

Plant Senescence: The Role of Volatile Terpene Compounds (VTCs)

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Abstract

Senescence is a natural, energy-dependent, physiological, developmental and an ecological process that is controlled by the plant's own genetic program, allowing maximum recovery of nutrients from older organs for the survival of the plant, as such; it is classified as essential component of the growth and development of plants. In some cases, under one or many environmental stresses, senescence is triggered in plants. Despite many studies in the area, less consideration has been given to plant secondary metabolites, especially the role of VTCs on plant senescence. This review seeks to capture the biosynthesis and signal transduction of VTCs, the physiology of VTCs in plant development and how that is linked to some phytohormones to induce senescence. Much progress has been made in the elucidation of metabolic pathways leading to the biosynthesis of VTCs. In addition to the classical cytosolic mevalonic acid (MVA) pathway from acetyl-CoA, the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, originating from glyceraldehyde-3-phosphate (GAP) and pyruvate, leads to the biosynthesis of isoprenoid precursors, isopentenyl diphosphate and dimethyl allyl diphosphate. VTCs synthesis and emission are believed to be tightly regulated by photosynthetic carbon supply into MEP pathway. Thus, under abiotic stresses such as drought, high salinity, high and low temperature, and low CO₂ that directly affect stomatal conductance and ultimately biochemical limitation to photosynthesis, there has been observed induction of VTC synthesis and emissions, reflecting the elicitation of MEP pathway. This reveals the possibility of important function(s) of VTCs in plant defense against stress by mobilizing resources from components of plants and therefore, senescence. Our current understanding of the relationship between environmental responses and senescence mostly comes from the study of senescence response to phytohormones such as abscisic acid, jasmonic acid, ethylene and salicylic acid, which are extensively involved in response to various abiotic and biotic stresses. These stresses affect synthesis

and/or signaling pathways of phytohormones to eventually trigger expression of stress-responsive genes, which in turn appears to affect leaf senescence. Comparison of plant response to stresses in relation to patterns of VTCs and phytohormones biosynthesis indicates a considerable crosstalk between these metabolic processes and their signal to plant senescence.

Keywords

Senescence, Abscission, Volatile Terpene Compounds, Phytohormone, Plant, Stress, Plant Secondary Metabolites

1. Introduction

Plant senescence is deemed as a complex, highly regulated, developmental phase in the life of a plant with a consequence of a coordinated degradation of macromolecules and a subsequent benefit of component mobilization from other parts of the plant [1]. In studying leaves, needles and other plant organs, senescence has been known to occur in normal development and usually comprises of the cessation of photosynthesis, disintegration of chloroplasts, breakdown of leaf proteins, loss of chlorophyll, and removal of amino acids in those plant parts [2]. Rapid expansion of the organ, integration of nitrogen and carbon, and the synthesis of protein constitute the initial phase of the true-life span of a plant organ and it allows the organ to reach its maximum photosynthetic potential [1]. It becomes important for the plant to initiate the next phase of development once the organ is at its maximum photosynthetic potential since the organ at this stage is beneficial for the plant. At this phase, there is high carbon accumulation and consistently low protein turnover. From this point, both internal and external conditions can initiate senescence, where there is a massive relocation of carbon, nitrogen, and minerals to other developing parts of the plant [1]. In plant leaves, the common physical indicators of senescence most often occur in a much later stage than the actual onset, but are usually characterized when the mesophyll tissue begins to lose its greenness and turn to yellow or red. The color change is due to both preferential degradation of chlorophyll compared to carotenoids and synthesis of new compounds, such as anthocyanins and phenolics [3], and then the ultimate consequence of senescence, which may or may not result in organ abscission [2].

In addition to the programmed type of senescence, the degradation of macromolecules and mobilization of cellular component from leaves can also occur in response to external environmental stresses. Unlike animals that can avoid harsh environmental conditions by movement, plants must respond rapidly to deteriorating environmental conditions. Common among plants, they respond by the removal of the parts of the plant that are not essential. A diseased leaf will senesce, die and drop off the plant, thus helping to prevent the spread of disease and allowing the rest of the plant to continue in its development [1]. Nitrogen

deficiency, light limitation, and drought stress will initiate the onset of senescence, which will result in an early seed development or reduce photosynthetic requirements, allowing a plant to survive throughout the stressful period [4]. However, most plants under a particular environmental stress are able to reverse the senescence process up to a point when favorable environmental conditions are attained [5]. This is a major difference between environmentally caused and natural programmed senescence.

