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Physiological Changes Following Pollination of *Dendrobium* Pompadour Flowers

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ABSTRACT One of the most astounding phenomena in many flower species is pollination-induced senescence, where longevity of long-lived flowers is reduced and the functional life of the flowers are terminated. Ethylene which has long been implicated as the precursor of this phenomenon signals for other accompanying physiological changes such as perianth closure, discolouration, petal thinning, loss in flower weight and water uptake. *Dendrobium* Pompadour is one such orchid that undergoes pollination-induced senescence. In this study, it was found that the longevity of *Dendrobium* Pompadour flowers was significantly reduced by pollination. Furthermore, the quality of pollinated flowers was also significantly reduced when compared to unpollinated *Dendrobium* Pompadour.

ABSTRAK Kelayuan yang dirangsang oleh pendebungaan merupakan salah satu fenomena yang unik dalam kebanyakan spesies bunga di mana jangka hayat bunga berkurangan dan fungsi organ dihapuskan. Etilena, gas yang telah lama diimplikasikan sebagai penyebab fenomena ini, turut mengisyaratkan perubahan-perubahan fisologi lain seperti kucupan kelopak bunga, pengurangan ketebalan kelopak bunga dan pengambilan air serta penyahwarnaan. *Dendrobium* Pompadour merupakan salah satu orkid yang mengalami kelayuan yang dirangsang oleh pendebungaan. Dalam kajian ini, didapati jangka hayat bunga *Dendrobium* Pompadour berkurangan akibat pendebungaan. Selain itu, kualiti bunga yang didebungakan turut berkurangan berbanding dengan bunga yang tidak didebungakan.

(Dendrobium Pompadour, ethylene, pollination, senescence)

INTRODUCTION

The attractiveness and the longevity of many flowers are often eliminated due to pollination. Scientists have established that pollination in flowers such as digitalis [1], carnations [2], petunias [3] and orchids [4] results in a cue for floral degradation, abscission and senescence. This cue was found to be in the form of endogenous ethylene that triggers biochemical and physiological changes in floral organs, ultimately rendering the organs obsolete.

Ethylene production in these flowers is that of a climacteric pattern where a burst of ethylene coincides with the onset of petal senescence. Work done by Woltering et al. [5] elicited that ethylene expression was modulated from the receptive stigma and subsequently translocated to the petals and the sepals. As is observed in natural senescence, pollination-induced senescence is characterized by physiological changes such as perianth withering and hyponasty, colour change, thinning of petal, water and weight loss [6, 7]. In most flowers, senescence is characterized by the in-rolling of petals that eventually results in permanent flower closure. Permanent flower closure has been documented as a distinct characteristic of pollination- induced senescence in different species of orchids such as in *Arachnis* [8], *Phalaenopsis* [9] and *Dendrobium* [10, 11]. *Dendrobium* Pompadour is one such flower that is ethylene-sensitive and undergoes pollination-induced senescence. The most obvious physiological change observed in *Dendrobium*

Pompadour is the complete closure of the perianth. Alongside this movement are other physiological changes such as weight loss, thinning of petals and discolouration. The experiments carried out are designed to profile the physiological changes that occur during the pollination-induced senescence of *Dendrobium* Pompadour.

MATERIALS AND METHODS

Plant material

Dendrobium Pompadour flowers aged 5 to 10 days after full bloom were obtained from the glasshouse of University of Malaya. Flowers were hand-pollinated by placing the pollinia onto the stigma. Individual flowers were cut at the proximal end of the peduncles and placed in 20 ml water vials containing distilled water. Ten flowers were pollinated and another ten remained unpollinated (control). Physiological changes were observed and recorded from the beginning of the experiment (day 0) until vase life of flowers were deemed terminated. To show the visual changes, photographs were also taken at each time point. Experiments were maintained at room temperature (26 ± 2° C). All experiments were carried out three times.

Ethylene measurement

Individual, weighed flowers were placed in 30 ml Corex tubes covered with air tight rubber stoppers. The tubes were sealed for two hours daily and 1 ml gas samples were withdrawn from the tubes for ethylene determination by Hewlett-Packard 5890 Gas Chromatograph fitted with a Flame Ionization Detector (FID).

