Ethylene Production and Leaflet Abscession in *Mèlia azédarach* L.¹

PAGE W. MORGAN AND JAMES I. DURHAM

Department of Plant Sciences and Texas Agricultural Experiment Station, Texas A & M University, College Station, Texas 77843

ABSTRACT

Ethylene production or content was compared to leaflet abscession in detached, compound leaves of *Mèlia azédarach* L. In late autumn, when abscession was progressing from basal leaves upward, the oldest leaves both produced ethylene at the highest rates and abscessed their leaflets first. When C2H4 levels were measured in intercellular air removed immediately after leaves were harvested, C2H4 levels were also highest in basal leaves and declined progressively in more apical leaves. Levels as high as 1.8 microliters C2H4 liter⁻¹ air were observed. Earlier in the season groups of leaves demonstrated a pattern of sequential initiation of abscession from base to apex, but the peak rates of C2H4 production followed an opposite trend, being highest in the youngest leaves. Peak rates of C2H4 production occurred after the initiation of leaflet abscession and presumably are related to either the auxin content or a climacteric-like, autocatalytic phase of C2H4 production not directly involved in the initiation of abscession. In these experiments, the early abscession of the older leaflets reflects their greater sensitivity to C2H4, presumably due to lower auxin content. C2H4 production rates in all experiments, with rare exceptions, exceeded 3 microliters per kilogram fresh weight per hour at least 24 hours before leaflet abscession reached 10%. This achieving of a threshold internal C2H4 level is viewed as an initiating event in leaflet abscession. Hypoboric conditions, to facilitate the escape of endogenous C2H4, delayed abscession compared to controls, and termination of hypoboric exposure allowed a normal progression of abscession as well as normal C2H4 synthesis rates. All of the data indicate that C2H4 initiates leaflet abscession in intact but detached leaves of *Mèlia azédarach* L. The seasonal patterns observed suggest that C2H4, in concert with those hormones which govern sensitivity to C2H4, regulate autumn leaf fall in this species.

There is considerable evidence indicating that ethylene is a natural regulator of leaf abscession (1). Much of this work has involved the induced abscession of debladed petioles from explants prepared from seedlings, and thus it did not come directly to grips with the question of whether intact leaves make C2H4 at abscession-inducing rates as they naturally senesce and separate from the plant. Jackson and Osborne (13) found that the C2H4 production of abscession zones from leaves of different ages correlated with their state of senescence. Senescing leaves produced more C2H4 than green ones, but leaves that absceded during harvest showed a declining pattern of C2H4 production. Beyer and Morgan (8, 23) determined both C2H4 production rates of detached cotyledons and C2H4 concentrations in air extracted from cotyledons imme-

¹ A contribution of the Texas Agricultural Experiment Station. This research was supported in part by National Science Foundation Grant GB-31306.

Received for publication October 2, 1979 and in revised form March 3, 1980

MATERIALS AND METHODS

These experiments were conducted with leaves of *M. azédarach* L., China tree, collected from individual trees growing in College Station, Texas, during the autumn months of 1972, 1973, and
1974. Large branches, usually with 12 to 20 leaves each, were detached in the morning, and the cut ends were placed in water, transported to the laboratory where leaves were detached by breaking at the leaf abscission zone, recut under water, and placed with their petioles in flasks containing water. Leaves in flasks were enclosed under 12-l bell jars with top openings and jars were sealed to glass plates with silicone stopcock lubricant. Enclosure was completed by 1:00 PM on the day of harvest; data collection began the following day (day 1 in all figures) allowing time for dissipation of wound ethylene before containers were closed. Jars were maintained in the laboratory under room lighting and constant temperature (22 C).

Two types of experiments were conducted; in one type, groups of leaves equal in age and position on the branch were selected and subjected to various treatments. In the other type of test, leaves from one or two large branches were grouped sequentially from apex to base and the effect of relative age or position on the pattern of C2H4 production and on leaflet abscission was observed.

Bell jars were removed at 8:00 AM and 4:30 PM daily, leaves were shaken lightly, leaflet abscission was recorded, and bell jars were reattached to base plates. During the first series of experiments (Figs. 1 and 2), jars were sealed during the day and C2H4 was determined by withdrawing duplicate 1-ml air samples at various times and injecting them into a Beckman model GC 72-5 gas chromatograph equipped with a 60- to 80-mesh activated alumina column (180 × 0.31 cm) and hydrogen flame ionization detector. The jars were left open at the top between 5:00 PM and 8:00 AM. In all subsequent experiments, bell jars were maintained open during the day, they were closed at 5:00 PM, and C2H4 production was determined at 8:00 AM daily (Figs. 3–5). Data were calculated as μl C2H4/kg fresh weight in the container (original weight less weight of leaflets removed) per h.

