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Patterns of Ethylene Production in Senescing Leaves[1]

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ABSTRACT

Changes in the patterns of ethylene production, chlorophyll content, and respiration were studied in relation to the senescence of intact leaves and leaf discs. The primary leaves of pinto bean, which abscise readily during natural senescence, and tobacco and sugar beet leaves, which do not abscise, were used. A decrease in the rate of ethylene production and respiration, during the slow phase of chlorophyll degradation, was observed in leaf-blade discs cut from mature leaves and aged in the dark. During rapid chlorophyll loss both ethylene production and respiration increased and then decreased. These climacteric-like patterns were shown by leaf discs of all three species. Discs taken from leaves that had been senescing on the plant also showed a climacteric-like rise in ethylene production but not in respiration, which decreased continuously with leaf age. Climacteric-like patterns in the rise of ethylene and respiration for leaf discs were also shown by the petioles of both bean and tobacco leaves. This indicates that the rise of ethylene and respiration is characteristic of the general process of senescence in leaves and is not restricted to the abscission process. In contrast to the ethylene-forming systems in climacteric fruits and many flowers, the one in leaves declines sharply in the early stages of senescence. The subsequent rise of ethylene production appears to be associated with the rapid phase of chlorophyll breakdown, and may indicate the final stage of the senescence process during which ethylene could be actively involved in inducing leaf abscission.

Ethylene is known to be involved with both Chl degradation in leaf blades and with the abscission process (9, 24, 25). Beyer (6) demonstrated that the leaf blade is the primary receptor site for exogenous ethylene in leaf abscission. Aharoni (1) found that the principal site of ethylene production in response to a short period of water stress was also the blades rather than the petioles of leaves. McAfee and Morgan (21) found that ethylene levels in cotton leaf petioles were two to six times as high as those in leaf blades. Less attention was paid to the changing pattern of ethylene production by leaf blades during their senescence. Some studies in which ethylene production by the whole leaf was measured revealed a decrease in ethylene production with leaf age (12, 13, 20, 23), but other studies showed an increase (5, 8). McGlasson *et al.* (22) recently showed that ethylene production in tomato leaves decreased and then increased in a pattern characteristic of the climacteric in fruits. This study did not distinguish between the contribution of the tomato petiole and leaf blade to the ethylene production pattern observed. Since abscission is directly concerned with the petiole it seemed important to determine patterns of ethylene production in leaf blades and petioles in abscising and nonabscising leaves during senescence. In this study we examined patterns of ethylene production by senescing leaf blades and by leaf petioles with and without abscission zones. The capacity of leaves at various ages to produce ethylene in response to IAA and kinetin was also studied.

MATERIALS AND METHODS

Tobacco (*Nicotiana tabacum* L. cv. Xanthi), pinto beans (*Phaseolus vulgaris* L.), and sugar beet (*Beta vulgaris* L. cv. Saccarifera) were grown in a greenhouse under natural lighting at temperatures between 20 and 30 C. Details on age and location of the leaves used are described in the legends of tables and figures.

Leaves were washed in running tap water, surface-sterilized by soaking for 30 s in a 0.5% (v/v) dilution of commercial sodium hypochlorite in water, and rinsed several times in sterile distilled H_2O. All subsequent handling of the tissue involved sterile technique.

Discs 1 cm in diameter were cut from leaf blades with a corkborer and were floated abaxially down under cool-white fluorescent light for about 3 h in open Petri dishes containing 20 ml of H_2O or hormone solution. This preincubation, carried out with all tissues, allowed wound ethylene to subside. Samples of leaf discs were placed on filter paper in 9-cm Petri dishes (16 discs) or in 50-ml Erlenmeyer flasks (12 discs) to which 4 ml or 2 ml, respectively, of the test solution was added. Weights of 12 leaf discs of either tobacco, bean, or sugar beet were about 180 mg, 220 mg, or 280 mg, respectively. For additional protection against bacterial contamination, streptomycin (100 μg/ml) and penicillin (100 units/ml) were added to the test solution (15). Preliminary experiments verified that these antibiotics at the concentrations used did not affect each of the parameters measured: ethylene production, respiration, and Chl content. The flasks were sealed with rubber serum caps and incubated in darkness at 28 C.

