J Plant Growth Regul (1989) 8:309–317

Journal of
Plant
Growth
Regulation

Interrelationship Between Ethylene and Growth Regulators in the Senescence of Lettuce Leaf Discs

Nehemia Aharoni

Department of Fruit and Vegetable Storage, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel

Received January 10, 1989; accepted June 2, 1989

Abstract. The interrelationship between ethylene and growth regulators in the senescence of romaine lettuce (*Lactuca sativa* L.) leaves was studied. Gibberellic acid (GA_3), kinetin, and 3-indoleacetic acid (IAA) retarded chlorophyll loss from leaf discs which were floated on hormone solutions. Abscisic acid (ABA) and ethephon enhanced chlorophyll loss and antagonized the senescence-retarding effect of GA_3 and kinetin. A high concentration of IAA (10^{-4} M) caused accelerated chlorophyll loss, whereas a similar concentration of kinetin neither retarded nor promoted chlorophyll loss. The ineffectiveness of IAA and kinetin at their supraoptimal concentrations in retarding leaf senescence was related to increased production of ethylene induced in the treated leaf discs. GA_3 was the most effective in retarding chlorophyll loss and did not stimulate ethylene production at all. The senescence-enhancing effect of ABA was not mediated by ethylene. However, the moderately increased production of ethylene, induced by relatively high concentrations of ABA, could act synergistically with the latter to accelerate chlorophyll loss. It is proposed that the effectiveness of exogenously applied hormones, both in enhancing and retarding senescence, is greatly affected by the endogenous ethylene concentration of the treated plant tissue.

Gibberellins and cytokinins have been shown to retard the senescence of leaves in a wide range of species, whereas ABA accelerates senescence (Aharoni et al. 1975; Noodén 1988; Noodén and Leopold 1978; Van Staden et

Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel, No. 2571-E, 1988 series.

al. 1988). Evidence based on the changes in the level of these endogenous hormones (Aharoni and Richmond 1978; Chin and Beevers 1970) suggest that these hormones play an important role in the regulation of leaf senescence.

In some species, the senescence-enhancing effect of abscisic acid (ABA) can be completely overcome by the application of either gibberellic acid (GA_3) or cytokinin (Aspinall et al. 1967; Back and Richmond 1971). In other species, these hormones only partially overcame the ABA-enhanced effect (Beevers 1968; Back and Richmond 1971). Furthermore, some reports have shown that GA_3 and cytokinins were ineffective in retarding leaf senescence and, in some instances, even an enhancing effect was observed (Dennis et al. 1967; Noodén 1988; Noodén and Leopold 1978). The different results obtained indicate that the effectiveness of the hormones is dependent on species, leaf age, experimental system used (i.e., intact versus excised tissue), and experimental conditions. Although leaf discs provide a convenient system to study the relationship between hormones, it should be noted that wounding of leaf tissue causes increased ethylene production (Burg 1962; Lieberman 1979). Other experimental conditions, such as water stress, also caused increased production of ethylene, which varied in extent from species to species (Aharoni 1978; Yang and Hoffman 1984). Stimulation of ethylene production was also found in vegetative plant tissues following treatment with 3-indoleacetic acid (IAA), kinetin, GA_3, or ABA (Abeles and Rubinstein 1964; Aharoni et al. 1979; Burg and Burg 1968; Lieberman 1979; Morgan and Hall 1964; Yang and Hoffman, 1984). Ethylene was found to accelerate plant senescence (Abeles 1972; Aharoni and Lieberman 1979; Burg 1968; Kader 1985; Lieberman 1979; Mattoo and Aharoni 1988; Osborne 1968) and was capable of antagonizing some physiological effects caused by auxins, gibberellins, or cytokinins (Lieberman 1979). Ethylene was reported to act synergistically with ABA in leaf senescence and abscission (Lieberman 1979; Mattoo and Aharoni 1988).

In the present study we examined the interaction between ethylene and other growth regulators in the senescence of both leaf discs and detached leaves of lettuce.

Materials and Methods

Plant Material

Leaves were harvested from either 9- or 18-week-old romaine lettuce plants (*Lactuca sativa* L. cv. 'Hazera Yellow') grown under field conditions from November to May. Young expanding leaves, fully expanded mature leaves, and green old leaves, classified as described by Aharoni and Richmond (1978), were used.

