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Ethylene Production and Leaflet Abscission in *Mèlia azédarach* L.[1]

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ABSTRACT

Ethylene production or content was compared to leaflet abscission in detached, compound leaves of *Mèlia azédarach* L. In late autumn, when abscission was progressing from basal leaves upward, the oldest leaves both produced ethylene at the highest rates and abscised their leaflets first. When C_2H_4 levels were measured in intercellular air removed immediately after leaves were harvested, C_2H_4 levels were also highest in basal leaves and declined progressively in more apical leaves. Levels as high as 1.8 microliters C_2H_4 liter^{-1} air were observed. Earlier in the season groups of leaves demonstrated a pattern of sequential initiation of abscission from base to apex, but the peak rates of C_2H_4 production followed an opposite trend, being highest in the youngest leaves. Peak rates of C_2H_4 production occurred after the initiation of leaflet abscission and presumably are related to either the auxin content or a climacteric-like, autocatalytic phase of C_2H_4 production not directly involved in the initiation of abscission. In these experiments, the early abscission of the older leaflets reflects their greater sensitivity to C_2H_4, presumably due to lower auxin content. C_2H_4 production rates in all experiments, with rare exceptions, exceeded 3 microliters per kilogram fresh weight per hour at least 24 hours before leaflet abscission reached 10%. This achieving of a threshold internal C_2H_4 level is viewed as an initiating event in leaflet abscission. Hypobaric conditions, to facilitate the escape of endogenous C_2H_4, delayed abscission compared to controls, and termination of hypobaric exposure allowed a normal progression of abscission as well as normal C_2H_4 synthesis rates. All of the data indicate that C_2H_4 initiates leaflet abscission in intact but detached leaves of *Mèlia azédarach* L. The seasonal patterns observed suggest that C_2H_4, in concert with those hormones which govern sensitivity to C_2H_4, regulate autumn leaf fall in this species.

There is considerable evidence indicating that ethylene is a natural regulator of leaf abscission (1). Much of this work has involved the induced abscission 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 C_2H_4 at abscission-inducing rates as they naturally senesce and separate from the plant. Jackson and Osborne (13) found that the C_2H_4 production of abscission zones from leaves of different ages correlated with their state of senescence. Senescing leaves produced more C_2H_4 than green ones, but leaves that abscised during harvest showed a declining pattern of C_2H_4 production. Beyer and Morgan (8, 23) determined both C_2H_4 production rates of detached cotyledons and C_2H_4 concentrations in air extracted from cotyledons immediately after they were removed from plants. C_2H_4 rose significantly when progressing in physiological age from green to moderately senescent cotyledons and then declined somewhat in those which were fully chlorotic. Auxin transport capacity of petioles declined parallel to the rise in C_2H_4. When levels of C_2H_4 similar to the highest ones occurring naturally were applied to younger plants, abscission was induced and auxin transport capacity was reduced.

A role of C_2H_4 in natural leaf abscission is also supported by evidence linking the gas with induction of fruit dehiscence of cotton, pecan, okra, and squirting cucumber (15–18, 23). Fruit dehiscence is a specialized abscission process and, if C_2H_4 plays a role in fruit dehiscence, similar activity would be suspected in leaf abscission.

Some data do not support the proposal that ethylene initiates leaf abscission of intact plants. Water stress increased C_2H_4 production by detached citrus leaves but not by those on the tree (6). Water stress often promotes abscission (21). Water stress promoted C_2H_4 production by petioles of mature leaves on intact cotton plants (20), but no promotion was found under similar circumstances with cotyledonary petioles of seedlings (12). In cotton seedling explants (19), in contrast to findings with bean and citrus explants (5, 14), there was not a consistent peak of C_2H_4 produced just prior to abscission. The only C_2H_4-abscission correlation found was between wound C_2H_4 production during the first 12 h and abscission at the end of that period (19). Thus, although it might seem safe to concede that C_2H_4 initiates abscission of leaves on intact plants, such a role is not, in fact, established.

It would be desirable to monitor the production of C_2H_4 by intact leaves attached to plants as they senesce and abscise, but the amount of C_2H_4 produced is very small and there are many other technical difficulties. As an alternate approach, we used complete leaves from *Mèlia azédarach* L., China tree. The large, compound leaf has many leaflets, a fleshy petiole, rachis, rachilla, and multiple abscission zones. Leaves may form 200 to 300 abscission zones each; in addition to detaching leaflets, separations form between segments of rachilla, rachis, and petioles. The abscission of individual leaflets from an intact, although detached, leaf was judged by us to be a somewhat more complete or natural system than an abscission zone explant. Wounding during detachment should damage proportionally less cells than in an abscission zone explant. We report here correlative data consistent with the hypothesis that C_2H_4 does initiate leaflet abscission in *M. azédarach* L. and thus is probably the signal for initiation of the total foliar abscission sequence on the intact plant. A preliminary report has appeared (23).

