp.**

Anthophora collect only the nectar from the flower of *A. sericea.* Eventhough their foraging behavior is more or less similar to those of *Megachile*, but the keel has never pressed downwards and uncovered the stigma and anthers during their visits. Moreover, they often suck the nectar from the lateral side of the flower by

cling on the vexillum rather on the wings. This genus can be found from around 10:00 hour -15:00 hour on sunny day.

***Mylabris* sp.**

From the present investigation, it seems that the oil beetles feed on the flower. They usually bites and cuts the wings, keel, anther and may eat pollen grains to. Many pollen grains can be found sticking on hairs on their heads and bodies. This species always found in the morning.

***Chilades* sp.**

Chilades pandava collect only nectar from the flower. They land on the wing and move directly towards the nectary guide and insert their proboscis down into the path at the base of the vexillum and suck for the nectar. Like Anthophora, they never make the stamen and pistil exposed from the keel. Thus, no parts of anther or stigma can touch their bodies.

***Nectarinia* sp.**

This sunbird is usually only found in rainy days, in the morning or in the evening. They perch on the axis of the inflorescence and project their beaks directly to the base of the vexillum and suck the nectar without any contact to the wings and keel.

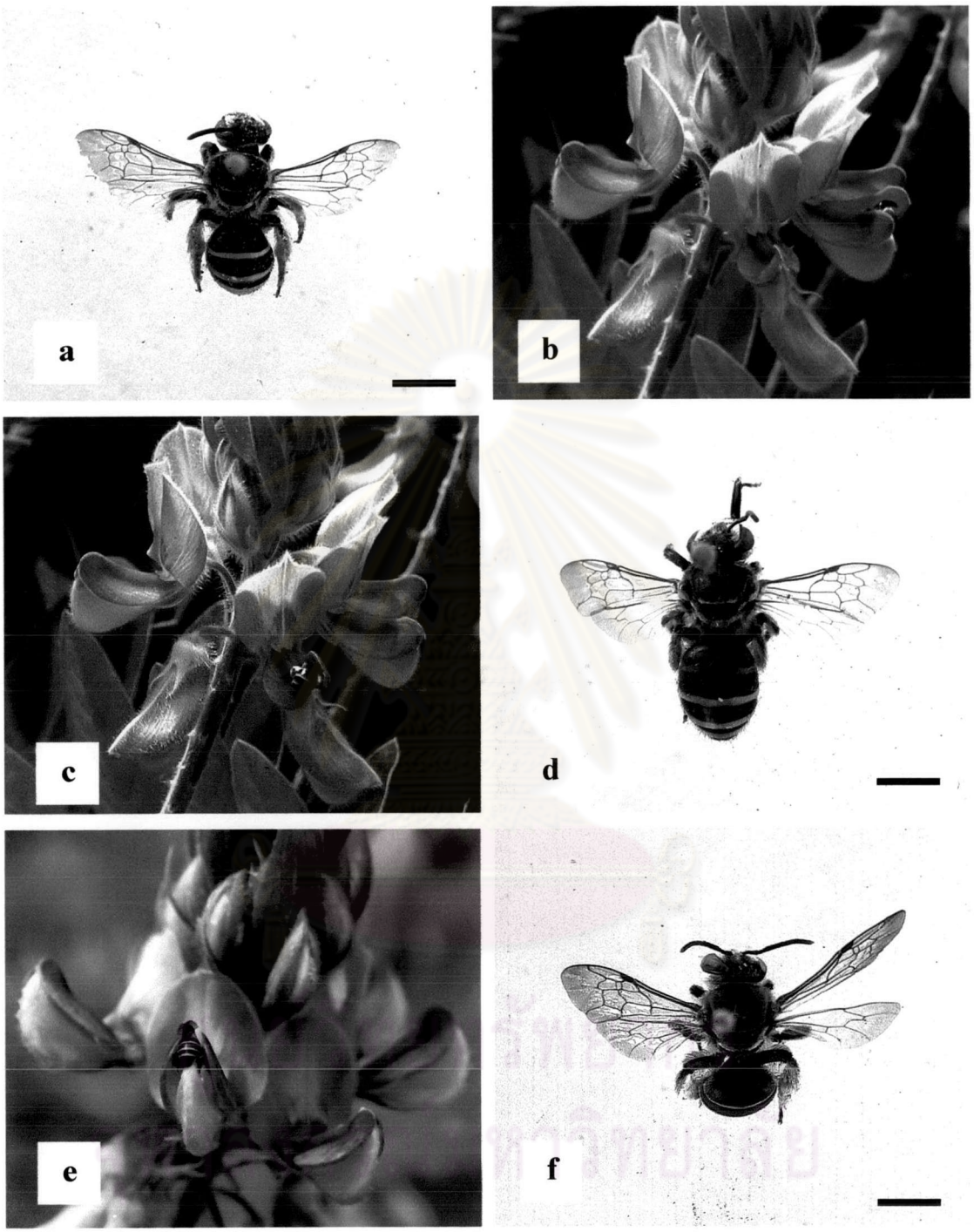


Figure 5.73 *Nomia elliotii*; **a** bar = 3 mm, **b** the bee with pollen loads forcing an entry into a flower and **c** leaving the flower, **d** *Nomia iridescens*, bar = 4 mm, **e** the bee also forcing into a flower, **f** *Nomia* sp. with pollen grains on both hind legs, bar = 4 mm.

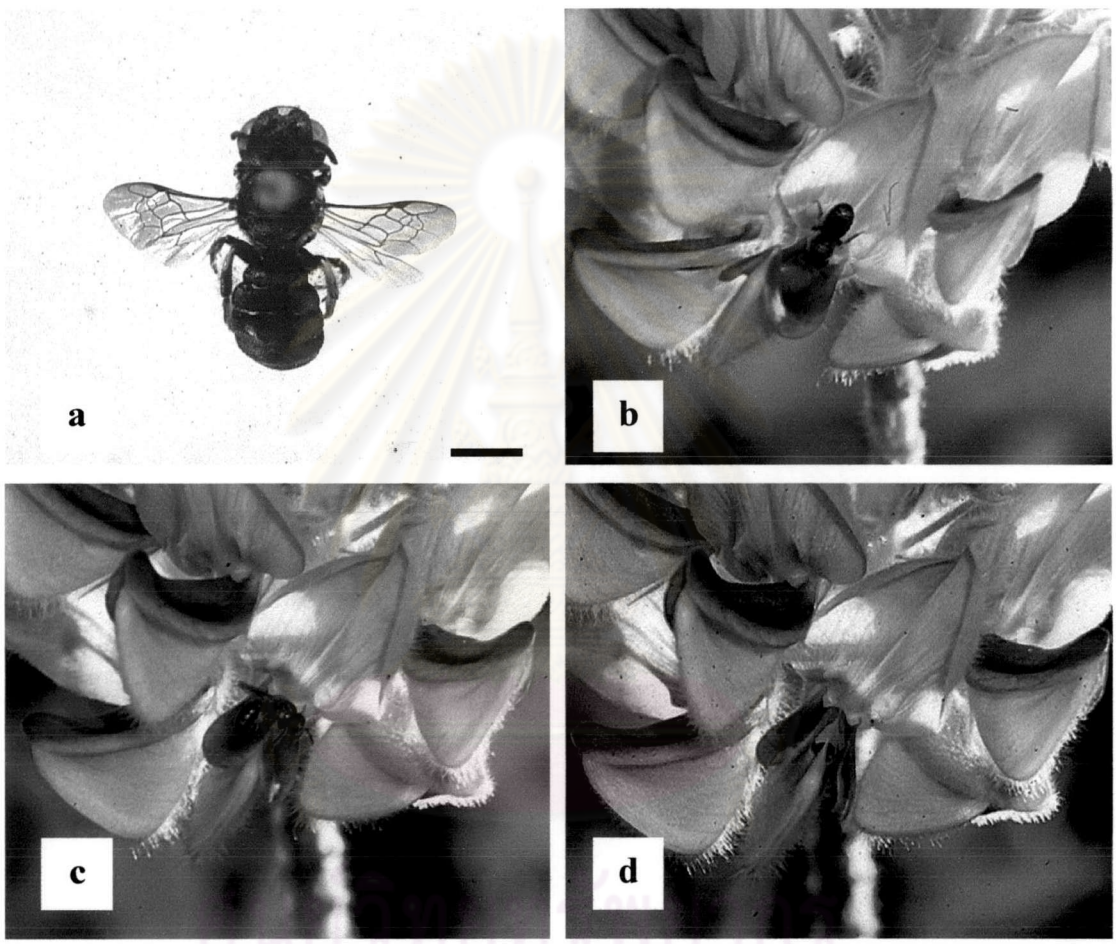


Figure 5.74 *Pithitis smaragdula*, a small brilliant metallic green bee (**a**), bar = 2 mm; bee can collect nectar from the out side of the flower (**b**), and forcibly enter the flower to collect pollen (**c**, **d**).

