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A Review of the Research on the Mechanism of Ethylene in Plant Leaf Senescence

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Abstract: ***Leaf senescence represents the final stage of plant development, involving nutrient redistribution and cellular degradation. Ethylene, a gaseous hormone, plays a pivotal role in regulating this process. Despite extensive research, gaps persist in understanding ethylene's contribution to leaf senescence comprehensively. This review synthesizes existing literature to elucidate ethylene's signalling cascade, interaction with other hormones, and response to environmental stress. Key findings highlight the nuanced relationship between ethylene, leaf age, and environmental factors, emphasising the need for further investigation to optimize plant health and agricultural productivity.***

Keywords: leaf senescence, plant development, ethylene, phytohormone, signal transduction, environmental stress, age-related changes

1. Introduction

Senescence is regarded as being the last step in the developmental process of a plant or part of a plant. This process is not limited to death, however, but is rather a more complex process that involves the aggregation, followed by the distribution, of existing nutrients to the other parts of the plant as a mechanism to optimise fitness, ultimately concluding with cellular death. Leaf senescence, and its importance, can be seen with the changing of the seasons. As deciduous trees experience the transition from summer to autumn to winter, they must make adequate preparations before their leaves yellow and fall. The example of leaf senescence is particularly of interest as it is usually compared to the process of ageing. Still, with situations involving plants, both annual and perennial, they may begin to senesce due to a level of environmental stress instead, such as dropping temperatures for the scenario above or droughts, infections, and weather, as well as other internal factors (Gill et al., 2015).

This explains the fact that endogenous factors or exogenous factors do not solely regulate the initiation of senescence, but instead, it is often by a combination of the two. Numerous phytohormones, both positive and negative regulators, alongside senescence-associated genes (SAGs), are responsible for controlling when a plant senesces (Jing et al., 2005). While these components collectively control the timing of senescence, one major inducer stands out: ethylene, due to the intricacies in its signal transduction pathway that result in extensive amplification by the release of this one versatile hormone.

However, despite the abundance of studies addressing ethylene's role in leaf senescence, gaps still remain. Existing research has well-explored the details of ethylene signalling, but a comprehensive synthesis that simultaneously compares the general variation with plant species and leaf age, impact of environmental stress, and evaluates potential for future research on several facets is lacking. Hence, a clear question emerges: **How does ethylene contribute to leaf senescence?** This essay aims to amalgamate the countless studies done on this topic, shedding light on the variations and commonalities present regarding the aspects impacting and impacted by ethylene-induced senescence and contribute to a holistic understanding of the role of ethylene in the elaborate process of leaf senescence.

2. Methodology

In order to assimilate the extensive existing knowledge from scientific literature and generate appropriate insights into the mechanisms regulating ethylene-induced senescence in leaves, the key databases from which the research papers and scientific journals were sourced include but are not limited to ScienceDirect, PubMed, and PNAS. These were chosen for their reputed scientific accuracy and the range of subtopics covered due to the interdisciplinary nature of this topic, predominantly focused on the overlap of biochemistry and plant biology and physiology. The studies reviewed were chosen based on their relevance to the research question, with criteria for inclusion including conducting or citing experiments with ethylene manipulation, studying the specifics on organelles, proteins, and ligands among other elements involved in cellular communication applicable to ethylene signalling cascades, as well as one particularly chosen other phytohormone used as a comparison point, and investigating the effects on plant physiology as a result of ethylene-induced senescence. Any other pertinent research or review article adding additional details to the relationship between ethylene and leaf senescence was thoroughly evaluated by checking past citation analytics and similarities in keywords before inclusion.

A variety of plant species were considered to delineate both the consistencies and variations in the role of ethylene, but otherwise, studies on *Arabidopsis thaliana* were prioritised due to its widespread use as a genetic plant model (Chang, 2016) and to maximise the justification for comparability between papers. As the scope of research on ethylene and leaf senescence continually evolves, a challenge encountered during the secondary research process was determining the validity of dated studies. This was mitigated by focusing on the overlap between the information in these studies and melding it with contemporary knowledge from more recent studies, ensuring not to misinterpret or manipulate the data to support a subconscious hypothesis.