Average rates of ethylene production by leaf discs were determined by analysis of a gas sample withdrawn from the Erlenmeyer flasks with a hypodermic syringe. Ethylene was allowed to accumulate usually for 18 to 24 h. During enclosure for 24 h ethylene accumulated in a range of 0.02 to 0.05 μl/l in tobacco and sugar beet leaf discs, respectively. Although accumulation of ethylene in sealed containers might tend to accelerate senescence, the pattern of senescence and ethylene production was not altered in these discs as compared to detached intact leaves. After sampling, the flasks were flushed with sterile fresh air. Ethylene from intact tobacco leaves was allowed to accumulate for 3 h after samples of six leaves each had been enclosed in 900-ml glass jars containing 50 ml H_2O. Averate rates of ethylene production by whole de-

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tached petioles were determined from samples of three petioles each, enclosed for 20 h in 6-ml vials containing 0.5 ml H_2O. Average rates of ethylene production by petiole segments were determined from samples of two segments each, enclosed for 20 h in 3-ml vials containing 0.1 ml H_2O. Intercellular ethylene of intact tobacco leaves was extracted by the vacuum method as described by Beyer and Morgan (7) except that a vacuum of 50 mm Hg was employed for 3 min. Four to 6 ml of air was collected from 20 apical leaves, 20 expanding leaves, and 10 of each of the older types of leaves tested. Ethylene was analyzed by gas chromatography with a 30-cm alumina column and a flame ionization detector.

Rates of respiration were determined by measurement of CO_2 accumulated in the flasks. Samples were taken as described for ethylene and were analyzed with a model 29 Fisher gas partitioner.

The Chl content of leaves was used to indicate the stage of senescence, since it was found to be correlated with other modifications characteristic of leaf senescence such as decline in protein and RNA content (4). Chl was extracted from leaf discs with dimethylformamide (two discs in 3 ml solvent) for 48 h in darkness at 4 C. Preliminary experiments revealed that shaking improved Chl extraction. Chl level was determined spectrophotometrically at 665 nm and expressed in optical density (O.D.) units.

Three to four replicates were used for ethylene and respiration experiments. Six to eight replicates, each consisting of two leaf discs, were used for Chl determination. Rates of ethylene and respiration in tables and figures are average rates during the time periods indicated. Experiments were repeated at least twice and gave reproducible results. Representative experiments are presented.

RESULTS

Rates of Ethylene Production and Respiration by Leaves during Development and Senescence on the Plant. Internal concentration of ethylene in intact tobacco leaves, which was determined within 30 min after harvest, was highest in apical leaves, decreased during expansion, maturation, and early senescence, and rose during advanced stages of senescence (Fig. 1). A similar pattern of change was found for the rate of ethylene production by intact leaves (Fig. 1).

An increase in endogenous ethylene in senescing leaves was reported to be associated with the abscission process (5, 8). Therefore, we examined the changes in ethylene production by petioles and blades of senescing bean leaves, which abscise and senescing tobacco leaves, which do not. Also, we determined the changes in respiration rate and Chl content, which are known to characterize leaf senescence (26). For these experiments, we used leaf-blade discs and petiole segments taken from leaves that had been senescing on the plant. As tobacco leaves aged, the ethylene production of both petioles and blades rose to a maximum and then declined (Fig. 2). The ethylene rise began only after more

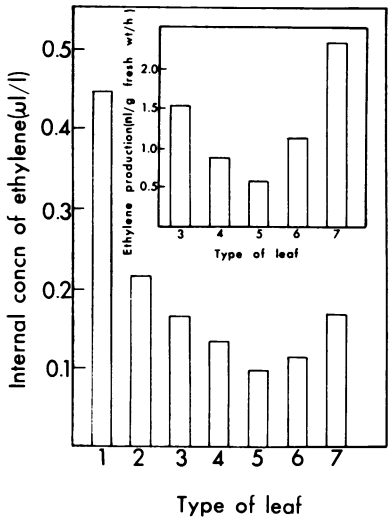

FIG. 1. Internal concentration of ethylene and average rate of ethylene production in intact tobacco leaves harvested at different ages. Leaves are numbered according to their position on the stem. 1: Apical expanding leaves; 2: older expanding leaves; 3: fully expanded mature leaves; 4: slight yellowing; 5: moderate yellowing; 6: advanced yellowing; 7: complete yellowing (basal leaves). Internal ethylene was extracted within 30 min after harvesting. After detaching, leaves were kept in wet perforated polyethylene bags for 24 h in darkness at 28 C, to maintain turgor and allow wound ethylene to subside. Ethylene production was measured after leaves were enclosed for 3 h in 900-ml glass jars.