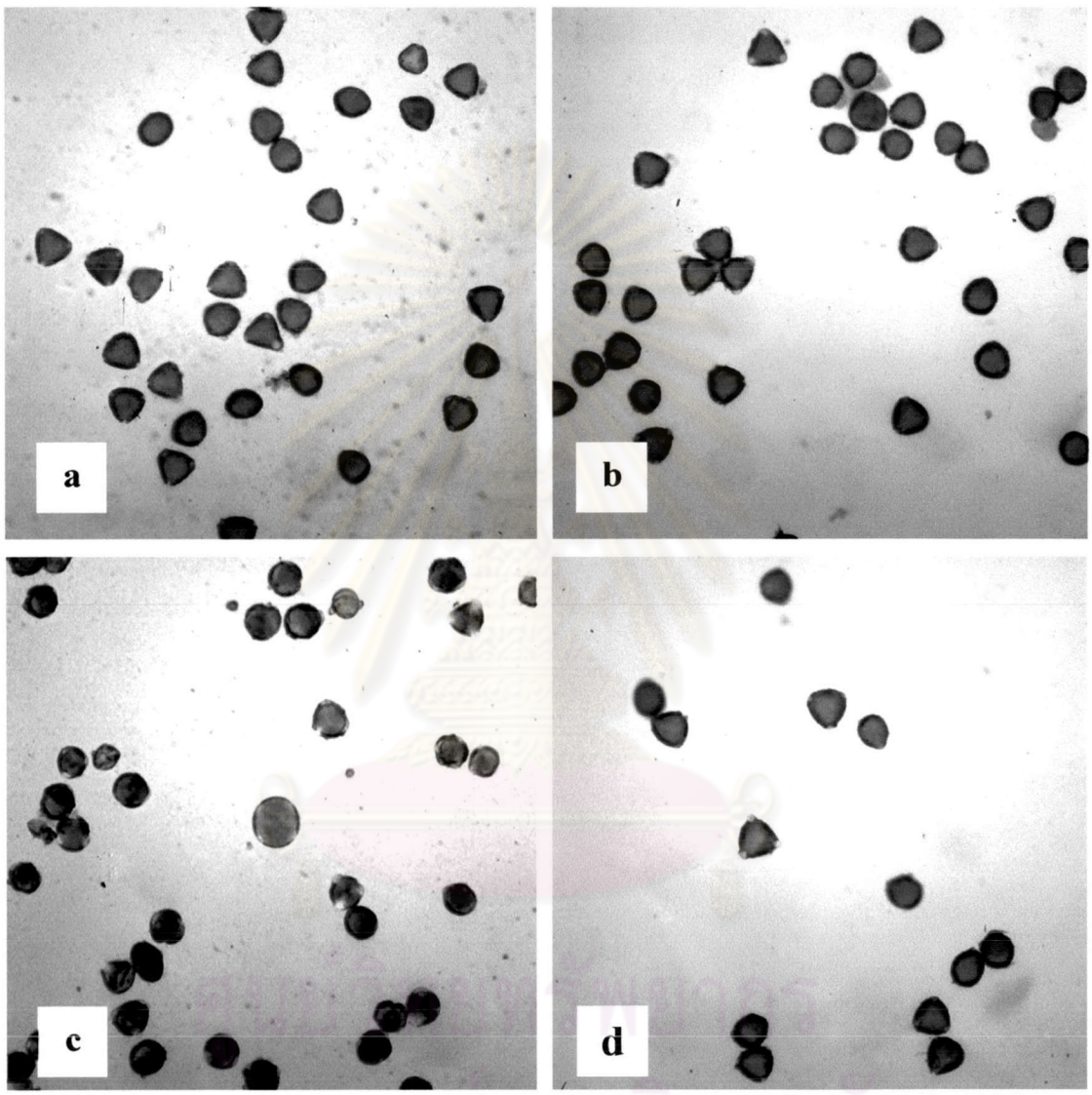


Figure 5.75 Bee loads; **a** pollen from *A. sericea*, **b** bee load from *Megachile velutina*, **c** bee load from *Nomia elliotii*, **d** bee load from *Pithitis smaragdula*, only bee load from *Nomia elliotii* pollen from the other species can be found (**c**).

