THE EFFECT OF AUXINS AND ETHYLENE ON LEAF ABSCISSION *OF FICUS BENJAMINA*

N.S. AL-KHALIFAH[1] and P.G. ALDERSON[2]
[1]*King Abdulaziz City for Science and Technology (KACST), P.O. Box 6086, Riyadh 11442, Saudi Arabia.* [2]*University of Nottingham, School of Biological Sciences, Sutton Bonington Campus, Loughborough, Leics., LE12 5RD, UK*

1. Abstract

Removal of part of the new fully expanded leaf lamina of *Ficus benjamina* cv. Exotica had no effect on abscission of the petiole, whereas removal of the whole lamina caused abscission within 48 hours. One drop (1 µl of 0.1 mgl^{-1}) of either indole acetic acid (IAA), indole butyric acid (IBA) or naphthalene acetic acid (NAA) applied to the petiole resulted in a delay in its abscission. IAA delayed the abscission for 5 days, whereas IBA and NAA treated petioles started to abscise after 3 days. By 14 days, comparable numbers of the IAA and NAA treated petioles had abscised, however less of the IBA treated petioles had abscised. The time of application of IAA to petioles in relation to removal of the distal part of the lamina also influenced the delay of abscission. No accumulation of ethylene was observed when the removed leaf laminas were held in sealed culture vessels. In contrast, ethylene accumulation occurred in vitro in sealed culture vessels containing shoots of *F. benjamina* on MS medium supplemented with 0.2 mgl^{-1} benzylaminopurine (BAP). Sealing and size of culture vessel significantly enhanced the percentage of leaves which abscised. Injection of ethylene into cultures immediately after sealing increased abscission, even when the ethylene inhibitor, silver thiosulphate, was present.

2. Introduction

Leaf abscission is a common response of plants to stress imposed by external or internal factors, and hormonal, nutritional and other physiological factors interact to control the onset and rate of abscission [1]. In *F. benjamina*, abscission has been related to changes in environmental conditions, e.g. water stress and low light intensity [10, 13]. Auxin (IAA) is the major growth hormone controlling abscission in many plants, and when the ability of leaves to produce auxin diminishes, i.e. when they become senescent, they tend to abscise [1]. Removal of the leaf lamina will lead to abscission of the remaining petiole, however this can be delayed by applying auxin to

the petiole. This paper reports a study of the role of auxin in the abscission of leaves of *F. benjamina* by removing its source (leaf lamina) and applying auxins as a substitute, and on the possible relationship with ethylene production by the system.

3. Materials and Methods

3.1. THE EFFECT OF PARTIAL OR COMPLETE REMOVAL OF THE LEAF LAMINA ON ABSCISSION OF THE PETIOLE

Plants of *F. benjamina* cv. 'Exotica' were grown in Levington M2 compost (Fisons UK) in pots in a glasshouse at 20-26°C until they were 40-50 cm in height, with supplementary lighting to provide a photoperiod of 16 hours and light intensity of >80 $\mu molm^{-2}s^{-1}$. Three new fully expanded leaves on each of four plants were used for each of the following treatments: (i) distal ¼ of the lamina removed, (ii) distal ½ of the lamina removed, (iii) distal ¾ of the lamina removed, (iv) the whole lamina removed and (v) the whole lamina removed and 1 µl of 0.1 mgl^{-1} IAA applied to the petiole stump. The incidence of petiole abscission was recorded for each treatment at intervals of 24 hours up to 120 hours as the cumulative number of petioles which had dropped or which dropped when lightly touched with forceps.

3.2. THE EFFECT OF TYPE OF EXOGENOUS AUXIN AND TIME OF APPLICATION ON ABSCISSION OF THE PETIOLE

Leaf lamina were removed leaving petioles which were treated with one drop (1 µl) of either IAA, IBA or NAA (all at 0.1 mgl^{-1}). Twelve petioles were treated with each auxin and twelve control petioles did not receive any auxin. Starting at 48 hours from setting up the treatments, the incidence of abscised petioles was recorded at 24 hour intervals for 14 days.

Using four plants, 1 µl of 0.1 mgl^{-1} IAA was applied to the petiole of twelve leaves 15 min, 30 min, 1 hour, 2 hours, 24 hours and 48 hours after removal of the lamina. These treatments were compared to a control (no auxin). Removed laminas were held in sealed culture vessels for 3 days to monitor ethylene accumulation, as described below.