3. Literature Review and Discussion

Ethylene-Induced Senescence Signalling Cascade

The hydrocarbon ethylene (C_2H_4) is both simple and gaseous—two features that prove to be of the utmost importance in initiating senescence. The fact that ethylene is gaseous makes it different from its counterparts, one of which are abscisic acids, as ethylene can easily diffuse from membrane to membrane. Since it is also hydrophobic and small (i.e. simple), transport proteins are not required to facilitate the transport of this hormone to target cells (Chang, 2016).

The synthesis of ethylene is at its peak when the leaves first start growing; then, the rate decreases once the leaf has fully developed, and the rate of ethylene production increases once again before the initiation of senescence, indicating early on that ethylene must play a role in first few stages of leaf development as well, which is to be further discussed in this review. Ethylene synthesis depends on what stage of development each leaf's tissues are individually at. This dependency highlights that not only is the release of ethylene dependent on the internal and external factors that cause a leaf to reach its final stage of development, but the production of ethylene similarly occurs in a localised manner to ensure that the outcome of the process is relative to each leaf's conditions. Therefore, the release of ethylene is often dependent on the sensitivity of each of these tissues. The signalling cascade responsible for ethylene production, illustrated diagrammatically in Fig. 1, starts with methionine being converted to S-adenosyl methionine (SAM) by SAM synthetase and SAM then being converted to the cyclic amino acid 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase, with a byproduct of S-adenosyl methanethiol. By ACC oxidase, ACC is oxidised to ethylene, hydrogen cyanide, and carbon dioxide, and methanethiol is converted back into methionine through the Yang cycle (Hegelund et al., 2017). From this, it can be deduced that this is a regenerative process, replenishing the methionine levels available for further ethylene synthesis if required, and if so, ensuring continuous and rapid ethylene synthesis throughout the first few stages of senescence.

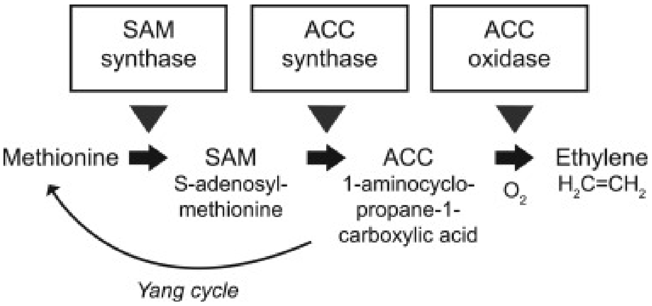


Figure 1: A "schematic overview of ethylene biosynthesis" (Müller et al., 2017)

Once ethylene has been synthesised, multiple pathways may arise depending on the presence, and if so, the severity of external pressures, as well as internal circumstances. For instance, during the beginning of its growth, a hypothetical young leaf was able to optimise its rates of photosynthesis and protein synthesis due to being located high up on the tall stem of the plant, with access to both an abundance of light energy and carbon dioxide and to nitrogen from the plant to which it belongs' extensive root system. Once the leaf in question had matured, it was able to continue to contribute to the high production of glucose and proteins even more so as it was now well-developed and the protein turnover was low. In theory, if this leaf presumably faced minimal external pressure from the environment and began to senesce solely due to nature running its course, perhaps explained by an overwhelming increase in the protein turnover levels as the leaf must regenerate its tissues eventually, the release of ethylene would now be stimulated. This situation illustrates the idea that endogenous factors alone can be enough to regulate the onset of leaf senescence.

Before the ethylene signal transduction pathway is taken to initiate senescence, the negative regulator protein CTR1 (constitutive triple response 1) remains bound to the ETR1 (ethylene response 1) receptor, located in the rough endoplasmic reticulum membrane. Since CTR1 phosphorylates a carboxyl-terminus when bound to ETR1, the activation of the EIN2 (ethylene-insensitive 2) enzyme, a positive regulator of senescence, is prevented. This presenescent interaction emphasises the regulatory role of CTR1 in controlling the initiation of senescence. Once the release of the ligand ethylene has been stimulated, it diffuses to bind to ETR1 receptors (Park et al., 2023). A copper cofactor allows for its high affinity binding, thus indicating that low concentrations of ethylene (as low as 0.2 nl l^{-1} in *Arabidopsis*) are enough to initiate leaf senescence (Azhar et al., 2023) and highlighting the sensitivity of ETR1 receptors to prompt a rapid response. As a result, CTR1 is freed from ETR1, resulting in the carboxyl-terminus' phosphorylation being halted and EIN2 being able to break down the carboxyl-terminus to the shorter amino acid chain CEND, which travels through the nuclear pores to stabilise EIN3 (ethylene-insensitive 3) in the nucleus (Xing et al., 2012). EIN3, being a transcription factor, causes numerous SAGs to leave the nucleus as mRNA, with key SAGs including chlorophyll catabolic genes that lead to the synthesis of enzymes that break down the chlorophyll present in the cells of the senescing leaf (Yang et al., 2022). This is one of the first outcomes of the ethylene signalling cascade, signifying the importance of the early relocation of starch molecules stored in the stroma of chloroplasts, concentrated in leaves' palisade mesophyll tissues.