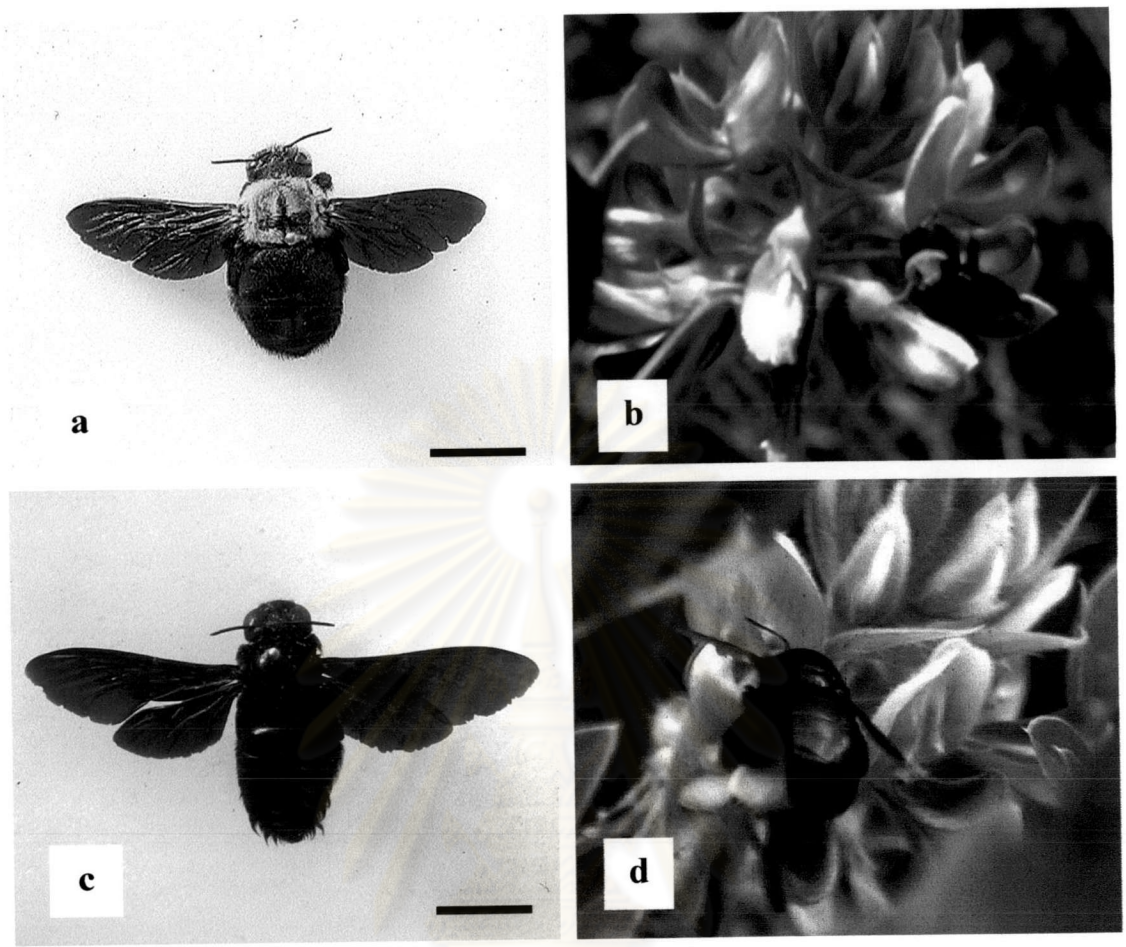


Figure 5.76 *Xylocopa aestuans* (**a**), bar = 10 mm; and *Xylocopa dissimilis* (**c**), bar = 11 mm, both bees collect only nectar from the flower (**b**, **d**).

Figure 5.77 *Trigona* sp. visiting the flower, this bee can collect pollen only from a flower biting opened by the others, bar = 2 mm.