Vase life

Changes in the appearance of the petals were recorded daily. The visual changes were observed according to three different stages. At stage I, flowers show fresh, deep purple perianth. At stage II, onset of upward petal movement occurs while at stage III, the perianth reaches full closure. At stage III, the vase life of the flowers is considered terminated.

Thickness

Thickness of petals was measured using a micrometer (Mitutoyo). Measurements were taken by placing the petal between the anvil and the spindle of the micrometer.

Colour measurements

The colour of the flowers was measured using a chromameter (Minolta CR-200). Measurements were done by placing the centre of the petals on the stage of the chromameter. The L*a*/b* colour system closely represents human sensitivity to colour. Thus, it can be used to measure the perceived colour of the flowers as they senesce. Lightness (brightness), hue (shade of colour) and chrome (colour saturation or vividness) are values of L*, a* and b* respectively. The L*a*/b* value is obtained using the formula:

$$\frac{L^* \times a^*}{b^*}$$

Water uptake

The difference between consecutive weighing of the vials plus solution (without the flower) was used to determine water uptake. Evaporative water loss was deemed negligible.

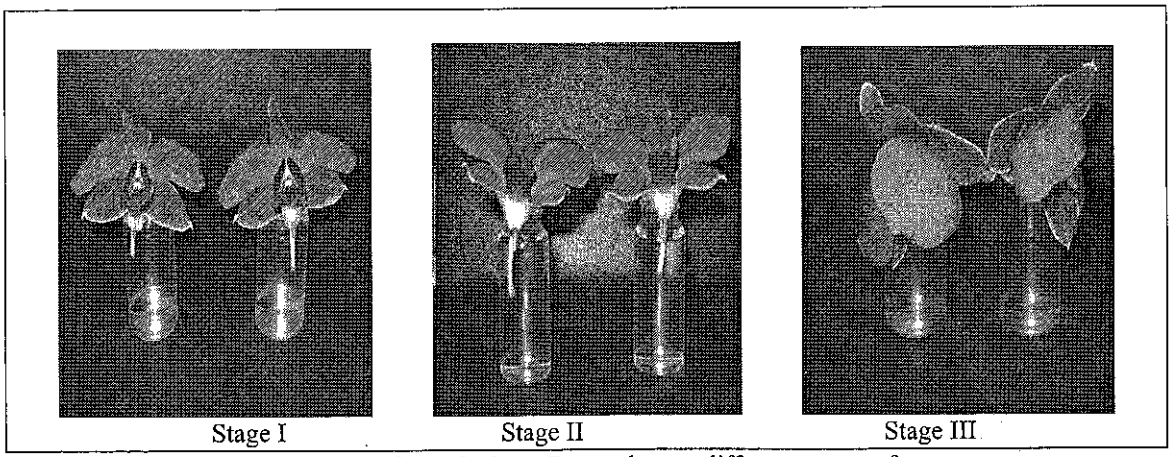

Figure 1. Perianth movement of *Dendrobium* Pompadour at different stages of senescence

RESULTS

From this study, it was observed that unpollinated and pollinated *Dendrobium* Pompadour held in distilled water exhibited a significant difference in flower longevity and stages of senescence (Table 1). All pollinated flowers exhibited Stage II on day I when the petals started to move upwards, and reached Stage III on day 2 (± 0.4). On the contrary, in the unpollinated flowers, Stage II and III were only exhibited on day 17 (± 0.7) and 19 (± 0.8) respectively.

Ethylene was detected in pollinated flowers as early as 6 hours after pollination (0.091 μl/g/h) and 12 hours after pollination, as the initial movement of the perianth was observed, peak ethylene production was obtained (0.481μl/g/h). Subsequently, ethylene production gradually decreased to zero after 36 hours. In unpollinated flowers, no ethylene production was detected throughout the experiment. It is therefore clear that the ethylene profile displayed by pollinated flowers followed that of a climacteric plant where a burst of ethylene coincides with the onset of petal senescence (Figure 2).