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

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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 C_2H_4 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 C_2H_4 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 C_2H_4 production was determined at 8:00 AM daily (Figs. 3–5). Data were calculated as μl C_2H_4/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 C_2H_4 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% O_2 (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 C_2H_4 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 C_2H_4 production, and the peak rates were progressively lower with age. An C_2H_4 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 C_2H_4 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 C_2H_4 by diffusion (10) and has become one diagnostic tool for the regulatory activity of native C_2H_4, we conducted several experiments in

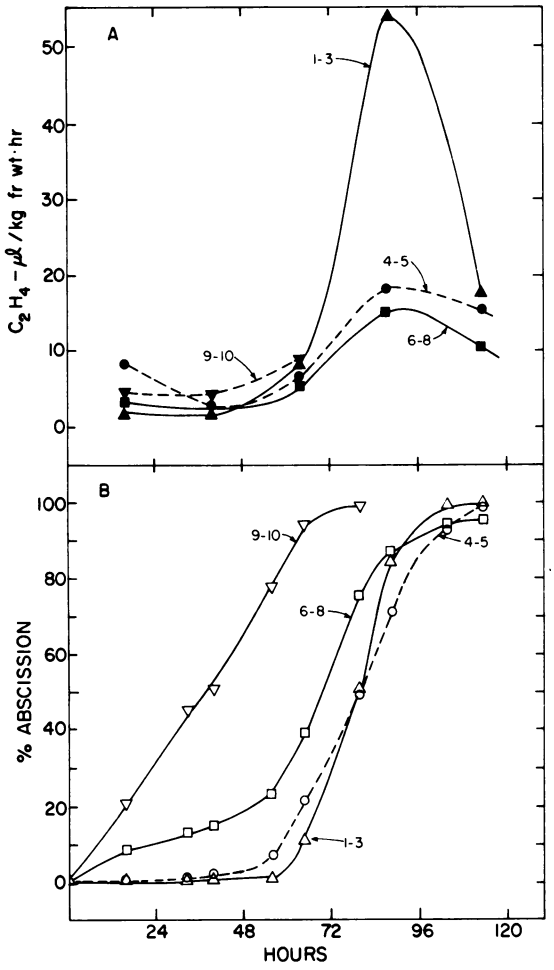

FIG. 1. Ethylene production rate (A) and abscission of leaflets (B) from detached leaves. Each group represents two adjacent leaves maintained in a bell jar with one being the most apical and 10 being most basal in location on the stem. Groups are combined and averages presented for clarity. Time zero is midnight of the day the leaves were harvested.

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 C_2H_4/l 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 C_2H_4 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,

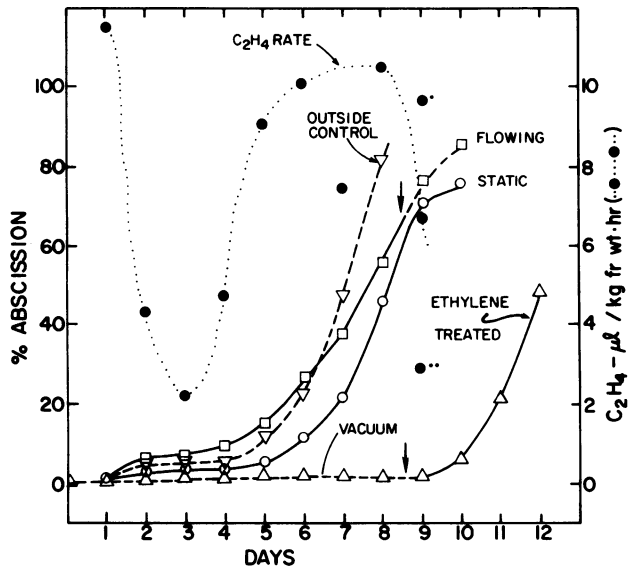

FIG. 2. Abscission of leaflets (open symbols) from equivalent groups of six detached leaves maintained unenclosed (outside control) or enclosed in bell jars subjected to reduced pressure (200 mm Hg, labeled vacuum), continuous air flow (200 cc/min), or static (closed) treatment. C_2H_4 production rates (solid symbols) are for the static bell jar. Arrows indicate termination of treatments, determination of C_2H_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 μl exogenous C_2H_4 air ("vacuum" jar only).