Reduced pressure (hypobaric) treatments were conducted as previously described (18). A vacuum regulator was set to maintain 200 mm Hg pressure by allowing air to continuously bleed into the bell jar (humidified by bubbling through water) and exit to the vacuum source. Controls were leaves not enclosed in bell jars, leaves in bell jars with equivalent air flow (200 ml/min), or leaves in bell jars closed (static) during the C2H4 collection periods and open at other times. Previous experiments showed no difference in cotton fruit dehiscence under hypobaric conditions whether ventilated with room air or 80% O2 (18); thus, room air was used in this study.

Except for Figure 1, data are plotted versus the date of collection without attempt to indicate the time of day that the samples were taken. Other experimental details are given in the legends to the figures.

RESULTS AND DISCUSSION

In an initial survey, we detached groups of successively older leaves and monitored leaflet abscission and C2H4 production with time (Fig. 1). As expected, the oldest leaves began to abscise leaflets first, and the initiation of abscission was consistently inversely correlated with age, the youngest leaves being the last to begin to abscise leaflets. The youngest leaves achieved the greatest peak of C2H4 production, and the peak rates were progressively lower with age. An C2H4 production rate of around 3 μl/kg fresh weight·h may be viewed as a threshold for induction of abscission (8, 11); thus, it is apparent that the rate for the oldest leaves were initially above that threshold rate and stayed above it for the course of the leaflet shedding process. The next oldest leaves (six, seven, and eight) were also producing C2H4 at or above the threshold rate for the entire experiment except at the second collection period when the rate was 2.2 μl/kg fresh weight·h.

Since reduced pressure facilitates the removal of C2H4 by diffusion (10) and has become one diagnostic tool for the regulatory activity of native C2H4, we conducted several experiments in which detached leaves were exposed to hypobaric pressure. In the first test, equivalent populations of leaves exhibited similar patterns of leaflet abscission whether they were open, enclosed in bell jars with constant air flow, or enclosed under static conditions (except for occasional opening to air out and test abscission) (Fig. 2). Abscission of leaflets under 200 mm Hg pressure was completely suppressed; therefore, as abscission in the controls neared completion, the hypobaric treatment was terminated and approximately 6 μl C2H4/I air was applied. Abscission began promptly and progressed at a rate similar to the other leaves. Ethylene production on days 4 and 5 in the static control was well above 3 μl/kg fresh weight·h, but abscission in this treatment did not rise above 10% until day 6. Leaves previously subjected to hypobaric pressure were producing C2H4 at about 3 μl/kg fresh weight·h on day nine and the leaves in the flowing system about 10 μl/kg fresh weight·h. A similar experiment was performed the following year; abscission of leaflets in the open and closed jars began on day 3 and reduced pressure (labeled vacuum) completely suppressed abscission (Fig. 3). First one, then the second, hypobaric pressure bell jar was returned to room pressure, after which abscission subsequently began and progressed in a normal fashion in each. Ethylene production in the closed bell jar was over 5 μl/kg fresh weight·h on day 2 and abscission began on day 3. Actually, the average production rate was 5 μl/kg fresh weight·h for the 15-h period (5:00 PM to 8:00 AM) ending on the morning of day 2, but abscission was not observed until the morning of day 3. Thus,

![Figure 1](https://example.com/fig1.png)

**Fig. 1.** Ethylene production rate (A) and abscission of leaflets (B) from detached leaves. Each group represents two adjacent leaves maintained in a bell jar with one being the most apical and 10 being most basal in location on the stem. Groups are combined and averages presented for clarity. Time zero is midnight of the day the leaves were harvested.
During the fall of 1973, a series of five experiments was conducted similar to the experiment reported in Figure 1. Progressively older leaves, those removed from the apex toward the base of one or more stems and grouped by relative age, were monitored for the progression of leaflet abscission and \( \text{C}_2\text{H}_4 \) production. Figure 4 illustrates several typical results. The progression of abscission was not always in orderly sequence from oldest to youngest leaves, especially among those relatively near the apex. This is also seen occasionally on the plant under natural conditions, especially in the early fall. The pattern of \( \text{C}_2\text{H}_4 \) production was generally similar to that previously seen in that it started low and then rose to a peak rate and declined as abscission near completion. Highest peak rates were often from some of the younger leaves and lower rates for older leaves (note the reversal of groups 2 and 4 in this regard). Especially when markedly older leaves were compared to obviously younger ones, as the leaves in group 5 in Figure 4, the peak \( \text{C}_2\text{H}_4 \) rate was relatively low. However, the rise in \( \text{C}_2\text{H}_4 \) production again preceded the initiation of rapid abscission. As detailed in Table I, all but one group of

**Fig. 2.** Abscission of leaflets (open symbols) from equivalent groups of six detached leaves maintained unenclosed (outside control) or enclosed in bell jars subjected to reduced pressure (200 mm Hg, labeled vacuum), continuous air flow (200 cc/min), or static (closed) treatment. \( \text{C}_2\text{H}_4 \) production rates (solid symbols) are for the static bell jar. Arrows indicate termination of treatments, determination of \( \text{C}_2\text{H}_4 \) production rate during next collection period (solid symbol with * is for flowing bell jar and symbol with ** is for “vacuum” jar), and exposure to 6 \( \mu \)l exogenous \( \text{C}_2\text{H}_4 \) air (“vacuum” jar only).