For many years, the role of VTCs in plant senescence has been debated, however in recent years several major roles of VTCs in plant development and survival have been discussed. Not only does VTCs serve as a feeding deterrent to insects and some herbivores [6], it is now well accepted that VTCs play a major role in plant senescence by keeping the plant healthy and also protecting it against environmental stresses that are known to cause plant death [7]. They are known to be synthesized by two pathways in the cytosol, endoplasmic reticulum, peroxisomes and plastids, and stored in glandular cells of leaves and resin ducts of needles [8]. VTC synthesis and plant senescence have been tied to photosynthesis of a plant since photosynthesis is reported to serve as a carbon source in initiating VTC biosynthesis [9]. However, there have been speculations of alternative carbon sources such as xylem and chloroplast in studies where plants showed reduced rates of photosynthesis but increased in VTC biosynthesis [10] [11]. Membrane destruction and cell deaths leading to organ senescence as a result of exposure of plants such as *Arabidopsis thaliana* to citral, peppermint, β -pinene, α -pinene, and camphene have confirmed the role of VTCs in plant senescence [12] [13] [14]. Postharvest studies have also shown that after trees such as balsam fir are cut, excessive synthesis and/or emission of VTCs such as β -pinene, β -Terpinene, Camphene and 3-Carene are induced prior to needle abscission [15].

Although the emission of plant VTCs are speculated to be dependent on both ethylene and jasmonic acid [16], especially when plants are under stress, little is known about the effect of VTCs on the synthesis of these phytohormones and *vice versa*, and how that relationship plays a role plant senescence. Despite all intensive research efforts, the role of VTCs in plant senescence is still not clearly understood. VTC synthesis and signaling pathways, its physiological role in senescence and the crosstalk between VTCs, phytohormones and plant senescence are discussed below.

2. Secondary Metabolites

Unlike plant primary metabolites such as chlorophyll, amino acids, nucleotides, simple carbohydrates and membrane lipids, the secondary metabolites are made up of a diverse array of organic compounds that differ in distribution and had earlier on appeared to have no direct function in plant growth and development (Taiz and Zeiger, 1998). For many years, the adaptive significance of most plant secondary metabolites was a mystery, until more recently, when it was suggested

that they help to protect plants against herbivores, infections by microbial pathogens and mechanical damage such as wounding [17] [18]. Secondary metabolites are made up of three distinct groups: Phenolics (aromatic substances), nitrogen-containing compounds (alkaloids), and terpenes [19]. In recent years, a lot of focus has been placed on the biosynthesis [17] [19] [20] and physiological role of volatile terpene compounds [6] [21] [22] because of its reported role in plant protection and development.

3. Biosynthesis and Distribution of VTCs

All terpenes are derived from the common precursor isopentenyl diphosphate (IPP), which are synthesized from primary metabolites through two different pathways: mevalonic acid (MVA) and 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways [19]. With the exception of plants, all other organisms such as bacteria [23], yeast [24] and animals [25] use only one of these pathways. Well studied in plants, the cytosolic mevalonic acid (MVA) pathway uses three molecules of acetyl CoA that are joined together stepwise to form mevalonic acid (Figure 1(a)) [17]. The key six-carbon intermediate is then phosphorylated, decarboxylated, and dehydrated to yield isopentenyl pyrophosphate (IPP) [20] (Figure 1(a)). The methylerythritol (MEP) pathway, also referred to as 1-deoxy-D-xylulose 5-phosphate (DXP) pathway uses the plastid-localized route to produce IPP. Pyruvate reacts with thiamine pyrophosphate (TPP) to yield a two-carbon fragment, hydroxyethyl-TPP, which condenses with glyceraldehyde