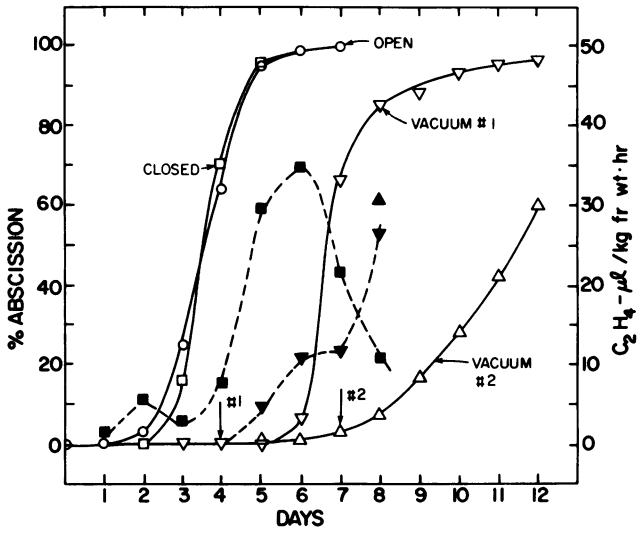

FIG. 3. Abscission of leaflets (open symbols) from groups of six equivalent leaves under bell jars maintained open, closed (static), or reduced pressure (200 mm Hg, labeled vacuum). C_2H_4 production rates shown in matching solid symbols. Hypobaric treatments were terminated at times indicated by arrows after which C_2H_4 production was monitored (appropriate solid symbols). Bell jars were closed from 5:00 PM until 8:00 AM daily, at which time C_2H_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 μl/kg fresh weight·h. After the first hypobaric pressure-treatment bell jar was returned to normal pressure on day 4, C_2H_4 production was about 5 μl/kg fresh weight·h on day 5, whereas abscission began on day 6. A third reduced pressure experiment produced similar results (data not presented).

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 C_2H_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 C_2H_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 neared 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 C_2H_4 rate was relatively low. However, the rise in C_2H_4 production again preceded the initiation of rapid abscission. As detailed in Table I, all but one group of

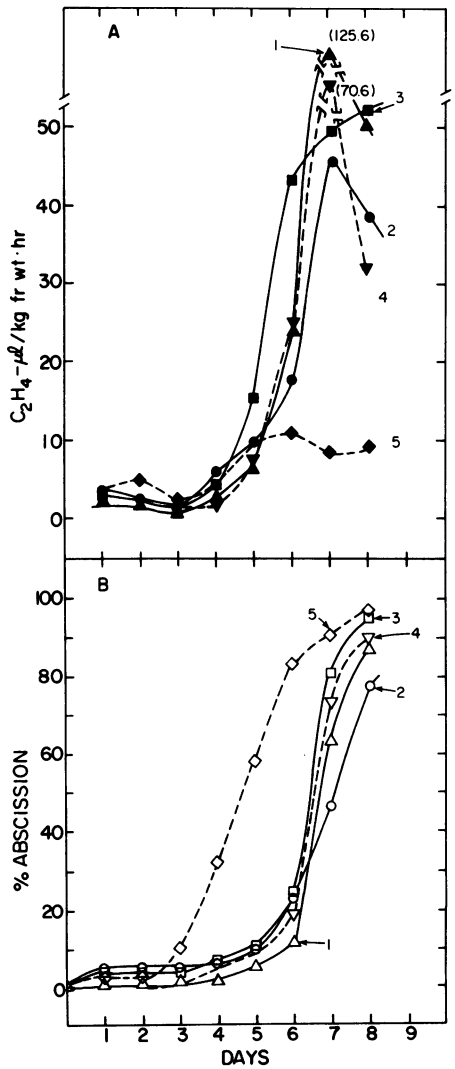

FIG. 4. C_2H_4 production rates (A) and leaflet abscission (B) from detached leaves maintained under bell jars. One to three leaves per container, all from same branch, arranged from most apical (No. 1) to most basal (No. 5) with a gap of eight leaves between groups 4 and 5. Peak C_2H_4 production rates for groups 1 and 4 are recorded in parentheses adjacent to the appropriate curves. Sampling schedule was the same as Figure 3.

Table I. *Relationship of Rate of Ethylene Production in Detached M. azédarach L. Leaves to the Time at Which 10% Leaflet Abscission Was Observed*

Experiment (Figure)	Sample (Group)	Time of 10% Abscission	C_2H_4 Production[a]	C_2H_4 Production Previous Day[b]
		day	*μl/kg fresh wt·h*	
4	1	6	23.7	6.5
	2	5	9.7	6.0
	3	5	15.2	4.5
	4	6	24.6	7.6
	5	3	2.3	5.0
5	1	8	35.1	10.9
	2	6	9.5	4.3
	3	5	16.1	10.2
	4	4	19.9	16.9

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

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

leaves was producing C_2H_4 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 C_2H_4 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, C_2H_4 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 C_2H_4 synthesis was somewhat depressed in the oldest *versus* the youngest leaves. All groups of leaves exceeded C_2H_4 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 C_2H_4 concentration was determined (7). During the early autumn (September–October), several such tests revealed either very little difference in C_2H_4 content with leaf age or that the youngest leaves contained the highest C_2H_4 levels (Table II). The highest C_2H_4 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 C_2H_4 in apical leaves had not changed too much from earlier observations, but basal leaves contained as much as 1.8 μl/l of C_2H_4 and frequently about 1.0 μl/l.