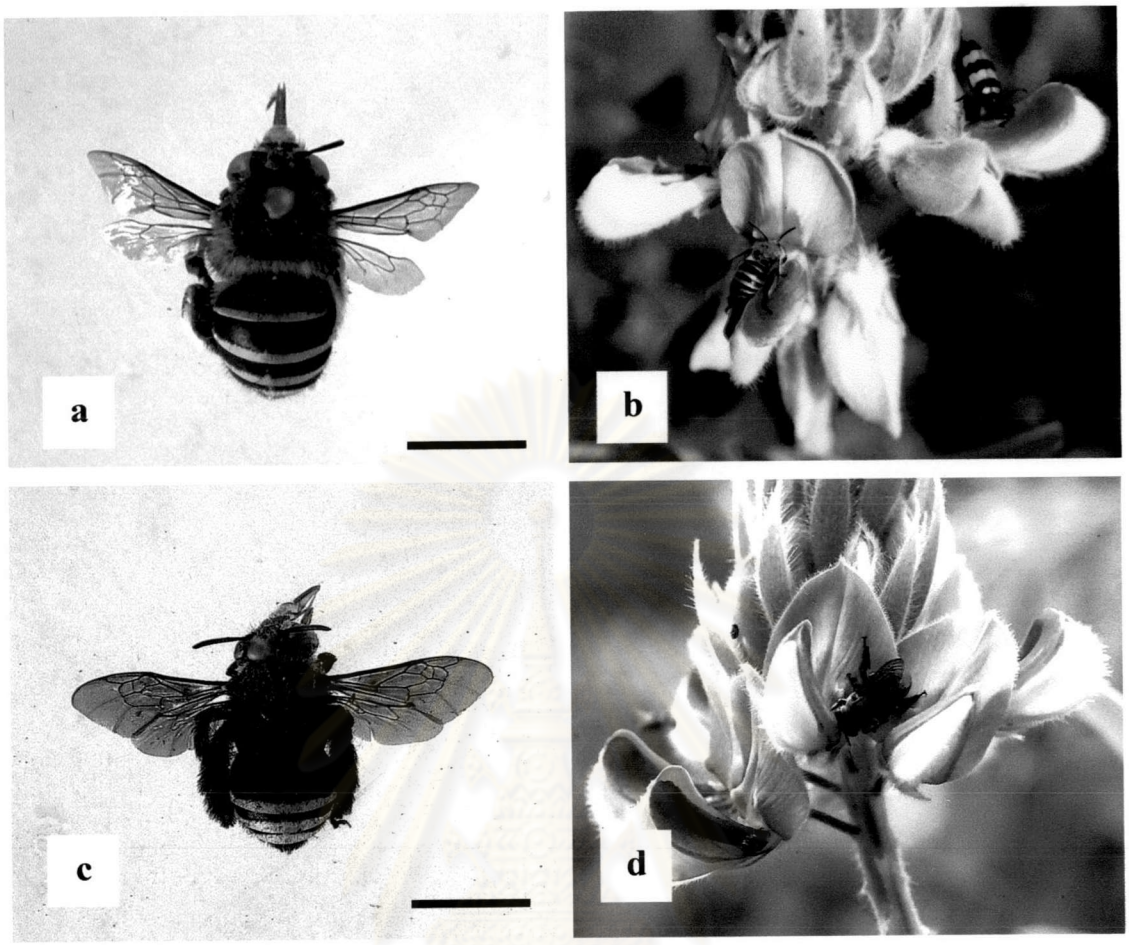


Figure 5.78 *Anthophora zonata* (**a**), bar = 6 mm, *A. crocea* (**c**), bar = 8 mm, both are similar in color and foraging behavior. They only sucked the nectar (**b**, **d**).