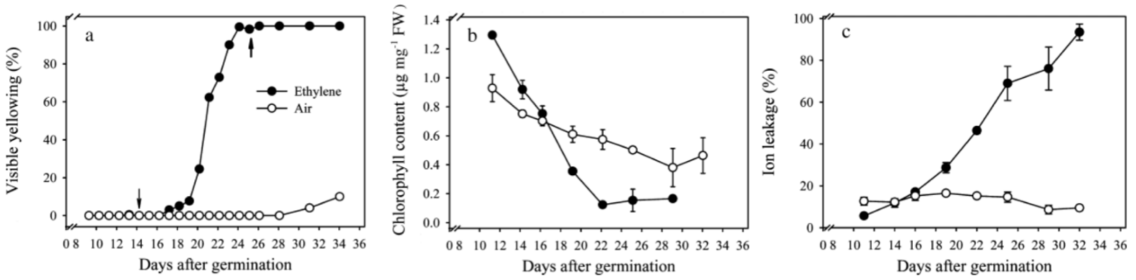
The breakdown of chloroplasts has proven to have a crucial advantage in preserving evolutionary fitness, as this allows the lipids and especially proteins forming them to be reprocessed, providing a plentiful source of nitrogen. This is followed by the breakdown of nuclei and mitochondria (Schippers et al., 2015), allowing the leaf to carry out respiratory functions and control gene expression before it is finally followed by the leaf beginning its degradation, allowing these assimilated nutrients to accumulate in other regions of the plant. These regions can range from younger leaves or new buds to aid them in their growth to bark and phloem tissues in perennial deciduous trees (Guo et al., 2021). The reutilisation of existing nutrients stresses the overall impact of ethylene signalling on resource allocation within the plant as this facilitates growth in specific regions that allow for the plant’s sustained development and ultimate fitness.

The Relationship Between Ethylene, Age, and Environmental Stress

The aforementioned signalling cascade focuses on the role of ethylene as a promoter of senescence. It must be noted that ethylene can also promote leaf growth instead, depending on the concentration of the hormone, relative timing of release, and the species of plant. As an example, the (2005) and (2008) Khan et al. investigations involved spraying ethephon, a compound that releases ethylene, on mustard (*Brassica juncea L.*) leaves. At low concentrations of ethephon, the surface area of the leaves increased and further ethylene synthesis was induced to promote this growth, whereas signs of senescence were only observed in the leaves when concentrations were high. For this reason, a general, proportional relationship between ethylene concentration and senescence rate cannot be established due to the number of circumstances needing to be taken into consideration. It is not out of the question that a graph with axes representing the senescence rate by ethylene concentration is linear, and it is undoubtedly incorrect to assume that there is no correlation between ethylene concentration and senescence rate. However, due to the presence of an optimum value of ethylene concentration and drastic variation between leaves’ external and internal circumstances of a particular plant, let alone a particular species, the relationship between ethylene *concentration* and the senescence induced by it is deeply complex and cannot be done justice with a graph or being described as positively or negatively correlated with the existing capacity for research.

These studies were carried out to illustrate the variability in ethylene’s function as both a senescence inhibitor and promoter, but they can also serve as evidence that the exogenous treatment of ethylene is enough to induce senescence, regardless of the environmental stress. Despite this, it would be incorrect to deduce from this information that the age of the plant can be disregarded; ethylene-induced senescence is dependent on age-related changes (ARCs) of individual leaves (Jing et al., 2005). Even if the plant is not ‘ageing’, though, if the plant is above a certain developmental age, senescence may be induced by exogenous ethylene application. This is evident in the (2002) Jing et al. investigation, where *Arabidopsis* cotyledons’ starting age in days was recorded and they were then consistently treated with ethylene for three days.