3.3. THE EFFECT OF SIZE OF CULTURE VESSEL AND PLANT DENSITY ON ETHYLENE ACCUMULATION

Single shoots of *F. benjamina* cv. 'Cleo' were cultured in vitro for 10 weeks in 70 ml glass jars containing 15 ml MS medium [8] supplemented with 0.2 mgl^{-1} BAP, or 3 shoots in 350 ml glass jars containing 40 ml of the same medium. At the end of this period, five of the 70 ml jars and five of the 350 ml jars were sealed with Nescofilm (Bando Chemicals, Tokyo) for four weeks, after which ethylene was monitored by taking a 1 ml sample from the head-space of each vessel and injecting it into a gas chromatograph (GC, PV 4500, Pye-Unicam, Phillips, UK) fitted with an alumina F1 JJ column (JJ Chromatography Ltd, Kings Lynn, UK) maintained at operating

temperature of 110°C and equipped with a flame ionisation detector heated to 130°C. Nitrogen and hydrogen were supplied as the carrier gases at flow rate of 40 ml min^{-1} and an air flow rate of 500 ml min^{-1} was used. The system was calibrated prior to each reading by injecting 1 ml of 10 ppm ethylene, i.e. ethylene at 1% in nitrogen [7]. The three treatments comprised normal 70 ml jars, sealed 70 ml jars and sealed 350 ml jars.

3.4. THE RESPONSE OF *F. BENJAMINA* CULTURES TO INTERNAL AND EXTERNAL SOURCES OF ETHYLENE

Shoot tips of 'Cleo' were sub-cultured individually in 100 ml glass jars containing 20 ml MS medium supplemented with 0.2 mgl^{-1} BAP and 0.5 mgl^{-1} GA3. In one treatment (STS), the medium was supplemented with 12.5 mll^{-1} silver thiosulphate (stock solution prepared by dissolving 5 g silver nitrate plus 0.85 g sodium thiosulphate in 1 litre of water). The cultures were grown for 8 weeks until there were between 4 and 8 leaves per jar. The numbers of green and abscised leaves were recorded, then 5 replicates of cultures with and without STS were sealed and the remainder were left unsealed. Eight weeks after sealing, the numbers of green and abscised leaves were recorded and ethylene was monitored. The culture vessels which had not previously been sealed were sealed, and 5 ml of 10 ppm ethylene was injected in three replicates of each of the four treatments based on the time of sealing and presence of STS, i.e. early versus late sealing and with and without STS. Three weeks after injecting the ethylene, the number of green and abscised leaves were recorded and the concentration of ethylene in culture vessels was monitored.

4. Results

Partial removal of the leaf lamina had no effect on abscission of the petiole (Fig. 1). Even retention of one quarter of the leaf lamina provided sufficient stimulus for the petiole not to be abscised. In contrast, removal of the whole leaf lamina caused 100% abscission within 72 hours. The addition of a drop of IAA delayed abscission for 120 hours, but it eventually reached 100% after 14 days.

All of the auxins tested delayed abscission compared to the control. IAA retarded abscission for 5 days, whereas IBA and NAA treated petioles started to abscise after 3 days. After 14 days, 78% and 89% of petioles had abscised in the IAA and NAA treatments respectively, however only 33% of the IBA treated petioles had abscised. All of the control petioles had abscised by day 5.

The application of auxin to petioles 15 min, 30 min, 1 hour and 2 hours after removal of the lamina delayed their abscission for 5 days, at which time 10% of each treatment had abscised (Fig. 2). The control petioles, and those treated after 24 hours or 48 hours, started to abscise within 3 days. Abscission increased with time showing a similar response for all treatments, however by day 14 the control treatment had reached 100% abscission, whereas the 15 min treatment showed at least 50%. Accumulation of ethylene was not detected in the sealed culture vessels containing removed laminas.

