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Short communication

Considerations for evaluating flower abscission in potted plants with multiple inflorescences—*Plectranthus* as a case study

G.D. Ascough, L.J. Rice, J. Van Staden*

Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

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Abstract

The ability to protect plants and flowers from the adverse affects of ethylene and sub-optimal transport conditions relies on a thorough understanding of the environmental triggers and subsequent downstream physiological and molecular processes that result in senescence or abscission. Characterising abscission is the essential starting point in this process. In this study we explore additional measures of abscission that provide insight into differential abscission patterns where open flowers or unopened buds are preferentially shed. We examine equations relating to the proportion of open or unopened flowers shed, as well as those that are available for abscission using simulated data. To test these equations, two varieties of potted *Plectranthus* were subjected to continuous darkness or placed under fluorescent lights. After 96 h, abscission data was calculated using equations. Cultivar P000603 was found to preferentially shed open flowers in both conditions, as indicated by differences in the number of open flowers abscised as a proportion of the total available for abscission. Cultivar P010509 shed open and unopened flowers at similar proportions. These additional measures of abscission enlarge our understanding of this intricate process by providing a more comprehensive and thorough approach to evaluate abscission.

Keywords: Ethylene; Flower abscission; 1-MCP; *Plectranthus*; Post-harvest

1. Introduction

Flower drop in potted plants is a major problem that affects agricultural, horticultural and floricultural markets throughout the world. Although it is a natural aspect of plant life and is a strictly regulated developmental sequence of events, it leads to a loss of quality and yield, and consequently, a reduction in profit for both the producer and the seller. The ability to control flower abscission therefore, is of great importance. Ethylene is the major factor promoting flower abscission in ethylene-sensitive species, but the effect of applied ethylene can be reduced or eliminated using various inhibitors of ethylene synthesis and action (Sisler and Serek, 1997, 1999). Flower abscission is controlled by both external environmental conditions and internal (timing and energy availability) mechanisms (Ascough et al., 2005).

Potted *Plectranthus* plants produce many inflorescences that develop at different rates. At any given time therefore, a single plant may have inflorescences that range from recently initiated meristems to those where all flowers are fully opened, and everything in between. The inflorescences are terminal and typically produce three to 12 ranks containing one–six flowers each. Flowers open from the base of the inflorescence upwards, with oldest flowers at the bottom and youngest flowers at the distal end. It may take several weeks for all the flowers in an inflorescence to open. We have previously shown a differential response to darkness in two cultivars in terms of flower abscission: one cultivar abscises open flowers when held in continuous darkness, while the other abscises unopened buds (Ascough and Van Staden, 2007). This presents an intriguing system to study flower abscission since both open and closed flowers were present in inflorescences during experimentation. Recent research on excised inflorescences shows that open and unopened flowers differ in their response to various environmental conditions (Cameron and Reid, 2001; Uthaichau

* Corresponding author.
E-mail address: rcpgd@ukzn.ac.za (J. Van Staden).

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et al., 2007; Abebie et al., 2008). We present here considerations that should be taken into account by examining different equations for calculating abscission by using some hypothetical data as well as some from studies on potted *Plectranthus*.

2. Measures of abscission

The abscission at a given time point is simply defined as the number of flowers that have been shed divided by the total number at the start of the monitoring period. Under natural conditions, open and/or unopened flowers can be shed in response to pollination, incorrect light intensity, elevated temperature, insufficient carbohydrate supply or because of an inherent timing mechanism (Reid et al., 2002; Marcelis et al., 2004; Zhao et al., 2005). Thus, total abscission (here designated A_t)=(total flowers abscised)/(total flowers at start).

A number of additional measures of abscission can be easily calculated if the appropriate data are collected during experimentation. For example, the proportion of open and unopened flowers of the total that have abscised will give insight into whether or not flowers in a particular state (unopened or open) are preferentially shed. However, this needs to be interpreted with care since this figure, in some cases, could depend on the initial proportion of open and unopened flowers. For example, consider four *Plectranthus* plants each containing a single inflorescence at different stages: inflorescence of plant A is immature and contains only unopened flowers; inflorescence of plant B contains one-third open flowers and two-thirds unopened flowers; inflorescence of plant C contains two-thirds open flowers and one-third unopened flowers; and inflorescence of plant D is mature and consists entirely of open flowers. If these plants were exposed to ethylene (2 μl/l) complete abscission occurs in 24 h. The number of open and closed flowers that abscised as a proportion of the total for each inflorescence would be identical to the proportion of these at the start of the experiment, since there is no preferential abscission in response to ethylene. Plants with multiple inflorescences representing a broad spectrum of maturities should therefore be selected when considering experimentation. Thus, the proportion of abscission for open flowers, A_t^o=(number of open flowers abscised)/(total flowers abscised); and for unopened flowers, A_t^u=(number of unopened flowers abscised)/(total number of flowers abscised).