Fig. 2: “The effect of ethylene on visible yellowing [graph **a**], chlorophyll content [graph **b**] and ion leakage [graph **c**] of *Arabidopsis* cotyledons... [that] were grown either continuously in air for the indicated days, or first in air until 3 days before the indicated days and then exposed to 10 $\mu l \, l^{-1}$ ethylene. ...The visible yellowing was observed on at least 100 plants for each data point and expressed as the percentage of yellow cotyledons versus the total number of cotyledons. Results for chlorophyll content and ion leakage were shown as mean ±SE of three replicates.” (Jing et al., 2002).



As observed by graph **a** in Fig. 2, only the ethylene-treated plants older than 24 days yellowed significantly (over 95%), while plants younger than 17 days showed no signs of yellowing, the hallmark of leaf senescence. To ascertain whether the impact of exogenous ethylene on inducing senescence was intrinsically linked to leaf age, the research extended beyond the visible indicators, incorporating measures of chlorophyll content and ion leakage as indicators of physiological changes. Referring to graph **b** in Fig. 2, a decline in chlorophyll content coincided with the progression of external yellowing, which can be compared to the earlier finding regarding the stimulation of ethylene release ultimately and first resulting in the catabolism of chlorophyll (Jing et al., 2002). Thus, the presence of ethylene, whether endogenous or exogenous, in leaves above a certain age, has the loss of chlorophyll as an early consequence. This deduction demonstrates that although the function of ethylene can vary drastically depending on several factors, a relationship between the presence of ethylene and leaf age can be established, given that the species of plant, the concentration of ethylene, and the conditions of each leaf are kept constant as in this investigation and other similar studies are conducted in laboratory settings.

Such settings are not achievable in the plants’ natural environment and such experiments are more challenging to conduct in these environments due to the variable soil conditions, unpredictable weather, and the plants’ involvement with the rest of the ecosystem. Despite this, conducting an investigation in the environment, or more likely a controlled version of actual environmental circumstances, may lead to resolving gaps present in existing research to determine to which extent environmental stress is an accelerator of leaf senescence and whether age is still the overriding factor affecting the process. Investigations on stress-induced senescence should aim to cover an array of circumstances separately, with suggestions including nutrient-deficiency-induced, severe-rainfall-induced, and pathogen-induced, focusing on the signalling cascades and responses by the plant of a particular species, recommending *Arabidopsis thaliana* as per the methodology section. Examining signalling cascades should prioritise the identification of ethylene involvement as the responses that occur to combating the stress the plant is facing should not be confused with the responses that are in an attempt to initiate senescence, which is the point of focus, especially as yellowing can be a sign of ethylene-induced senescence from ARCs alone and a sign of potassium deficiency, for example. The presence of severe environmental stress, particularly kinds where the photosynthetic rate drops below a sustainable threshold, should induce senescence earlier than it would for a plant with optimal settings for growth up until the point of ethylene application as per the existing information, so this can be used to form a hypothesis to be tested in future investigations.

According to graph **a** from Fig. 2, yellowing began around 31 days for the control cotyledons not exposed to ethylene exogenously. The yellowing around this time until the measured 34 days was below approximately 15% (Jing et al., 2002). Although two plot points are not satisfactory to use for extrapolation, it can be assumed that senescence-associated signs would be seen weeks after this; hence, ethylene is not only an initiator, but also an accelerator of senescence, allowing a leaf to begin degradation and assimilate nutrients elsewhere in the plant before premature defoliation can occur.

This comparison shows the connection between the visual manifestation of senescence and underlying physiological changes, but the leakage of ions can also be observed in graph **c** of Fig. 2, with there being a substantial increase in ion leakage following the breakdown of chlorophyll, indicating that the plasma membrane of senescing leaf cells breaks down soon after. Ion leakage should precede visible yellowing, however, as shown in graph **c** with the ion leakage percentage of ethylene-treated leaves being higher than the average leakage level, deduced from the plot points of leaves not treated with ethylene, starting from the age of 19 days (Jing et al., 2002). Thus, these findings present the sequence of significant outcomes of the ethylene-induced senescence signalling cascade in leaves as chlorophyll breakdown followed by cellular membrane damage, with yellowing occurring subsequently.