Observation on the colour changes of *Dendrobium* Pompadour showed that pollinated flowers experienced colour changes after 24 hours and continued until 96 hours (Figure 3). This was reflected by the increase in L*a*b readings which showed a value of - 62.55 in the beginning of the experiment and increased to – 41.47 at the end of the experiment. The increase of L*a*b readings was gradual and most obvious after 48 hours. Unpollinated flowers however, exhibited very little colour change or increase in L*a*b value throughout the experiment, measuring – 62.44 at the beginning of the experiment and increasing only to – 60.24 after 96 hours.

In both pollinated and unpollinated *Dendrobium* Pompadour, loss in petal thickness was observed. However, pollinated flowers exhibited an earlier and higher total loss in petal thickness compared to unpollinated flowers. As shown in Figure 4, pollinated flowers exhibited a drop in petal thickness after 24 hours while unpollinated flowers exhibited a drop in petal thickness only after 36 hours. Furthermore, pollinated flowers experienced a total loss of 0.14 mm in thickness whereas unpollinated flowers experience a total loss of 0.03 mm in thickness throughout the experiment.

Water uptake by pollinated flowers was significantly less compared to unpollinated flowers in this experiment (Fig 5). As early as after 24 hours, pollinated flowers exhibited a drastic drop in water uptake and this trend continued thereafter, where water uptake was close to zero. Water uptake by unpollinated flowers was more stable, showing small and gradual reduction in water uptake compared to pollinated flowers. At the end of the experiment, unpollinated flowers still exhibited water uptake significantly more than pollinated flowers.

Table 1. Stages of senescence and vase life of unpollinated and pollinated *Dendrobium* Pompadour

	STAGE I	STAGE II	STAGE III	VASE LIFE
Unpollinated	Day 0	Day 17 ± 0.7	Day 19 ± 0.8	19 days ± 0.8
Pollinated	Day 0	Day 1 ± 0	Day 2 ± 0.4	2 days ± 0.4

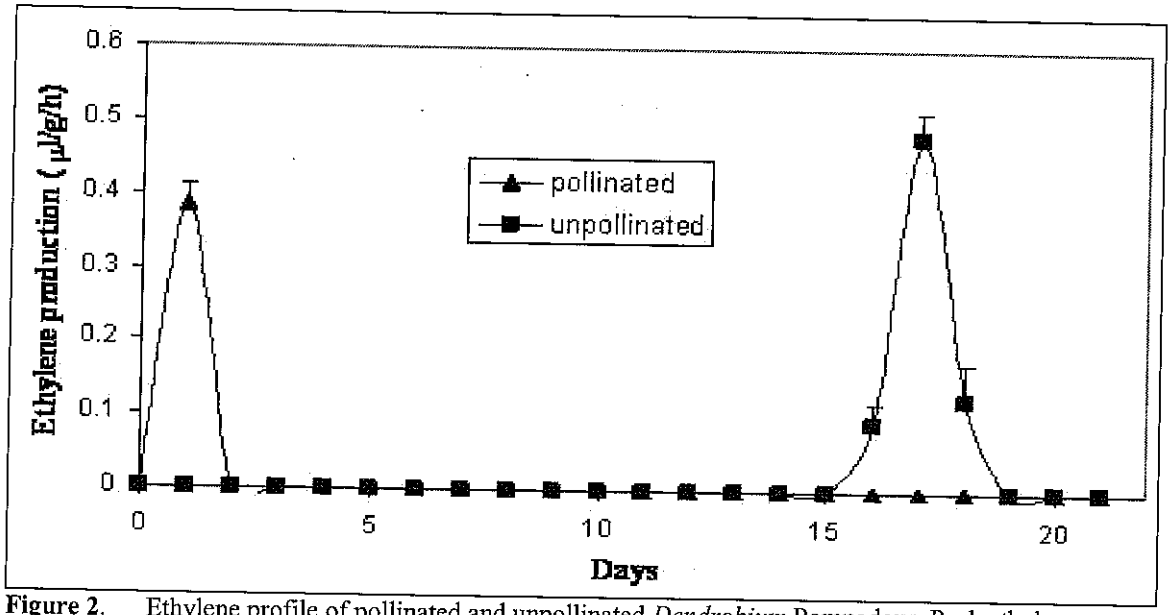

Figure 2. Ethylene profile of pollinated and unpollinated *Dendrobium* Pompadour. Peak ethylene production was observed on Days 1 and 17 for pollinated and unpollinated flowers respectively.