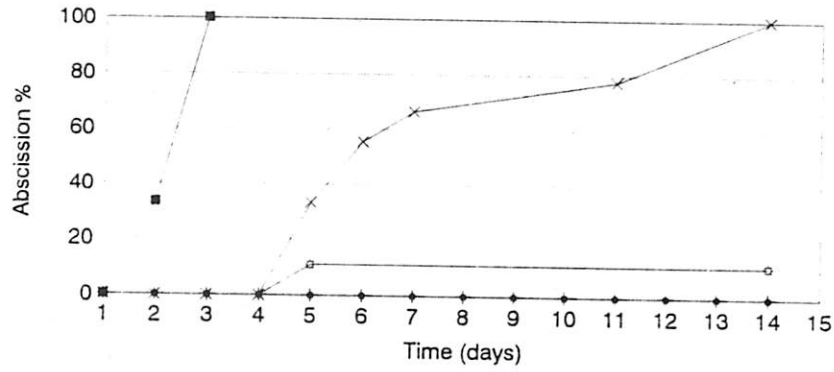

Figure 1. Effect of partial or complete removal of leaf lamina on the abscission of petioles in *F. benjamina cv.* "exotica" (n=12)
• ¼ lamina
+ 1/4 lamina
○ ¾ lamina
■ whole lamina
* whole lamina+IAA added

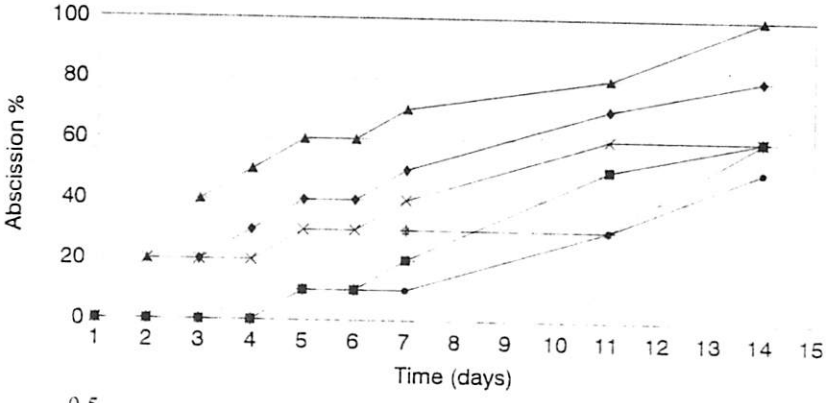

Figure 2. Effect of the time of auxin application after removal of leaf lamina on abscission of petioles in *F. benjamina cv.* "exotica" (n=12)
• 15 min
+ 30 min
○ 1 hour
■ 2 hours
* 24 hours
♦ 48 hours
▲ no auxin

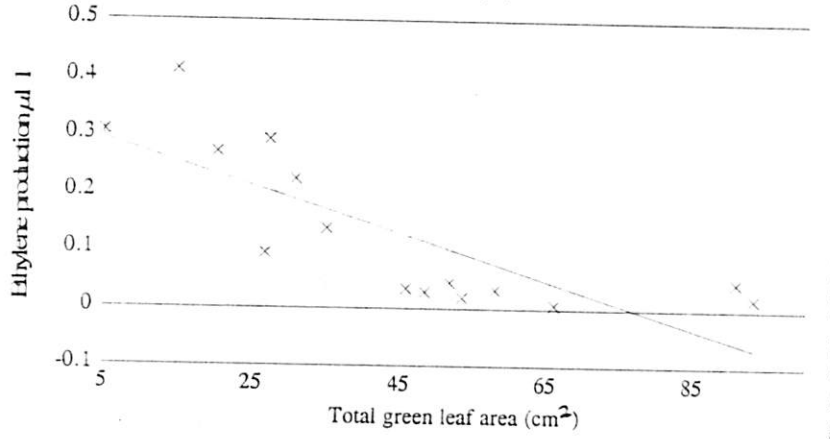

Figure 3. The relationship between ethylene production in the cultures of *F. benjamina* and green leaf area (Y'=0.0314058-0.0041X, R=0.614)