**Fig. 3.** Abscission of leaflets (open symbols) from groups of six equivalent leaves under bell jars maintained open, closed (static), or reduced pressure (200 mm Hg, labeled vacuum). \( \text{C}_2\text{H}_4 \) production rates shown in matching solid symbols. Hypobaric treatments were terminated at times indicated by arrows after which \( \text{C}_2\text{H}_4 \) production was monitored (appropriate solid symbols). Bell jars were closed from 5:00 PM until 8:00 AM daily, at which time \( \text{C}_2\text{H}_4 \) was determined, the containers were opened, and abscission was determined.

there was a considerable interval before first abscission occurred during which the average ethylene production rate exceeded 3 \( \mu \)l/kg fresh weight-h. After the first hypobaric pressure-treatment bell jar was returned to normal pressure on day 4, \( \text{C}_2\text{H}_4 \) production was about 5 \( \mu \)l/kg fresh weight-h on day 5, whereas abscission began on day 6. A third reduced pressure experiment produced similar results (data not presented).

**Fig. 4.** \( \text{C}_2\text{H}_4 \) production rates (A) and leaflet abscission (B) from detached leaves maintained under bell jars. One to three leaves per container, all from same branch, arranged from most apical (No. 1) to most basal (No. 5) with a gap of eight leaves between groups 4 and 5. Peak \( \text{C}_2\text{H}_4 \) production rates for groups 1 and 4 are recorded in parentheses adjacent to the appropriate curves. Sampling schedule was the same as Figure 3.
leaves was producing C2H4 at rates exceeding 9 μl/kg fresh weight-h during the night prior to the morning when total leaflet abscission first exceeded 10%. Further, all groups were exceeding 4.5 μl/kg fresh weight-h 24 h earlier. Group 5 was at 3 μl/kg fresh weight-h or higher on both days 1 and 2 and reached 10% abscission on day 3. The absence of an orderly progression of abscission and rates of ethylene production from basal leaves upward in Figure 4 suggests that most of the groups of leaves, except group 5, were similar physiologically.

As the autumn progressed and the time of rapid natural leaf fall approached, a very different pattern of C2H4 production was observed (Fig. 5). The leaves began abscising leaflets in definite order from the oldest to the youngest; but, with the exception of the first day, C2H4 production was highest in the oldest leaves and progressively less during the interval of abscission initiation in younger groups of leaves. Again, the peak of C2H4 synthesis was somewhat depressed in the oldest versus the youngest leaves. All groups of leaves exceeded C2H4 production rates of 10 μl/kg fresh weight-h the day before 10% abscission occurred, except group 2 which had a rate of 4.3 (Table I).

The following year, a slightly different approach was used. Branches were harvested and brought to the laboratory, and leaves were removed immediately. The internal air was removed by vacuum and its C2H4 concentration was determined (7). During the early autumn (September–October), several such tests revealed either very little difference in C2H4 content with leaf age or that the youngest leaves contained the highest C2H4 levels (Table II). The highest C2H4 level observed was 0.59 μl/l. Later a different trend was noted (Table II, Nov. 15 and 19) and verified subsequently by several assays on four different dates. Abscission of leaflets was occurring from the basal leaves and progressing rapidly up the stems. Basal leaves had higher levels of ethylene than apical leaves. The level of C2H4 in apical leaves had not changed much from earlier observations, but basal leaves contained as much as 1.8 μl/l of C2H4 and frequently about 1.0 μl/l.

The two patterns of internal levels of C2H4 (Table II) appear to be related to the change in patterns of C2H4 production with the progression of the season noted the previous year (compare Fig. 4 to Fig. 5). Thus, it appears that, as the time of rapid natural defoliation approached in the fall, elevated levels of internal C2H4 or rising rates of C2H4 production occur first in the most basal leaves, which in turn abscise their leaflets first. After leaflet abscission, the rachilla and rachis segments separate and eventually the petiole-abscission zone develops and separation occurs.