Sterilization

Leaves were washed in running tap water, surface-sterilized by soaking for 20 s in a 0.5% (vol/vol) sodium hypochlorite commercial solution, and rinsed several times in sterile distilled H_2O.

Growth Regulators

Kinetin (Calbiochem, USA) was dissolved in distilled H_2O for 20 min at 120°C in an autoclave. GA_3, IAA (Sigma, USA) and ethephon (2-chloroethylphosphonic acid; Amchen, USA, 68-250, 48% active ingredient) were dissolved in sterile distilled H_2O. (+)-ABA (Hoffman-La Roche, Switzerland) was dissolved in a minute quantity of absolute ethanol and then diluted with sterile distilled H_2O. The solutions of ABA and all comparable controls contained the same concentration of ethanol (0.2%).

Leaf Discs

Leaf discs, 9 mm in diameter, were punched from the distal third part of the leaf blades. Eighteen leaf discs, which were taken from different leaves, were floated abaxially on a 20-ml solution of the various growth regulators in sterile petri dishes. The leaf discs were incubated in darkness at 25°C for 3 or 4 days. Chlorophyll was extracted in dimethylformamide (three leaf discs in a 4-ml solution) for 48 h in darkness at 4°C (Back and Richmond 1971; Moran and Porath 1980). The chlorophyll level was determined spectrophotometrically at 665 nm and expressed in absorbance values. Each treatment included six replicates, each consisting of three leaf discs. In the ethylene experiment (Fig. 2) leaf discs were floated for 2 h on the hormone solutions; thereafter, each 12 leaf discs were placed abaxially on filter paper and moistened with 2-ml hormone solution in a 100-ml sterile flask. The flasks, kept in darkness at 25°C, were sealed with rubber serum caps, and the ethylene was allowed to accumulate for a 19-h period. Ethylene production was determined in three replicates by an injection of 1 ml of air, sampled from each flask, to a Packard gas chromatograph equipped with a 1800 × 6 mm activated alumina column and a flame ionization detector.

Detached Leaves

Detached leaves were allowed to senesce in darkness in a ventilated chamber (100% relative humidity, 25°C). The leaves were kept vertically in 2200-ml open-glass jars. Growth regulators were applied to detached leaves immediately after harvest by immersion in an aqueous solution for 30 min, or by immersion followed by continuous petiole feeding throughout the next 18 h of incubation. Ethylene production rates were determined in three replicates, each consisting of three leaves, which were enclosed in a jar for a 90-min period. Chlorophyll was extracted from detached leaves by the following method. Weighed samples of leaf blades were homogenized with cold 80% acetone (5 g/80 ml) in a Sorvall 'Omni Mixer.' The homogenate was filtered through a Buchner funnel and washed several times with cold acetone. The filtrate was brought to equal volume and after appropriate dilution the level of chlorophyll was measured spectrophotometrically. Two replicates, each consisting of 5 g of leaf blades, were sampled randomly.

Results and Discussion

Effect of Growth Regulators on Ethylene Production and Chlorophyll Retention by Leaf Discs

GA_3 and kinetin retarded chlorophyll loss from lettuce leaf discs, which were excised from either young or old leaves, whereas ABA accelerated it (Fig. 1). Dose–response curves of GA_3 and kinetin in retarding chlorophyll loss have two phases with an optimal concentration of 10^{-7} M or 10^{-6} M. Kinetin was more effective than GA_3 in retarding senescence of old leaf discs (Fig. 1B) but was less effective in young leaf discs (Fig. 1A). In the latter discs, kinetin at supraoptimal concentrations of 10^{-4} M did not delay chlorophyll loss at all. ABA was more effective in enhancing senescence of old leaf discs than young leaf discs (Fig. 1). The dose–response curve of ABA in young leaf discs shows two phases: a moderate response to increasing concentration from 10^{-9} M to 10^{-7} M; and a sharp response to increasing concentration from 10^{-6} M up to the maximal concentration tested, 10^{-4} M (see also Fig. 4).

The interrelationship between ethylene and other growth regulators was examined in discs from young lettuce leaves exhibiting vigorous growth. Such leaves produced ethylene at higher rates than older leaves (Aharoni, unpublished observations), as previously reported for other kinds of leaves (Hall et al. 1957; McAfee and Morgan 1971; Osborne 1968).