Regulation of Senescence

Ethylene alone cannot fully regulate the intricate process that is senescence. A combination of hormones results in the intertwining of signalling cascades that, with evolution, has resulted in a finely-tuned cellular communication operation. Although senescence relies heavily on external factors to allow the efficient reallocation of nutrients to take place, a plant and its leaves cannot merely senesce each time it is faced with environmental changes that may impact its physiology and photosynthetic rate.

Abscisic Acid (ABA) is involved in the response specific to drought, closing the stomata of mature leaves to maintain water potential. ABA and ethylene are inhibitory to each other, so once the release of ABA has been stimulated, it results in the inhibition of ethylene synthesis. Therefore, chlorophyll loss has been delayed so that during a time as dire as drought, the photosynthetic output can be maximised. Combined with the preservation of water, the odds of the plant in question's survival will have increased, reducing the likelihood of the leaf needing to senesce. Further elaborating on the irregular nature of ethylene, deepwater rice (*Oryza sativa*) seedlings that have adapted to being submerged in water and surviving with low concentrations of oxygen with evolution experience a delay in senescence and chlorophyll catabolism upon the release of ethylene. Ethylene instead acts as an inhibitor of senescence with this species of plant to promote the growth of shoots underwater and increase the plant's capability of tolerating flooding in extreme cases, further emphasising the heterogeneous properties of ethylene (Jakubowicz & Nowak, 2010).

Nonetheless, ethylene is not the only multifaceted hormone; ABA can often act as an inducer of senescence, with experiments comparable to the (2002) Jing et al. investigation and (2005) and (2008) Khan et al. investigations among others showing similar results for ABA as they did with ethylene. For instance, the (2003) Yang et al. study showed that ABA is released at the beginning of senescence in leaves and, predictably, the exogenous treatment of ABA results in speeding up the process. As for how ethylene and ABA differ, given that both can act as positive regulators and negative regulators of senescence, this is dependent on the situation and species of the plant. It is apparent that the two always act antagonistically to each other, but it is still unknown whether the signal transduction of these two ligands ever consolidates or acts side-by-side (Cheng et al., 2009), thus indicating potential for future research. Since ethylene and ABA are both capable of inducing growth in a leaf and vice versa, investigations that analyse both of their signalling cascades where each acts as an inducer of growth, inhibitor of growth, inducer of senescence, and inhibitor of senescence and which acts as the positive regulator and which as the negative in whichever developmental process is being considered can delineate if and under which circumstances a convergence point arises. This has the benefit of enhancing the contextual understanding of senescence regulation to lead to a more comprehensive model that can be applied to agricultural settings to optimise crop yield, manage plant growth, and enhance stress tolerance. Moreover, identifying such points could open avenues for targeted interventions, genetic modifications, or specific hormone applications to regulate plant development as needed for situations like drought and flooding to optimise environmental conditions and prevent premature senescence when it is not necessary.

4. Conclusion

It is evident that ethylene contributes greatly to leaf senescence. As for how, this depends on the multiple factors this essay delved into. However, it can be concluded that ethylene is an activator of senescence in leaves once the leaves have lived past a certain age or have experienced an extreme level of environmental pressure, rapidly degrading cellular contents, salvaging the nutrients that can be repurposed within the plant, and leading to the yellowing of leaves. Ethylene contributes to senescence in an accelerating manner when leaves are treated exogenously with it, still iterating that the leaves must be over a specific age. Such an emphasis on age is placed due to both the dependence on ARCs of individual leaves and the fact that ethylene may function as a senescence inhibitor and promote leaf growth instead in plants of particular species, leaves below the age threshold, or concentrations of ethylene that are either too high or too low. This serves as a reminder of the nuances that exist to tightly regulate senescence, and why these nuances exist. Although the outcome is largely positive with the conservation of nutrients, helping younger parts of the plant accelerate their growth and leading to a cycle of increased nutrient provision to sinks within the plant, avoiding the premature senescence of leaves that have gone through environmental stress but are not old enough to be considered "ageing" should be a plant's regulatory system's priority. As detailed by this essay, initiating and carrying out senescence involves extensive signal transduction, which takes more energy than it is worth for a leaf whose purpose is largely to photosynthesise, synthesise proteins, and control water potential, making it an asset to the plant more than a burden. Premature senescence undermines the ultimate goal of an organism: to survive; hence, having research that investigates the inhibitors of senescence is as equally as important as the promoters, as well as understanding the circumstances under which ethylene acts as a promoter and as an inhibitor, to optimise, on an agricultural and environmental scale, leaf and overall plant health.

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