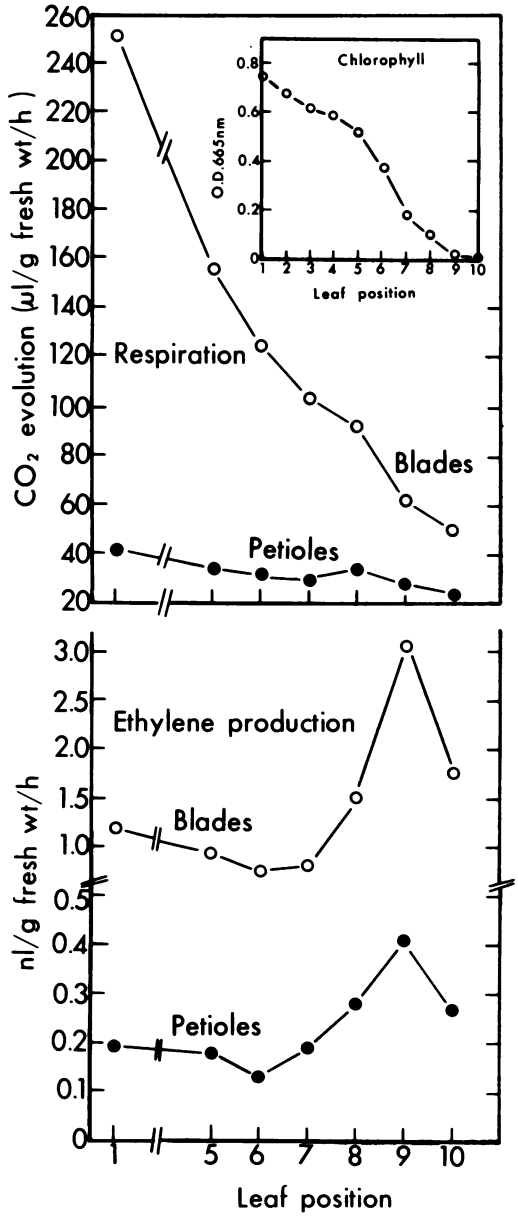

FIG. 2. Changes in ethylene production, respiration rate, and Chl content of leaf discs and petioles cut from tobacco leaves at various ages. Leaves are numbered according to their position on the stem in intervals of one leaf. 1: Fully expanded mature leaves with dark green color; 10: basal leaves with complete yellowing. For measurement of evolved ethylene and CO_2, leaf discs and petiole segments (about 4-cm length) were enclosed for 24 h in darkness at 28 C after a 3-h period to allow dissipation of wound ethylene production. Chl contents of the fresh leaf discs are shown in the inset.

than 50% of the initial Chl had been lost. Rates of ethylene production were much higher in blades than in petioles. Respiration decreased rapidly in blades and slowly in petioles without any appreciable rise during the course of senescence. The patterns of both ethylene production and respiration shown by the bean leaves (Fig. 3) were generally similar to those shown by tobacco except that: (*a*) respiration rates of both blades and petioles rose slightly during aging (35 or 40 days after sowing); (*b*) the difference between minimum and maximum rates of ethylene production was about 9-fold in bean petioles compared with only about 3-fold in tobacco petioles.

To determine whether petioles of tobacco and bean differed further, we measured rates of ethylene production by segments along their petioles. In beans proximal segments containing abscission zones as well as the distal one produced more ethylene than middle segments (Fig. 4). In tobacco ethylene production by petiole segments was reciprocally associated with Chl content (Fig. 4).

Rates of Ethylene Production and Respiration during Senescence of Leaf Discs. The sequence of changes in ethylene production and respiration was studied with discs from fully expanded mature leaves of tobacco and sugar beet allowed to senesce in the dark. The curves of Chl loss are characterized by a moderate-loss phase (phase I) followed by a rapid-loss phase (phase II), (Fig. 5). Both respiration and ethylene began to increase to a maximum near the transition of phase I to phase II. In all instances, respiration peaked 1 day before ethylene did. Changes in ethylene production, rate of respiration, and Chl loss were temperature-dependent. Decreasing the temperature by 8 C had a relatively slight effect on Chl loss in sugar beet leaf discs but delayed respiration and ethylene maxima by 1 day. However, the temperature reduction noticeably retarded Chl loss in tobacco leaf discs and delayed ethylene and respiration maxima by 9 days.

Changes in Rate of Ethylene Production in Leaves at Various Ages in Response to Exogenous Hormones. To determine whether the decrease in ethylene production during the early stage of leaf senescence was caused by a deficiency of either endogenous auxin or cytokinin, we examined leaf discs to which we had applied IAA and kinetin. The discs were taken from bean plants at different ages. Table I shows that the capacity of leaf discs to produce ethylene in response to added hormones sharply declined with leaf age.