Comparison of the percentages of chlorophyll loss with the rate of ethylene production induced by each hormone concentration indicates that induced ethylene was indeed involved in the action of the hormones (Fig. 2). GA_3, which did not stimulate ethylene production, was the most effective compound in retarding chlorophyll loss. Kinetin was the most active ethylene stimulator and induced ethylene production at a concentration as low as 10^{-7} M. Increasing the kinetin concentration above 10^{-6} M markedly accelerated the rate of ethylene production which reached approximately 26 $\mu l\ kg^{-1}\ h^{-1}$ in response to 10^{-4} M kinetin. This rate of ethylene production was about fivefold greater than that of untreated leaf discs. In this experiment, kinetin reduced slightly chlorophyll loss only at 10^{-8} M, whereas at higher concentrations it neither retarded nor promoted chlorophyll loss. It seems that the age of the plant from which the leaves were taken is an important factor influencing the effectiveness of kinetin to retard senescence. Thus, kinetin at concentrations of 10^{-7} M and 10^{-6} M retarded senescence in young leaves from 18-week-old plants (Fig. 1) but was ineffective in young leaves taken from younger 9-week-old plants (Fig. 2). It is possible that the ineffectiveness of cytokinins to retard senescence in the young leaves of Brussel sprouts (Dennis et al., 1975) may also be associated with the increased production of ethylene by the treated leaves. IAA markedly accelerated ethylene production when applied at concentrations above 10^{-6} M. At lower concentrations IAA retarded chlorophyll loss, whereas at higher concentrations IAA accelerated chlorophyll loss. The senescence-enhancing effect of ABA was observed when applied at concentrations of 10^{-8} M and 10^{-7} M, levels which did not induce ethylene production. However, it seems that the accelerated chlorophyll loss from leaf discs in response to 10^{-4} M ABA could be related to the increased stimulation of ethylene.

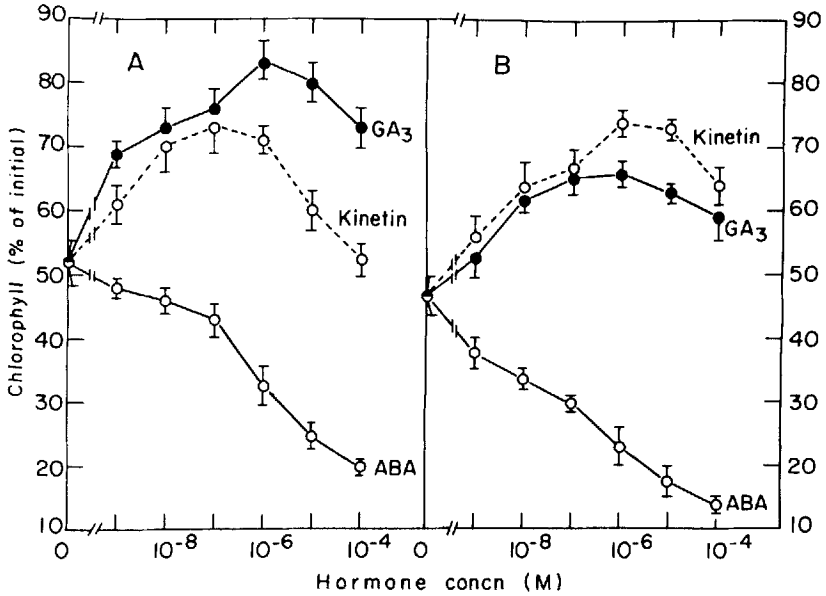

Fig. 1. Effect of increasing concentrations of GA_3, kinetin, and ABA on chlorophyll (A_{665}) retention by lettuce leaf discs. **A** Young expanding leaves. **B** Old leaves. Leaves were harvested from 18-week-old plants. Leaf discs were incubated in petri dishes for 4 days. Vertical lines represent the SE.