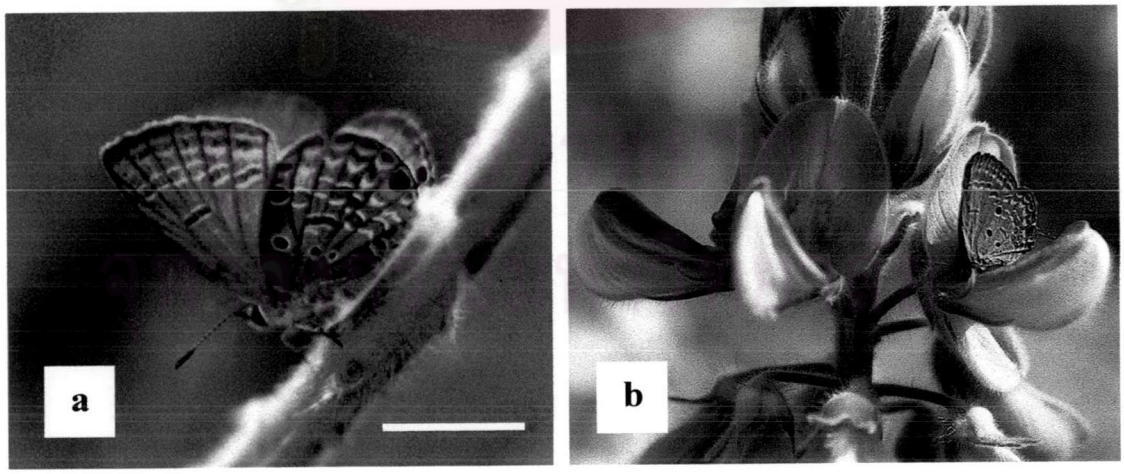


Figure 5.79 *Chilades pandava* (**a**), this butterfly also only sucked the nectar (b), bar = 6 mm.

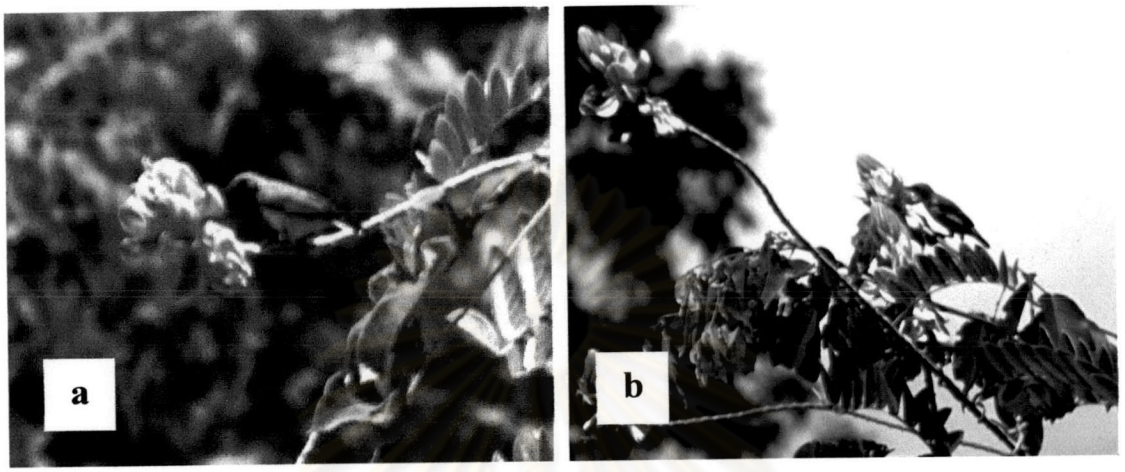


Figure 5.80 *Nectarinia sperata*, non-pollinator, the female purple-throated sunbird, usually found in the rainy day, in the morning or in the evening, fond of nectar in the flower of *A. sericea*. It is always seen busily feeding on nectar.