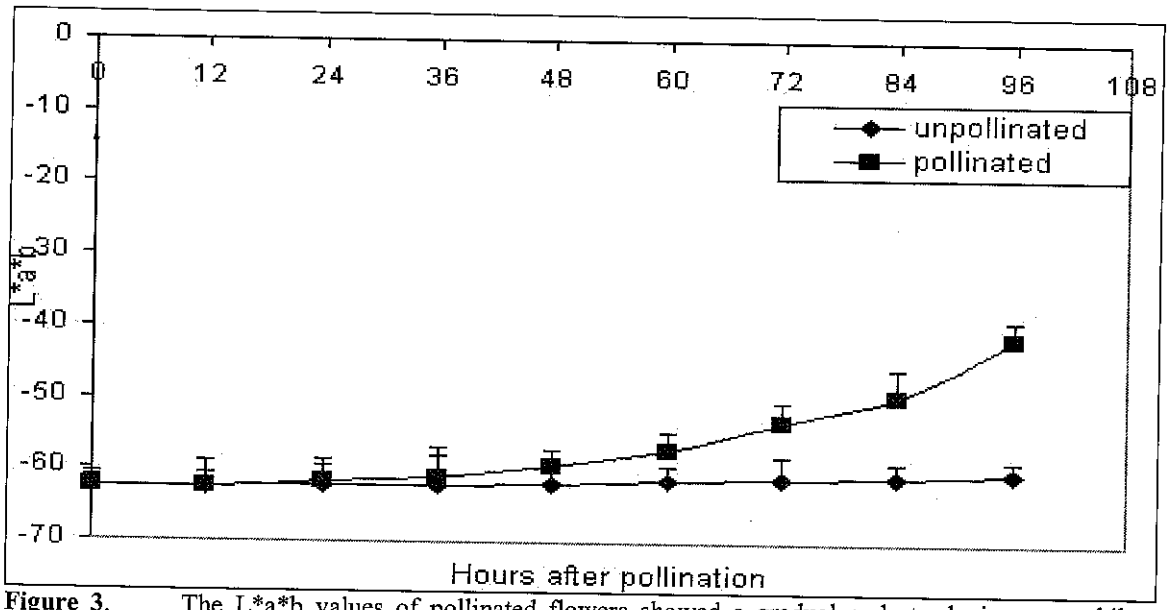

Figure 3. The L*a*b values of pollinated flowers showed a gradual and steady increase while unpollinated flowers showed negligible change