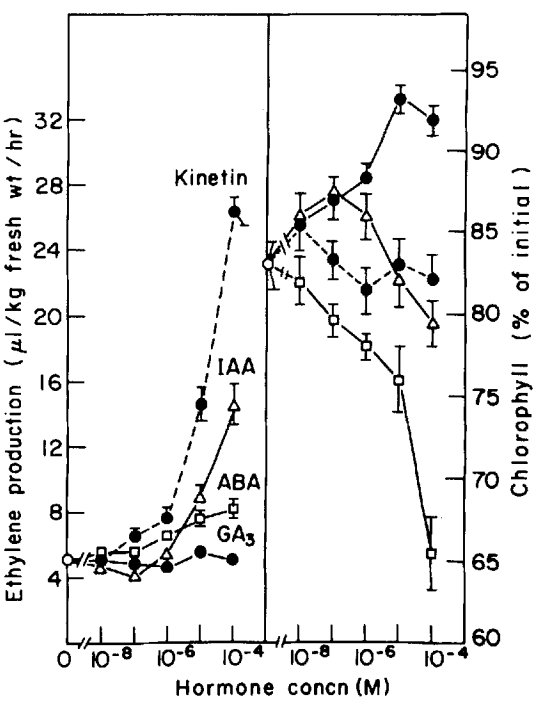

Fig. 2. Effect of increasing concentrations of GA_3, kinetin, IAA, and ABA on ethylene production and chlorophyll (A_{665}) retention by lettuce leaf discs excised from expanding young leaves. Ethylene was allowed to accumulate for the first 19 h of incubation. Leaf discs obtained from 9-week-old plants were incubated for 3 days in sealed flasks which were ventilated daily. Vertical lines represent the SE.

Interaction of Growth Regulators in the Senescence of Leaf Discs

GA_3 and kinetin at 10^{-5} M, as expected, retarded chlorophyll loss from lettuce leaf discs excised from mature leaves (Fig. 3). The ethylene-releasing chemical, ethephon, enhanced chlorophyll loss and reversed the senescence-retarding effect of both GA_3 and kinetin.

Whether or not GA_3 and kinetin are capable of completely overcoming ABA-enhanced senescence depends on the leaf species studied (Aspinall et al. 1967; Back and Richmond 1971; Beevers 1968; Manos and Goldthwaite 1975). Interactions between ABA and either GA_3 or kinetin affecting chlorophyll loss from lettuce leaf discs are depicted in Fig. 4. Both GA_3 and kinetin, which were applied at 10^{-5} M, completely reversed the senescence-enhancing effect of low concentrations of ABA (10^{-9} M to 10^{-7} M) but only partial reversion occurred when higher concentrations of ABA were used (Fig. 4). It should be noted that the inability of GA_3 and kinetin to completely reverse the ABA effect was observed at concentrations in which ABA induced ethylene production (Fig. 2), indicating that ethylene could act synergistically with ABA to antagonize the GA_3 or kinetin effect in retarding leaf senescence. It should be noted that supraoptimal concentrations of GA_3 did not induce ethylene (Fig. 2). This indicates that, for GA_3, and maybe for kinetin also, factors such as toxicity in addition to ethylene are probably involved in the reduced effectiveness of these hormones when applied at supraoptimal concentrations.

Effect of Growth Regulators on Ethylene Production and Chlorophyll Retention by Detached Young Leaves

The experiments described above were carried out with leaf discs. It has long been known that wounding of the leaf tissue causes increased metabolism, including ethylene production (Burg 1962). In further experiments the effect of growth regulators on chlorophyll retention was examined with detached young leaves allowed to senesce in ventilated systems to prevent the accumulation of ethylene in ambient atmosphere. The rate of ethylene production by young untreated leaves, which was measured 2 days after detachment, was approximately 1 $\mu l\ kg^{-1}\ h^{-1}$. This rate of production is one fifth of that which was produced by leaf discs excised from similar leaves (Fig. 2). Pretreatments by dipping the young leaves in solutions of ABA and IAA immediately after detachment, have only a slight effect on the rates of ethylene production as well as on the level of chlorophyll (Table 1). Treatments with ABA and IAA by dipping followed by an 18-h petiole feeding with the hormone solutions, caused an increase in both ethylene production and chlorophyll loss. Cathey (1968) and Milborrow (1974) have already shown that the effectiveness of exogenous ABA is relatively low in undamaged tissues, unless applied continuously at high concentrations. Application with kinetin effectively blocked chlorophyll degradation in spite of increased ethylene production. This treatment was as effective as the GA_3 treatment, which did not stimulate ethylene production.