These data reveal that there is a relationship between the abscission of leaflets from intact (but detached) China tree leaves and the rate of production of C2H4 by the entire leaf. By necessity, the experiments dealt with populations of leaves and/or leaflets and cannot define the internal C2H4 content of a specific abscission zone. Although the system of abscission zones was intact, the leaves themselves were detached from the tree. Keeping in mind these qualifications, we conclude that ethylene produced by the leaf seems to initiate leaflet abscission because: (a) C2H4 production rises (to rates judged in other work to represent a threshold for activity in physiological processes such as abscission and

<table>
<thead>
<tr>
<th>Experiment (Figure)</th>
<th>Sample (Group)</th>
<th>Time of 10% Abscission</th>
<th>C2H4 Production</th>
<th>C2H4 Production Previous Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1</td>
<td>6</td>
<td>23.7</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5</td>
<td>9.7</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>15.2</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6</td>
<td>24.6</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3</td>
<td>2.3</td>
<td>5.0</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>8</td>
<td>35.1</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6</td>
<td>9.5</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>16.1</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>19.9</td>
<td>16.9</td>
</tr>
</tbody>
</table>

* Data are average rates of ethylene production during the night collection period ending the morning (day) that abscission reached 10%.

* Data are average rates of ethylene production during the night collection period ending the morning 24 h before the morning that abscission reached 10%.

---

**Table II. Concentration of Ethylene in Air Which was Vacuum-extracted from M. azedarach L. Leaves**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of Ethylene at Date of Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oct. 9a</td>
</tr>
<tr>
<td></td>
<td>Nov. 15b</td>
</tr>
<tr>
<td></td>
<td>Nov. 19c</td>
</tr>
<tr>
<td>1 (apical)</td>
<td>0.24</td>
</tr>
<tr>
<td>2</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>0.07</td>
</tr>
<tr>
<td>4 (basal)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* Single branch, six to 12 leaves per sample.
* Average of two branches, two to four leaves per sample.
* Average of four branches, two to four leaves per sample.
growth inhibition) before abscission exceeds 10% of the leaflets (Table I); (b) hypobaric pressure treatment, which should hasten removal of C₂H₄, prevents or delays abscission (Figs. 2 and 3); (c) just prior to rapid natural leaflet abscission both C₂H₄ levels and the tendency to abscise increase from apical to basal leaves on a branch (Fig. 5 and Table II); (d) during rapid abscission the oldest, most basal leaves consistently abscise first (Fig. 5); and (e) internal C₂H₄ levels of about 1 μl/l air (Table II) equal or exceed exogenous levels necessary to defoliate another species (8). Since leaflet abscission resumed when leaves kerp₄ under hypobaric pressure were returned to room pressure or room pressure plus C₂H₄ (Figs. 1 and 2), the hypobaric condition must have simply removed internal ethylene and not removed the capacity of leaflets to abscise.

The observed tendency for the youngest leaves to exhibit the highest peak rate of C₂H₄ production (Figs. 1 and 4) probably is not associated with the initiation of abscission. These peaks occurred well after abscission had been initiated and were 10-fold or more higher than the general threshold for physiological activity. The peak of C₂H₄ produced during abscission may be equivalent to the peak of C₂H₄ produced during fruit ripening. As has been well established in fruit ripening, the preclimacteric rise in C₂H₄ initiates ripening while the climacteric peak parallels or follows ripening (9, 25). If this analysis is correct, leaf groups 1, 2, and 3 produced the highest peak rate of C₂H₄ in Figure 1 because they were youngest and highest in auxin content (24) or C₂H₄ substrate, but the lower levels of C₂H₄ in the older leaves (groups 9 and 10) were adequate to initiate abscission of leaflets. The peak rates of C₂H₄ production would express the auxin content or relative age, whereas the sequence in abscission would reflect the natural sensitivity to C₂H₄ and the sequence in which threshold initiating levels are achieved.

While this paper was in preparation, Aharoni et al. (2–4) published evidence that C₂H₄ participates in senescence of tobacco leaves. Evidence includes the fact that ethylene production increases before the onset of chlorosis and the ability of the C₂H₄ synthesis inhibitor, aminoethoxyvinylglycine, to prevent chlorosis. They emphasize that C₂H₄ is a regulator of leaf senescence and thus is not limited to a role in leaf abscission since tobacco leaves do not abscise. We would emphasize that a general role as an inducer of leaf senescence does not preclude a more specific role in abscission induction in those cases where leaves do abscise in the normal progression of senescence events toward completion. Based on the present data and those of Aharoni et al. (2–4), one would conclude that C₂H₄ induces leaf senescence without abscission in tobacco and leaf senescence, which includes abscission, in M. azédarach L.

LITERATURE CITED

25. Pratt HK, JD Goeschl 1968 The role of ethylene in fruit ripening. In F Wightman, G Setterfield, eds, Biochemistry and Physiology of Plant Growth Substances. The Runge Press Ltd., Ottawa, Canada, p 1295