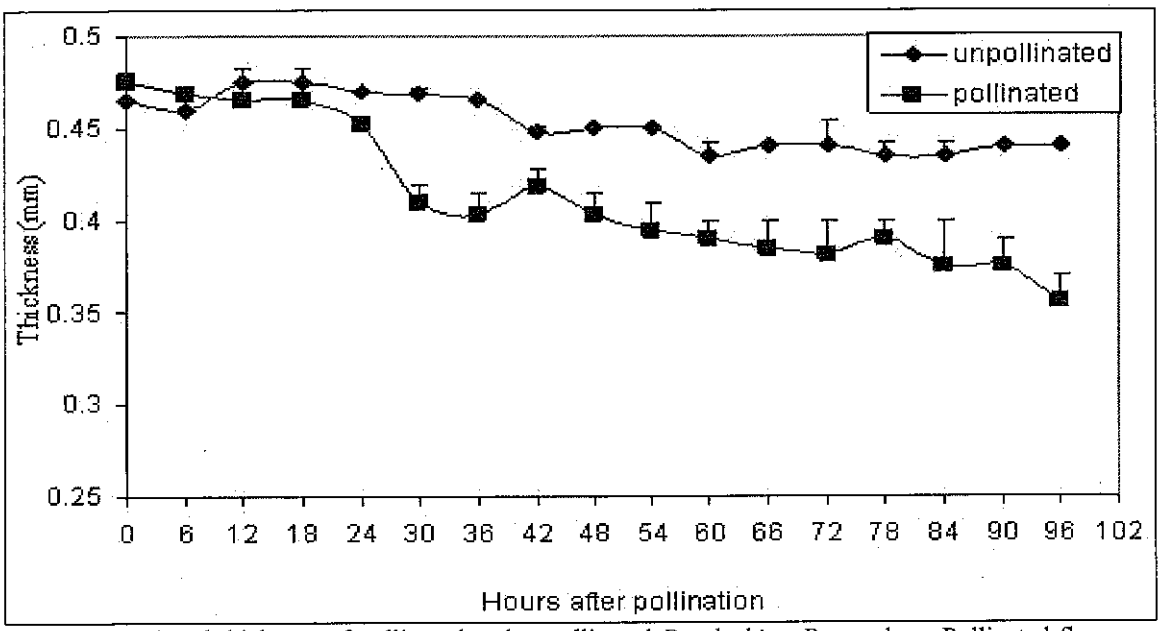

Figure 4. Petal thickness of pollinated and unpollinated *Dendrobium* Pompadour. Pollinated flowers showed a rapid thinning of the petals while unpollinated flowers showed a slower thinning of the petals.

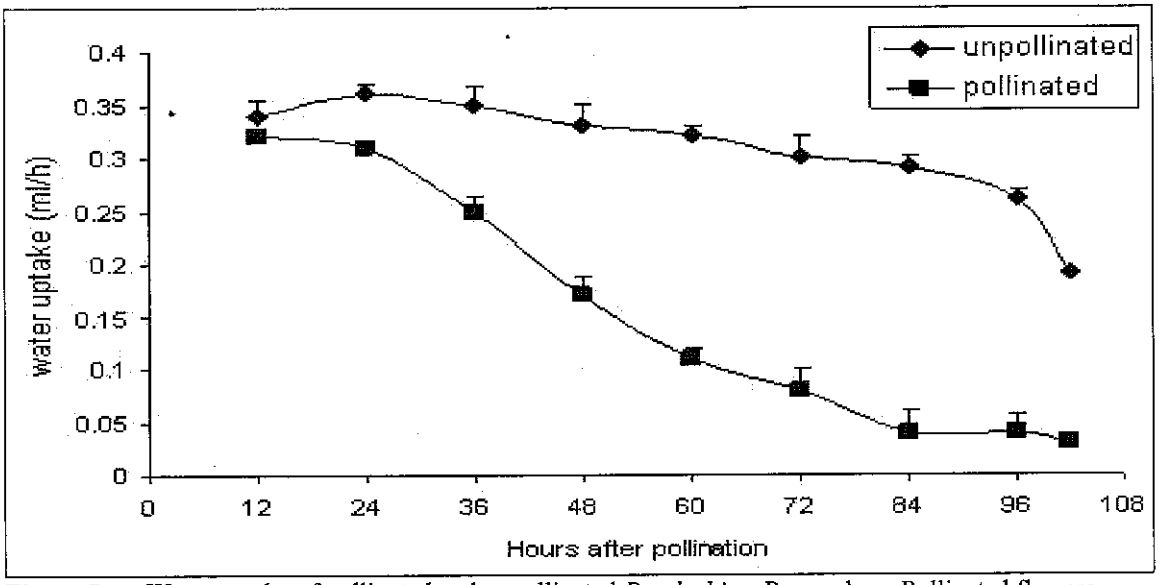

Figure 5. Water uptake of pollinated and unpollinated *Dendrobium* Pompadour. Pollinated flowers showed a drastic drop in water uptake after 24 hours while unpollinated flowers showed a slower and gradual reduction in water uptake.

DISCUSSION

Early investigations on pollination-induced senescence in *Dendrobium* Pompadour carried out by Nair and Tung [12], Ketsa and Luangsuwalai [10] and Ketsa and Rugkong [4] established that *Dendrobium* Pompadour is ethylene sensitive and pollination in this cultivar would necessarily hasten the process of senescence. The results from this experiment are agreeable to that of previous studies thus confirming the pollination-induced senescence that occurs in *Dendrobium* Pompadour. Pollination in this experiment elicited an induction of endogenous ethylene production, which acted as a signal for consecutive physiological changes, including inward movement of the perianth, change in colour,

thinning of petal and loss of fresh weight. This phenomenon has been observed and reported in other cultivars as well such as in carnations [13], and petunia [14].