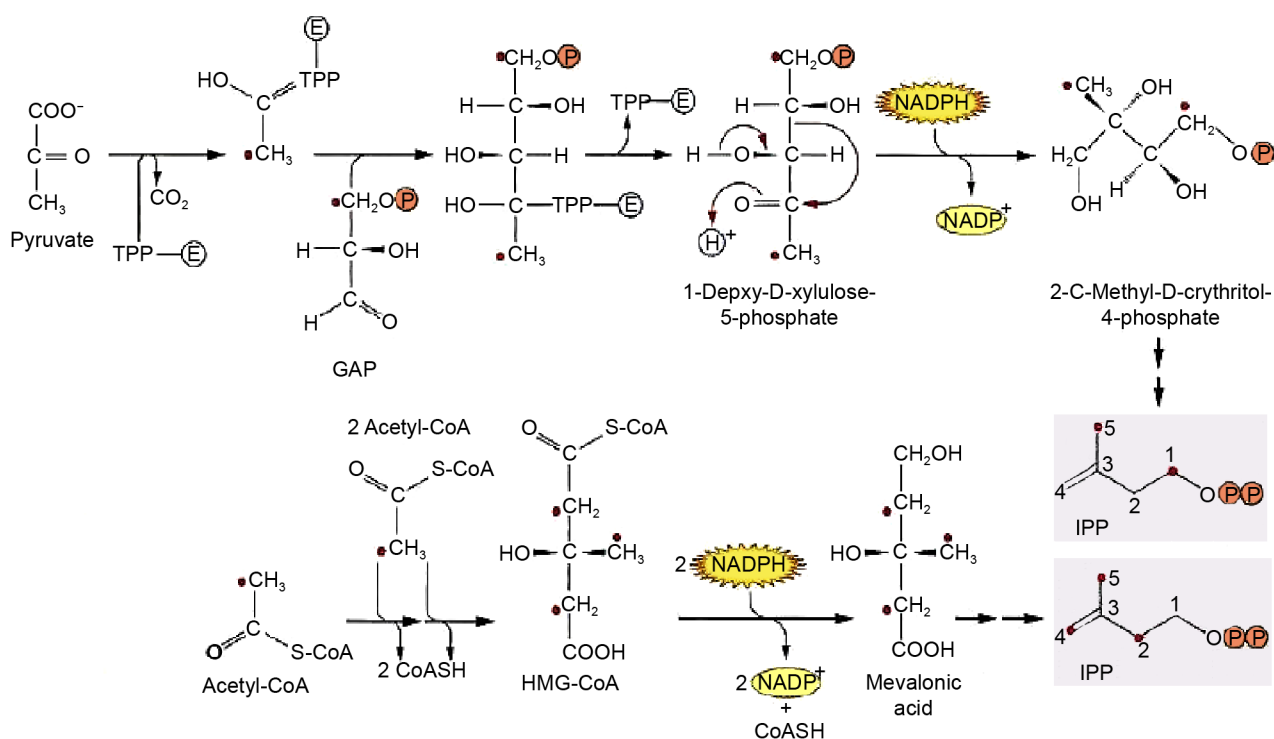


Figure 1. (a) Mevalonic acid (MVA) and (b) 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways for the formation of IPP. Adopted from Buchanan *et al.* (2000).

3-phosphate (GAP). TPP is then released to form a five-carbon intermediate, 1-deoxy-D-xylulose 5-phosphate, which is rearranged and reduced to form 2-C-methyl-D-erythritol 4-phosphate and subsequently transformed to yield IPP (**Figure 1(b)**) [19]. Inhibition of IPP by either mevastatin (inhibitor of IPP from MVA pathway) or fosmidomycin (inhibitor of IPP from MEP pathway) is known to obstruct terpene biosynthesis [26].

IPP and its isomer, dimethylallyl pyrophosphate (DPP), are the activated five-carbon building blocks of terpene biosynthesis that join together to form larger molecules (Figure). First, IPP and DPP react to give geranyl pyrophosphate (GPP), the ten-carbon precursor of all the monoterpenes (two C₅ units). Monoterpenes are best known as components of volatile essence of flowers and essential oils of herbs and spices, which make up as much as 5% of plant dry weight [17]. Common examples are pinene (α and β), nerol, citral, camphor, menthol, limonene and myrcene. Linked to another molecule of IPP, GPP can then give rise to a 15-carbon compound farnesyl pyrophosphate (FPP), the precursor of all the sesquiterpenes (three C₅ units). Like monoterpenes, many sesquiterpenes are found in essential oils and have also been found as phytoalexins, antibiotic compounds produced by plants in response to microbial challenge, and anti-feedants to discourage herbivore with examples as nerolidol and farnesol. Addition of yet another molecule of IPP gives the 20-carbon compound geranylgeranyl pyrophosphate (GGPP), the precursor of the diterpenes (four C₅ units). Examples are phytol₂, a hydrophobic side chain of chlorophyll, gibberellin hormone, and resin acids of conifer and legume species. Furthermore, FPP and GGPP can dimerize to give the triterpenes (C₃₀) and the tetraterpenes (C₄₀), respectively (**Figure 2**) [19].