Another measure of abscission is the number of flowers in a particular stage (unopened or open) that have abscised as a proportion of the total number of flowers in that stage that were available for abscission. This may be explained more clearly by examining some generated data in Table 1 below.

In the first three Treatments, the percentage A_t at the end of the experiment is 33.3%. In the first case, equal numbers of open and unopened flowers have abscised, thus A_t^o and A_t^u are the same: 50%. The number of flowers that abscised as a proportion of the available is defined: A_v^o=(number of open flowers abscised)/(number of open flowers available for abscission); and A_v^u=(number of unopened flowers)/(number of unopened flowers available for abscission). The denominators in these two equations are not necessarily equal to the number of open or unopened flowers at the start. By virtue of the fact that potted plants continue to grow as normal plants during experimentation, unopened flowers will open during the course of an investigation. This then increases the number of open flowers available for abscission and decreases the number of unopened flowers available for abscission. This conversion of one flower type to another must be taken into account calculating A_v values. Now, A_v^o=(number of opened flowers abscised)/(number of open flowers at start+number unopened flowers converted); and A_v^u=(number of unopened flowers abscised)/(number of unopened flowers at start−number of conversions). Table 2 shows the calculated values for these measures of abscission.

Table 1
Generated data from hypothetical flower abscission experiments

Treatment	Flowers at start				Flowers abscised		
Number	Open	Unopened	Total	Conversions	Open	Unopened	Total
1	10	20	30	5	5	5	10
2	10	20	30	10	5	5	10
3	10	20	30	5	9	1	10
4	10	20	30	5	2	8	10
5	10	20	30	0	10	20	30

If Treatments 1 and 2 are compared, it is noted that A_t values are the same with 33.3% abscission. Similarly, A_t^o and A_t^u values are 50% for both Treatments 1 and 2, indicating that equal numbers of open and closed flowers were abscised. When A_v^o values are compared, a lower percent of opened flowers available for abscission were shed in Treatment 2 compared to Treatment 1. Conversely, a higher proportion of available unopened flowers were shed in Treatment 2 compared to Treatment 1. This is because the number of unopened flowers that were converted to open flowers increased, thus increasing the conversion percentage.

Although the A_t values and conversion values are the same for Treatments 3 and 4, the plants in Treatment 3 are preferentially shedding open flowers while those in Treatment 4 are preferentially shedding unopened flowers (Table 2). This observation was only made possible by calculating the A_t^o, A_t^u, A_v^o and A_v^u values.

Taking into account the number of unopened buds that are converted to open flowers during the experiment improves the estimate of the abscission. For example, if Treatment 3 is examined, the value of A_v^o is 60%, in other words, 60% of the open flowers available for abscission were shed. However, if the number of abscised open flowers was divided by the number of open flowers at the start, the value would be 90%. Using this second method incorrectly inflates this measure of abscission because it is conceivable that this value could exceed 100%, which would be unrealistic. Thus, recording and using the number of unopened buds converted to open flowers is crucial to evaluate abscission accurately.

Experiment 5 is a simulated positive control treatment where plants are exposed to ethylene at a level that stimulates rapid and complete abscission (2 μl/l). A_t^o and A_t^u values are in the same

Table 2
Measures of abscission (%) calculated from generated data of Table 1

Treatment Number	A_t	A_t^o	A_t^u	A_v^o	A_v^u	Conversion
1	33.3	50	50	33.3	33.3	25
2	33.3	50	50	25	50	50
3	33.3	90	10	60	6.7	25
4	33.3	20	80	13.3	80	25
5	100	33.3	66.7	100	100	0

ratio as the number of open and unopened flowers at the start of the experiment, while A_v^o and A_v^u show that complete abscission of all available flowers in a particular stage have abscised (Table 2).