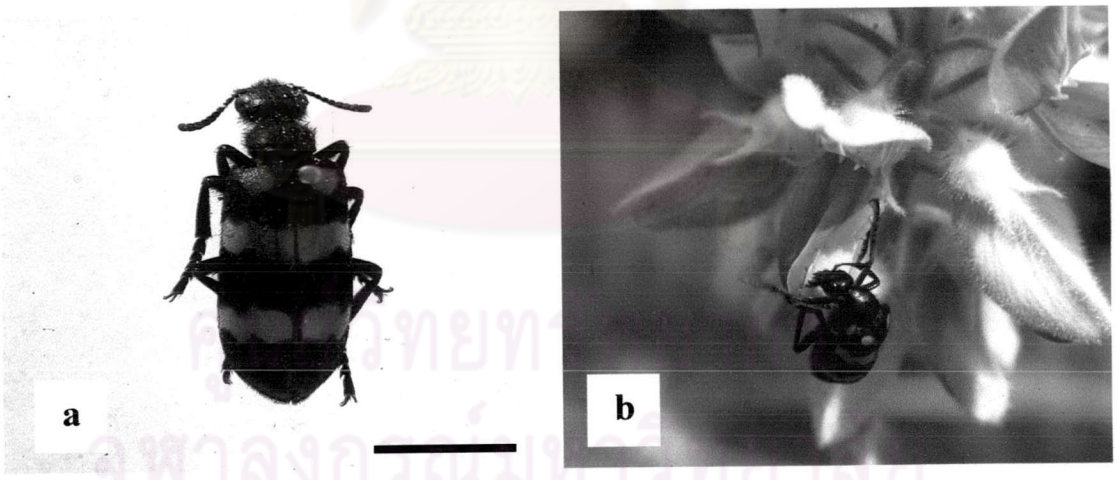


Figure 5.81 *Mylabris phalerata*, oil beetles, a serious plant pest of *A. sericea*, feed on the flower. It was found only at Sakaerat Environmental Research Station, bar = 7 mm.

Fruit Setting Studies

1. Pollinator-Exclusion Experiment

Pod set was not performed when insect pollinators were excluded from inflorescences at Sakaerat Environmental Research Station as it was mentioned before in topic 5.2.3.1 (Table 5.4). Insects capable of tripping the floral mechanism were presence at all study sites. It can be concluded that pollinators conducted an importance role in pod setting of *A. sericea.*

2. Fruit setting Related to Plants Distance

In this study it was found that inflorescences with pod in 3 plants which has the nearest others within approximately 20 meters was 65.92% and average pod numbers in inflorescence were 1.06. Whereas in farther 3 plants, which the nearest others was approximately 300 meters, percentage inflorescence with pods was lower, it was 32.25% and average pod numbers in inflorescence were 0.49 (Table 5.7).

3. Percentage Fruit Set, Percentage Seed Set, and Average Seed Mass

From random selected 30 plants at Sakaerat Environmental Research Station in 2000, 421 inflorescences, 78 infructescences with a total of 92 pods and 180 seeds were found. Hence percentage of infructescence was 18.53, and as it was mentioned before in 5.1.2.1 that the average floret in florescence was 228.93, therefore percentage of pod setting was 0.10. The percentage of seed set was determined in the same as those in the percentage of fruit set, total ovules number and total seed number were used in calculation, the average ovule number in this species was 2.01, thus percentage of seed set was also 0.10.

In 2001, 292 inflorescences, 71 infructescences with a total of 83 pods and 165 seeds were found, percentage of infructescence was 24.32, percentage of pod setting was 0.12, percentage of seed set was 0.12.

Table 5.7 Percentage of infructescence and average of pod production per plant in *ex situ* study site in Pharphayom District, Phatthalung Province in 2002.

Distance from nearest plant (m)	Mean percentage of infructescence	Sig.	Mean pod number in infructescence	Sig.
20	65.92	0.001	1.06	0.001
300	32.25		0.49	

Comparing with the data from 6 plants in *ex situ* study site in Pharphayom District, Phatthalung Province in 2002, in this study 565 inflorescences, 283 infructescences with a total of 449 pods and 901 seeds were found. Hence percentage of infructescence was 50.09, percentage of pod setting was 0.35 and percentage of seed set was also 0.35.

There was significant difference on inflorescence and fruit production per plant. Plants in natural habitat at Sakaerat Environmental Research Station produced fewer racemes; they also yielded fewer pods than at Pharphayom District. Within fruits, seed per pod is two; there were no significant site-to-site differences, fruit with lack of fertilization or seed abortion almost not found.

There was slightly difference on average seed weight, it was 1.39 $\pm$ 0.13, 1.38 $\pm$ 0.17 and 1.41 $\pm$ 0.22 g in samples form Sakaerat in 2000 and 2001 and seed samples from Pharphayom District, Phatthalung Province.

Table 5.8 ANOVA of seed weight of *Afgekia sericea* from 3 plant samples.

Source	Sum of Squares	df	Mean Square s	F	Sig.
Between Groups	1.23E-02	2	6.17E-03	0.44	0.65
Within Groups		87	1.41E-02		
Total		89			

Figure 5.82 Pod setting in a cluster of 20 meters plants (**a**, **b**) and in a 300 meters far plant (**c**) in the study sites in Pharphayom District Phatthalung Province in 2002.