The two patterns of internal levels of C_2H_4 (Table II) appear to be related to the change in patterns of C_2H_4 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 C_2H_4 or rising rates of C_2H_4 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.

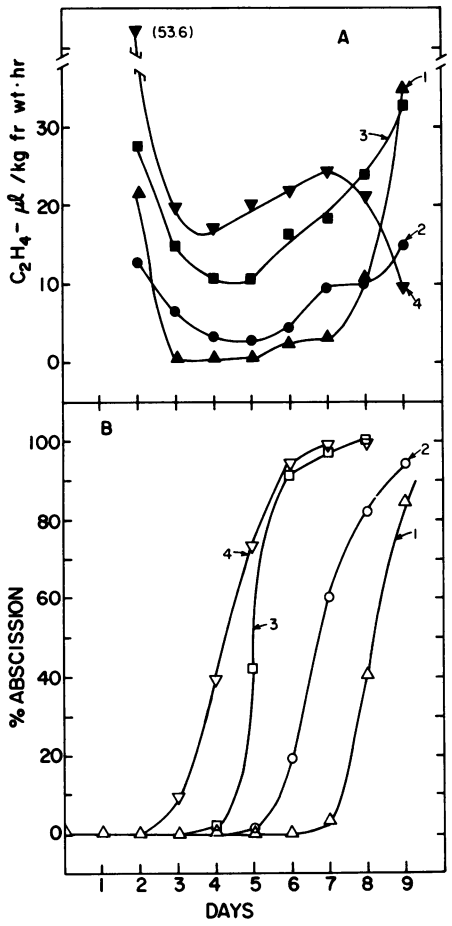

FIG. 5. C_2H_4 production rates (A) and leaflet abscission (B) from detached leaves maintained under bell jars near the time of rapid, natural defoliation in late autumn. Six leaves in each container, three in succession from each of two branches labeled from most apical (No. 1) to most basal (No. 4). Sampling schedule was same as Figure 3.

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

Sample	Amount of Ethylene at Date of Harvest		
	Oct. 9[a]	Nov. 15[b]	Nov. 19[c]
		μl/l air	
1 (apical)	0.24	0.12	0.23
2	0.15	0.19	0.64
3	0.07	0.38	0.78
4 (basal)	0.08	0.83	

[a] Single branch, six to 12 leaves per sample.

[b] Average of two branches, two to four leaves per sample.

[c] Average of four branches, two to four leaves per sample.

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 C_2H_4 by the entire leaf. By necessity, the experiments dealt with populations of leaves and/or leaflets and cannot define the internal C_2H_4 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*) C_2H_4 production rises (to rates judged in other work to represent a threshold for activity in physiological processes such as abscission and

growth inhibition) before abscission exceeds 10% of the leaflets (Table I); (*b*) hypobaric pressure treatment, which should hasten removal of C_2H_4, prevents or delays abscission (Figs. 2 and 3); (*c*) just prior to rapid natural leaflet abscission both C_2H_4 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 C_2H_4 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 kept under hypobaric pressure were returned to room pressure or room pressure plus C_2H_4 (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 C_2H_4 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 C_2H_4 produced during abscission may be equivalent to the peak of C_2H_4 produced during fruit ripening. As has been well established in fruit ripening, the preclimacteric rise in C_2H_4 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 C_2H_4 in Figure 1 because they were youngest and highest in auxin content (24) or C_2H_4 substrate, but the lower levels of C_2H_4 in the older leaves (groups 9 and 10) were adequate to initiate abscission of leaflets. The peak rates of C_2H_4 production would express the auxin content or relative age, whereas the sequence in abscission would reflect the natural sensitivity to C_2H_4 and the sequence in which threshold initiating levels are achieved.

While this paper was in preparation, Aharoni *et al.* (2–4) published evidence that C_2H_4 participates in senescence of tobacco leaves. Evidence includes the fact that ethylene production increases before the onset of chlorosis and the ability of the C_2H_4 synthesis inhibitor, aminoethoxyvinylglycine, to prevent chlorosis. They emphasize that C_2H_4 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 C_2H_4 induces leaf senescence without abscission in tobacco and leaf senescence, which includes abscission, in *M. azédarach* L.

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