Over the years, studies have reported the synthesis of hemiterpenes, monoterpenes and diterpenes *via* the MEP pathway while the MVA pathway synthesizes sesquiterpenes. However, most studies have reported the MEP pathway to be a major contributor to the synthesis of sesquiterpenes [27] [28]. In previous years, the possibility of metabolic crosstalk between both biosynthetic routes through IPP had been speculated [29] [30], however in the recent times, gene co-expression networks have revealed that there is no *en bloc* transcriptional regulation of all genes encoding MVA and MEP pathway enzymes. Therefore, VTCs synthesis *via* MVA and MEP pathways may be independently regulated [31].

Synthesis of VTCs in plants occurs at different organelles in the plant cell. Those synthesized through the MVA pathway occurs in the cytosol, endoplasmic reticulum, and the peroxisomes of the plant cell [32] [33]. However, it is reported that the production of sesquiterpenes through the MVA pathway take place in the mitochondria [31] [34]. On the other hand, VTC biosynthesis through the MEP pathway is reported to be in the plastids such as the chloroplast, although the mechanisms for efficient regulation of both pathways are not fully understood.

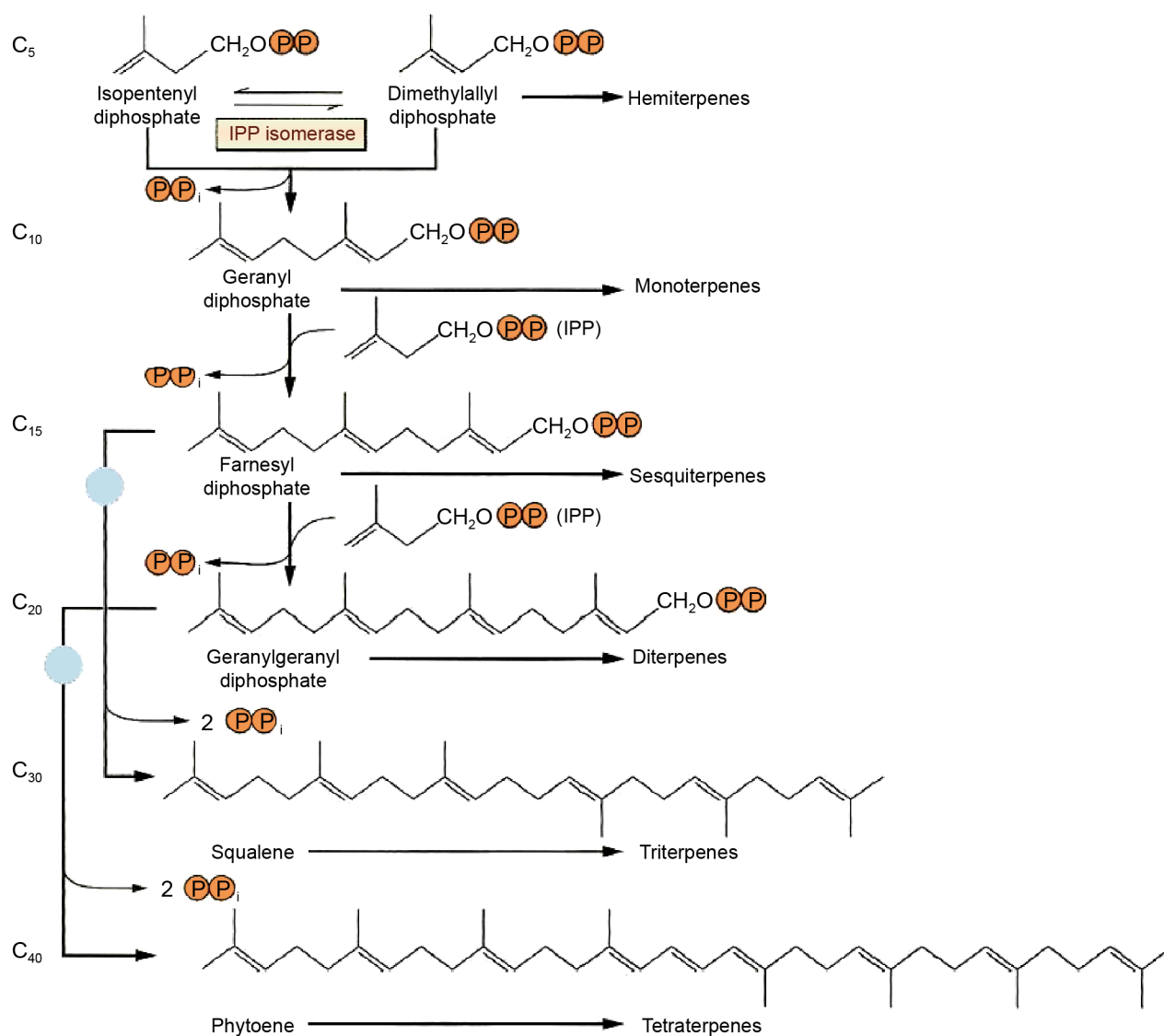


Figure 2. The major subclasses of terpenes. Adopted from Buchanan *et al.* (2000).

In plants, VTCs are stored massively in both internal and external structures such as the glandular cells of leaves and the resin ducts of needles [8]. Nonetheless, direct emission of VTCs from the mesophyll is common in some tree species. For example, in deciduous leaves of tree species such as Fagaceae and Salicaceae with no specific structures for VTCs, emissions originate from mesophylls cells in a light and temperature dependent mode [35] [36]. In conifer needles of Norway spruce, emission comes from photosynthetic tissues and is superposed by a temperature dependent volatilization of terpenes from the resin ducts, while in Lamiaceae leaves release of VTCs is from their external glandular cells [9].