The ethylene profile of *Dendrobium* Pompadour displayed that of a climacteric plant. In many flowers, the initial response to pollination is an early increase in ethylene production by the stigma often followed by increased ethylene production from ovaries and petals. Ethylene production in pollinated *Dendrobium* Pompadour was detected as early as 6 hours after pollination, peaked 12 hours after pollination and subsequently reduced towards the end of the experiment. Ethylene is synthesized by two enzymes: 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS), which catalyzes the conversion of *S*-adenosyl-l-Met into ACC, and ACC oxidase (ACO), which converts ACC into ethylene [15]. Studies of these two enzymes in both natural and pollination-induced senescence have shown an inter-organ translocation of the two enzymes originating from the stigma [16]. Furthermore, high concentrations of ACC content in the column have been detected compared to that of the petal or sepal, suggesting that the bulk of ethylene production is in the stigma [17]. This pattern of tissue-specific activity is consistent with the hypothesis that the gynoecium senses pollination of the flower and then propagates this information throughout the flower by synthesizing a translocated signal that must travel to other floral organs to induce ethylene biosynthesis in distal regions [18] [19]. The mechanism of this translocation of signal, however, is yet to be clearly established, although some studies have suggested hormonal [13] [20] and electrical [21] [22] signals to be involved.

The closure of the perianth is a mechanism developed in several orchid species and cultivars to protect the pollinated stigma and eliminate flower attraction to pollinators [23]. The synthesis of ethylene is concomitant with the initial inward movement of petals that subsequently results in full closure of the perianth, a distinct process that characterizes the senescence of *Dendrobium* Pompadour flowers and a number of orchid species. Perianth closure initiated by pollination in *Phalaenopsis* was prevented by ethylene inhibitors, thus indicating the involvement of ethylene in the regulation of the perianth's inward movement [9]. In this experiment, the onset of petal closure was initiated 2 hours after pollination, coinciding with the ethylene peak and continues even as the ethylene production reduces. Thus, it would be safe to extrapolate that ethylene triggers the onset of petal closure but need not be synthesized throughout the senescing process.

Colour changes have been documented in a number of ethylene sensitive species that also experience pollination-induced senescence [24] [25]. Colour change following pollination is an evolution in orchids that aims to eliminate floral attractiveness to pollinators [26]. In this experiment, pollination resulted in the flower petals of *Dendrobium* Pompadour fading from a bright crimson purple to a lighter shade of purple. This colour change is observed visually through the naked eye and is further confirmed by results obtained from the L*a*b readings. The loss in colour is a result of a decrease in anthocyanins, the major pigments present in this cultivar which contributes to its purple colour [27]. In the study on the physiology of orchids done by Avadhani [23], the destruction of anthocyanins is reported to occur in many orchid species following pollination. In studies done by Gori [24], this phenomenon was attributed to the production of endogenous ethylene that was further confirmed by the ability of ethylene inhibitors to prevent colour change in several species.

Pollination-induced senescence in *Dendrobium* Pompadour flowers also resulted in the thinning of petals and loss of fresh weight in flowers. In this experiment, pollinated flowers showed accelerated thinning of petals and loss in weight. This observation can be linked to the biochemical changes that occur throughout the senescence phenomenon. Increase in protease and hyrolytic enzymes have been reported in senescing flowers of *Sandersonia aurantiaca* resulting in the breakdown of protein and cell wall, which ultimately weakened the cell structure and degraded the flowers [28] Besides protein and structural destruction, the decrease in water content in senescing flowers has also been reported. The classic concept of water loss as a symptom of wilting has been consistent with a study done on senescing *Sandersonia aurantiaca* [7] and Petunia [3].

In conclusion, pollination in *Dendrobium* Pompadour orchids results in the production of ethylene that triggers a cascade of physiological changes in the floral organ. These changes

destroy the flowers' quality and eventually terminate the functional life of the flowers.

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