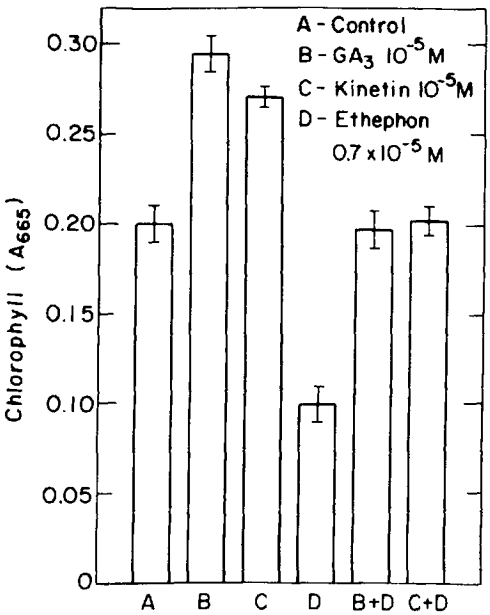

Fig. 3. Effect of ethephon on the senescence-retarding effect of GA_3 and kinetin in lettuce leaf discs. Leaf discs were excised from mature leaves (which were harvested from 18-week-old plants) and were incubated in petri dishes for 4 days. Vertical lines represent the SE.

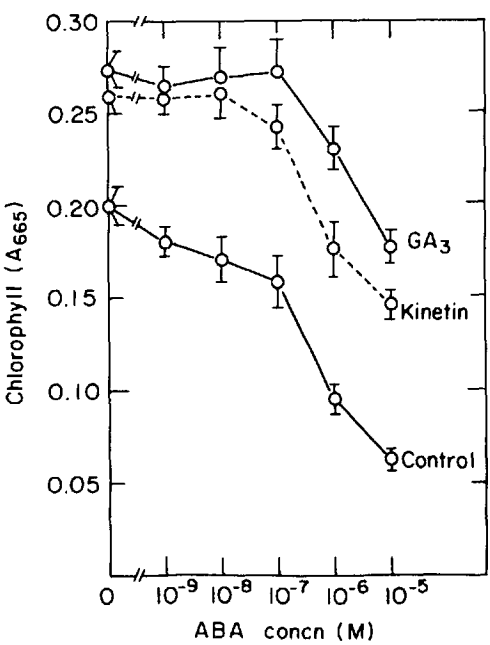

Fig. 4. Effect of increasing concentrations of ABA on the senescence-retarding effect of GA_3 and kinetin in lettuce leaf discs. Leaf discs were excised from mature leaves (which were harvested from 18-week-old plants) and incubated in petri dishes for 4 days. Concentration of both GA_3 and kinetin was 10^{-5} M. Vertical lines represent the SE.

Table 1. Effect of GA_3, kinetin, IAA, and ABA on ethylene production and chlorophyll retention by detached young expanding leaves of lettuce.

Treatment	Concentration (mg L^{-1})	C_2H_4 production ($\mu l\ kg^{-1}\ h^{-1}$)		Chlorophyll (A_{665})	
		Exp. 1	Exp. 2	Exp. 1	Exp. 2
H_2O		0.96 ± 0.02	1.08 ± 0.09	0.47 ± 0.03	0.42 ± 0.03
IAA	10	1.09 ± 0.12	2.48 ± 0.20	0.50 ± 0.04	0.35 ± 0.05
GA_3	10	0.80 ± 0.05	0.79 ± 0.04	0.55 ± 0.03	0.54 ± 0.03
Kinetin	5	1.99 ± 0.26	3.41 ± 0.21	0.56 ± 0.06	0.55 ± 0.05
ABA	10	1.07 ± 0.07	1.28 ± 0.14	0.45 ± 0.05	0.30 ± 0.04

Leaves were harvested from 9-week-old plants. Hormones were applied by a 30-min leaf immersion (Experiment 1) or by immersion followed by continuous petiole feeding for 18 h (Experiment 2). Rate of ethylene production was determined on the second day of incubation. The chlorophyll level was determined after 4 days of incubation.