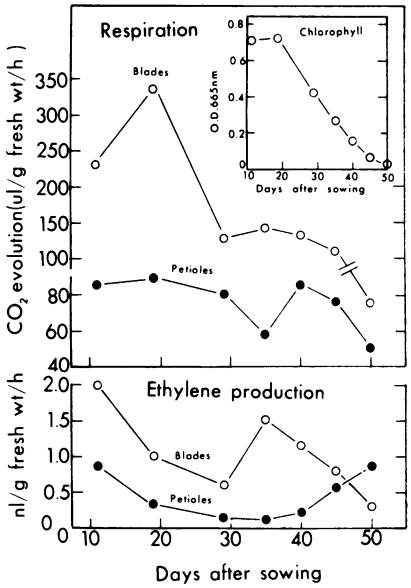

FIG. 3. Changes in ethylene production, respiration rate, and Chl content of leaf discs and petioles cut from primary pinto bean leaves at various ages. Leaves were harvested at the same date from plants which had been seeded at intervals of 5 to 10 days. Details are as described in Figure 2, except that the petiole lengths, which varied according to leaf age, ranged from 2 cm (11-day-old plants) to 5 cm (50-day-old plants). Chl content of the fresh leaf discs is shown in the inset. Beginning of abscission was observed 45 days after sowing.

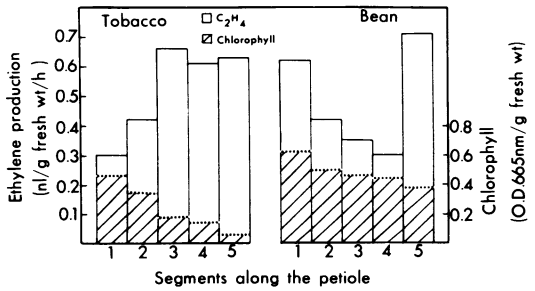

FIG. 4. Ethylene productions and Chl contents of petiole segments cut from tobacco and pinto bean leaves which had senesced and yellowed on the plant. Petioles were cut to five equal segments, 0.8 cm each for tobacco and 1 cm for beans. Segment 1: proximal end; segment 5: distal end.

DISCUSSION

Both ethylene production and rate of respiration decreased during leaf expansion, maturation, and phase I of senescence,

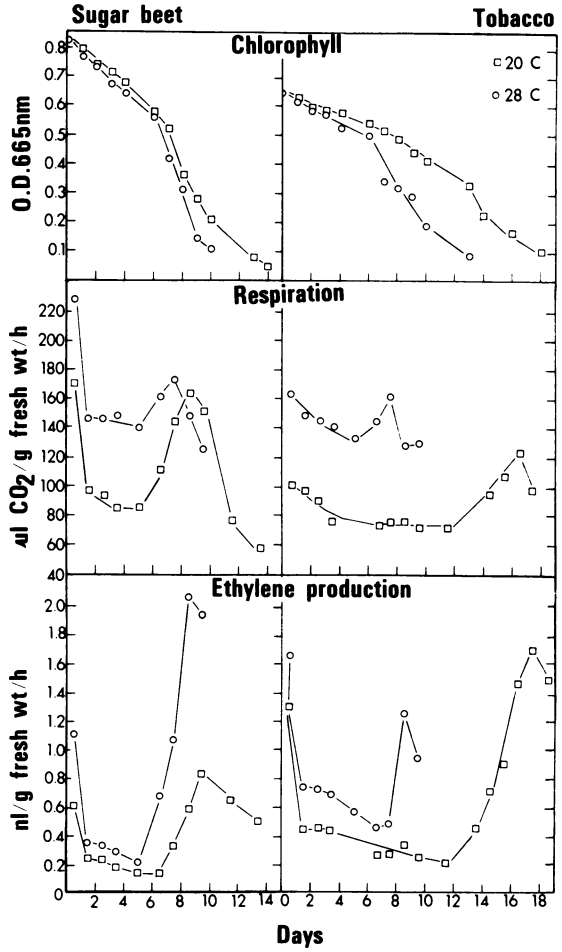

FIG. 5. Changes in ethylene production, respiration rate, and Chl content of sugar beet and tobacco leaf discs during senescence in darkness at either 20 C or 28 C. Leaf discs were cut from fully expanded mature leaves. Chl content was determined in leaf discs which had been senescing in Petri dishes.