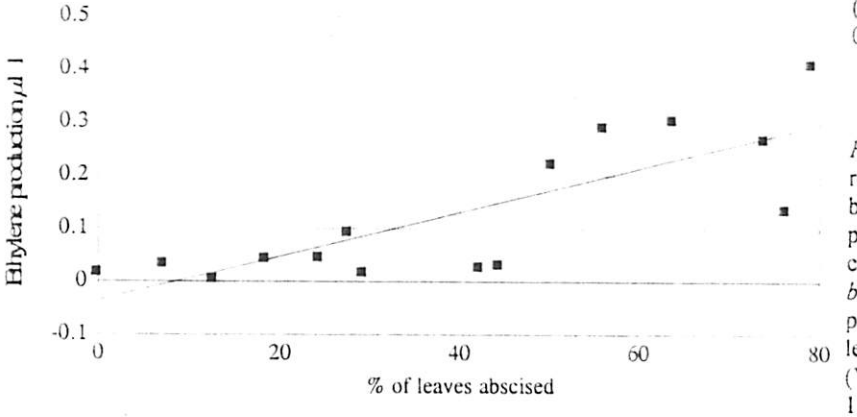

Figure 4. The relationship between ethylene production in the cultures of *F. benjamina* and the percentage of leaves abscised (Y'=0.0358+0.004 173X, R=0.647)

The ethylene concentration in sealed 70 ml culture vessels was significantly higher than in the unsealed vessels and was five times greater than in the sealed 350 ml vessels. During the period that the vessels were sealed, growth of the cultures (i.e. leaf number and leaf area) was promoted. At the end of the fourth week, abscission was stimulated resulting in a reduction in green leaf area, which was correlated with the increase in ethylene concentration (Fig. 3). However, the total area of the abscised leaves was not affected by ethylene concentration. The sealing and the volume of the vessel affected the percentage of leaves, which abscised, and the relationship with ethylene was significant ($p \leq 0.001$) (Fig. 4).

Sealing of the culture vessels did not increase the accumulation of ethylene in the STS treatments, as ethylene production was clearly inhibited by STS in the medium. The presence of STS removed any differences between the early and late sealed cultures as regards leaf production and leaf abscission. The injection of ethylene increased abscission only in cultures, which had been sealed late, with and without STS.

5. Discussion

Auxin appeared to be responsible for delaying or preventing the abscission of *F. benjamina* leaves. Auxins produced in the leaf lamina are transported through the petiole into the stem and, when the leaf ages or is exposed to removal of the lamina or physiological damage, the auxin status declines resulting in abscission [1, 3]. Removal of as much as three quarters of the leaf lamina of *F. benjamina* did not result in abscission, even after two weeks. This indicates that a small part of the leaf is capable of producing enough auxin to prevent abscission. Delaying leaf abscission by the application of auxin on the distal end of the petiole has also been reported for bean and Coleus [2]. The prolific growth of callus from tissues of *F. benjamina* in vitro supports the view that the plant is rich in auxins, and that, as soon as the transport of auxin is disturbed, abscission will occur.

The time of applying the auxin to the petiole after removal of the leaf lamina revealed that the abscission zone is "insensitive" to the absence of auxin for up to 2 hours. When auxin was applied at or after 24 hours, there was a delay in the abscission of some of the petioles as compared with the petioles not receiving any auxin. This suggests that the lag phase (Stage I) of the time course of abscission of *F. benjamina* leaves is less than 24 hours, after which the separation phase (Stage II) takes place depending on the availability of ethylene either as wound ethylene or applied ethylene, which is necessary to trigger and complete separation during the ensuing 24 hour period [9]. Complete separation of 100% of the control (no auxin) petioles of *F. benjamina* took between 72 and 96 hours. Osborne [9] reported that full separation of leaves may take 90 hours, and it is known that the duration of the lag phase is dependent on the auxin status of the tissue [5].

The abscission of leaves by plants of *F. benjamina* grown in a glasshouse and exposed to environmental stress is considered to be ethylene mediated [4, 11]. The accumulation of ethylene has been reported by Jackson *et al.* [6] for plants grown *in*

vitro and less so for plants grown *in vivo*. In the present study, sealing *in vitro* cultures of *F. benjamina*, even though it increased ethylene accumulation and the level of this accumulation decreased with the increase in volume of culture vessel, did not increase leaf abscission until the cultures had been sealed for a long time. This suggests that ethylene may not be the key factor in leaf abscission in *F. benjamina in vitro* or *in vivo*. In the presence of STS there was less accumulation of ethylene in cultures, however this did not prevent abscission. This supports the findings of Steinitz *et al.* [12], that STS did not reduce leaf abscission. It is possible that the conclusion reached by Addicott [1], namely that ethylene accumulation may follow abscission rather than preceding it, is more appropriate for *F. benjamina*, however further experimentation is required to prove this.

6. References

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