3. Studies on *Plectranthus*

In order to evaluate the efficacy of these measures of abscission, some preliminary experiments were initiated on *Plectranthus*. Cuttings of two *Plectranthus* cultivars, P000603 bearing pink flowers, and P010509 bearing purple flowers, were obtained from Dr. Gert Brits in Stellenbosch, South Africa. Cuttings were rooted in 12 cm pots containing a potting soil mix (two thirds pine bark and one third rough sand) and grown in a greenhouse with a maximum irradiation of approximately 100 μmol m^{-2} s^{-1}. An organic fertiliser (Nitrosol®, Efekto, South Africa) was applied to the soil every 10 days. Plants with inflorescences containing approximately one-third open flowers (at the transport stage) were transferred to the laboratory where the number of inflorescences, open and unopened flowers were recorded. They were placed inside sealed Perspex chambers (small: 288×438×250 mm, ca 31.5 l; medium: 138×538×300 mm ca 70.7 l; large: 488×636×350 mm ca 108.6 l) at 21±1 °C under a 12-h photoperiod of 12.6 hμmol m^{-2} s^{-1} radiant flux density. Alternatively, plants were placed in a dark room for continuous dark treatment under the same temperature conditions. Plants were observed every 24 h for 4 d and number of opened and unopened flowers that had abscised were recorded, as well as open and unopened flowers on the plant. During this time, plants were removed from the chambers and the chambers were cleaned and vented every day. Plants were then replaced and the chambers resealed. At least 3 plants were used for each treatment, and the experiment was repeated twice.

For both *Plectranthus* cultivars investigated, continuous darkness did not significantly alter any of the measures of abscission (Table 3). Although A_t and A_v^o values were higher for P000603 in the dark, these differences were not significant. The A_v^o and A_v^u values for P000603 under control and dark conditions are significantly different, indicating that open flowers are shed in preference to unopened flowers (Table 3). This is consistent with the hypothesis that flowers are shed when they become energetically unprofitable compared to the cost of opening a new one (Ashman and Schoen, 1994). For P010509, despite differences between A_t^o and A_t^u in control and dark conditions, A_v values are similar in all treatments, suggesting that it is equally likely for open and unopened flowers to abscise.

Significant differences were observed between cultivars in both control and dark conditions for A_t^o, A_t^u and A_v^o (Table 3). This strengthens the previous suggestion that P000603 preferentially abscises open flowers compared to P010509, and confirms earlier findings in these two cultivars that postulated differential control mechanisms for these varieties (Ascough and Van Staden, 2007).

In *Phlox paniculata* 'Rembrandt', ethylene had an adverse effect on the number of open flowers abscised from cut stems, but apparently had no affect on bud (unopened flower) abscission (Porat et al., 1995). Similarly, in *Pelargonium peltatum* 'Pink Blizzard', ethylene treatment had no effect on petal abscission when flowers were treated at stage-1 (petals not fully reflexed, prior to anthesis), but from the time of anthesis onwards, flowers were sensitive to ethylene (Cameron and Reid, 2001). In contrast, Uthaichau et al. (2007) found that cut, untreated *Dendrobium* inflorescences shed buds as opposed to open flowers, although buds were better able to withstand simulated shipping conditions (25 °C for 3 days in cardboard boxes) than flowers that had already opened. Molecular evidence for this observed differential abscission has been found. Analysis of the expression of auxin-inducible genes in *Cestrum elegans* showed that transcripts of *Ce-IAA5* accumulated in the abscission zone of unopened flowers, but not in the abscission zone, sepals or perianth of flowers that had already opened (Abebie et al., 2008). These studies clearly demonstrate that open and unopened flowers respond differently to environmental conditions, hence the need for additional measures of calculating and evaluating abscission.

Thus, by examining more closely the state of flowers in inflorescences of potted plants at the initiation of an experiment, and recording the state in which flowers are shed, additional measures of abscission can be calculated. These assist when analysing abscission and could provide novel experimental systems and directions for future studies.

Table 3
Measures of abscission (mean±se) in two *Plectranthus* cultivars held under control (fluorescent lights) and continuous dark conditions after 4 days

	A_t	A_t^o	A_t^u	A_v^o	A_v^u	Conversion
P000603						
Control	8.4±4.9	95.1±7.3	4.8±7.3	21.7±7.7	0.6±1.3	39±7.0
Dark	22.1±9.1	98.1±3.8	1.8±3.8	36.3±15.2	1.2±1.6	27.7±12.3
P010509						
Control	7.6±6.8	31.7±22.7	68.3±22.7	6.0±2.5	10.6±12.9	25.4±2.1
Dark	6.9±7.4	29.2±27.6	70.8±27.6	3.4±3.6	6.9±5.3	34.7±13.1

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