Table I. *Effects of IAA and Kinetin Applied at 10^{-5} M on Ethylene Production by Discs of Primary Pinto Bean Leaves Taken from Plants at Various Ages*

Treated leaf discs were floated in Petri dishes on hormone solutions under light for 3 h and then incubated in darkness in Erlenmeyer flasks to which hormone solutions were added. Ethylene was allowed to accumulate for 18 h. SE was about ± 10% of the means.

Age of Plant	Chl Content at Day Zero	Ethylene Production			
		Control	Kinetin	IAA	Kinetin + IAA
days	*O.D. 665 nm*	*nl/g fresh weight·h*			
12	0.628	1.16	2.15	79.92	66.94
23	0.665	0.92	2.46	9.22	29.37
35	0.173	1.30	1.57	4.44	8.19
45[a]	0.022	2.87	2.12	1.57	1.43

[a] Beginning of abscission.

which was characterized by moderate degradation of Chl. In leaf discs the period of rapid Chl degradation was accompanied by a rise in both respiration and ethylene, followed by decreases in both. Such climacteric-like patterns of ethylene and respiration were found when segments of tomato leaves were examined by McGlasson *et al.* (22), but they did not report the stage of senescence.

The pattern of ethylene production was similar in discs prepared from leaves which senesced on the plant (Figs. 1–3), and in leaf discs which were allowed to senesce in darkness (Fig. 5). The similarity indicates that the leaf disc is an appropriate experimental model system for studies of changes in ethylene production and perhaps the role of ethylene and its mode of action in leaf senescence. In contrast, the pattern of respiration in dark-senescing leaf discs (Fig. 5) differed from that of respiration in discs taken from leaves that had been senescing on the plant (Figs. 2 and 3). This difference in respiration patterns may be related to the difference in ^{32}P-labeling of respiratory metabolites, observed in yellow detached leaves as contrasted with yellow attached tobacco leaves (19). The yellow detached leaf discs showed considerably greater content of ATP and phosphoenolypyruvate than discs from yellow attached leaves, suggesting greater respiratory rates for the detached tissue.

Ethylene production rose climacterically both in leaf blades and leaf petioles with or without abscission zones (Figs. 2 and 3). In both pinto bean and tobacco leaves rates of ethylene production were markedly higher in the blades than in the petioles. These findings suggest that the increase in ethylene in leaves is not restricted to the abscission zone but is characteristic of the general process of leaf senescence. However, differences between tobacco and pinto bean with respect to the rates of ethylene production by segments along the petioles support the idea that ethylene does participate in the abscission process (8, 9, 14, 24).

IAA and kinetin were reported to induce ethylene production by young pea seedlings (11). Lau and Yang (16) found that the synergistic effect of kinetin on IAA-induced ethylene production by hypocotyl segments of mung bean was due to an influence of kinetin on IAA uptake and IAA metabolism. In our experiments the rate of ethylene production in response to IAA was highest in leaf discs taken from expanding bean leaves (Table I). Kinetin alone was less effective in inducing ethylene production, but acted synergistically with IAA to increase ethylene production markedly in discs from mature and old leaves (23- and 35-day-old plants, respectively). However, kinetin had no effect on ethylene production when added with IAA to discs from expanding bean leaves of 12-day-old plants. This phenomenon could be associated with the saturating endogenous levels of cytokinin and maximum activity of auxin in young, growing leaves. It is also possible that the actual rate of ethylene production, by leaf discs taken from young leaves and treated with either IAA or IAA plus kinetin, was inhibited by the accumulated ethylene (2). Ethylene concentrations in the flasks were as high as 5 μl/l. IAA and kinetin were also ineffective in stimulating ethylene production in old leaf discs, from 45-day-old bean plants. In these senescent leaves, exogenous IAA and kinetin slightly suppressed ethylene production. These findings (Table I) suggest that the regulation of the ethylene-forming system in very old tissues differs from that in younger tissues.

Auxin-like activity increased and then decreased in senescing tobacco leaves (27) as did ABA content in senescing bean leaves (10). The considerable rise in leaf ABA was associated with the phase of rapid Chl breakdown (3). Despite the ability of these hormones to influence ethylene production by leaves and fruit (17, 18), more evidence is needed to support the idea that the changes in the content of these hormones are directly related to the climacteric-like pattern in ethylene production during leaf senescence.

After comparing patterns of ethylene production with those of Chl content, we concluded previously that the climacteric-like rise in ethylene production occurs when leaves are in an advanced stage of senescence, and is probably caused by earlier events taking place in the course of leaf senescence. However, our current findings do not exclude the possibility that endogenous ethylene plays a considerable role in the regulation of leaf senescence, even in its early stage.

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