References

Abeles FB (1972) Biosynthesis and mechanism of action of ethylene. Annu Rev Plant Physiol 23:259–292

Abeles FB, Rubinstein B (1964) Regulation of ethylene evolution and leaf abscission by auxin. Plant Physiol 39:963–969

Aharoni N (1978) Relationship between leaf water status and endogenous ethylene in detached leaves. Plant Physiol 61:658–662

Aharoni N, Anderson JD, Lieberman M (1979) Production and action of ethylene in senescing leaf discs: effect of indoleacetic acid, kinetin, silver ion, and carbon dioxide. Plant Physiol 64:805–809

Aharoni N, Back A, Ben-Yehoshua S, Richmond AE (1975) Exogenous gibberellic acid and the cytokinin isopentenyladenine retardants of senescence in romaine lettuce. J Am Soc Hortic Sci 100:4–6

Aharoni N, Lieberman M (1979) Ethylene as a regulator of senescence in tobacco leaf discs. Plant Physiol 64:801–804

Aharoni N, Richmond AE (1978) Endogenous gibberellin and abscisic acid content as related to senescence of detached lettuce leaves. Plant Physiol 62:224–228

Aspinall D, Paleg LG, Addicott FT (1967) Abscision II and some hormone-regulated plant responses. Aust J Biol Sci 20:869–882

Back A, Richmond AE (1971) Interrelations between gibberellic acid, cytokinins and abscisic acid in retarding leaf senescence. Physiol Plant 24:76–79

Beevers L (1968) Growth regulator control of senescence in leaf discs of nasturium (*Tropaeolum majus*). In: Wightman F, Setterfield G (eds) Biochemistry and Physiology of Plant Growth Substances. Runge Press, Ottawa, pp 1417–1435

Burg SP (1962) The physiology of ethylene formation. Annu Rev Plant Physiol 13:265–302

Burg SP (1968) Ethylene, plant senescence and abscission. Plant Physiol 43:1503–1511

Burg SP, Burg EA (1968) Ethylene formation in pea seedlings: its relation to inhibition of bud growth caused by indole-3-acetic acid. Plant Physiol 43:1069–1074

Cathey HM (1968) Response of some ornamental plants to synthetic ABA. Proc Am Soc Hortic Sci 93:693–698

Chin TY, Beevers L (1970) Changes in endogenous growth regulators in nasturtium leaves during senescence. Planta 92:178–188

Dennis DT, Stubbs M, Coultate TP (1967) The inhibition of brussel sprout leaf senescence by kinins. Can J Bot 45:1019–1024

Hall WC, Truchelut GB, Leinweber CL, Herrero FA (1957) Ethylene production by the cotton plant and its effect under experimental and field conditions. Physiol Plant 10:306–317

Kader AA (1985) Ethylene-induced senescence and physiological disorders in harvested horticultural crops. HortScience 20:54–56

Lieberman M (1979) Biosynthesis and action of ethylene. Annu Rev Plan Physiol 30:533–591

Manos PJ, Goldthwaite J (1975) A kinetic analysis of the effects of gibberellic acid, zeatin, and abscisic acid on leaf tissue senescence in *Rumex*. Plant Physiol 55:192–198

Mattoo AK, Aharoni N (1988) Ethylene and plant senescence. In: Noodén LD, Leopold AC (eds) Senescence and Aging in Plants. Academic Press, San Diego, pp 241–280

McAfee JA, Morgan PW (1971) Rates of production and internal levels of ethylene in the vegetative cotton plant. Plant Cell Physiol 12:839–847

Milborrow BV (1974) The chemistry and physiology of abscisic acid. Annu Rev Plant Physiol 25:259–307

Moran R, Porath D (1980) Chlorophyll determination in intact tissue using N,N-dimethylformamide. Plant Physiol 65:478–479

Morgan PW, Hall WC (1964) Accelerated release of ethylene by cotton following application of indole-3-acetic acid. Nature 201:99

Noodén LD (1988) Abscisic acid, auxin and other regulators of senescence. In: Noodén LD, Leopold AC (eds) Senescence and Aging in Plants. Academic Press, San Diego, pp 329–355

Noodén LD, Leopold AC (1978) Phytohormones and the endogenous regulation of senescence and abscission. In: Lathem DS, Goodwin PB, Higgins TJV (eds) Phytohormones and Related Compounds: A Comprehensive Treatise. Vol. 2. Elsevier/North-Holland, Amsterdam, pp 329–369

Osborne DJ (1968) Ethylene as a plant hormone. In: Plant Growth Regulators. Monograph No. 31. Soc Chem Ind, London, pp 236–249

Van Staden J, Cook EL, Noodén LD (1988) Cytokinins and senescence. In: Noodén LD, Leopold AC (eds) Senescence and Aging in Plants. Academic Press, San Diego, pp 281–328

Yang SF, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol 35:155–189