4. Signal Transduction of VTCs

The MVA pathway for the cytosolic and mitochondrial VTCs cannot function

without essential precursors/enzymes. Initial precursor in the MVA pathway is known as acetoacetyl-CoA thiolase (AACT) and also known as acetyl-coenzyme A (CoA) C-acetyltransferase (EC 2.3.1.9) [31]. Acetoacetyl-CoA thiolase is believed to be encoded by a small gene family in plant, however, it functions to catalyze a Claisen-type condensation of two acetyl-CoA units to form acetoacetyl-CoA [37]. This reaction and precursor have been recognized as the first and important step in the classical MVA pathway for the synthesis of VTCs. Acetoacetyl-CoA is converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) using the enzyme HMG synthase (HMGS; EC 2.3.3.10) as a catalyst for the reaction. In most plant species, gene paralogs encode HMG synthase. Also, encoded in several paralogous genes in most plants is the enzyme, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR; EC 1.1.1.34). This enzyme catalyzes the reaction that converts HMG-CoA to mevalonic acid in two reduction steps with each requiring NADPH as the reducing equivalent [31]. This is the first committed step of terpene biosynthesis. In a plant, such as *A. thaliana*, the HMGR genes (*AtHMGR1* and *AtHMGR2*) code for three proteins (*AtHMGR1S*, *AtHMGR1L*, and *AtHMGR2*). It has been reported that these HMGR isoforms in plants are targeted to the endoplasmic reticulum with the same topology in the membrane and therefore, reinforces the theory that mevalonate is synthesized in cytosol [38] [39].

In the next two successive ATP-dependent reactions of converting mevalonic acid to mevalonic acid 5-phosphate (MVAP) and then to mevalonic 5-diphosphate (MVAPP), two enzymes, MVA kinase (MK; EC 2.7.1.36) and phospho-MVA kinase (PMK; EC 2.7.4.2) serve as catalyst to complete the reaction [19] [31]. In most plants, MKs and PMKs are encoded by single genes [31] and reported studies on *Arabidopsis thaliana* have shown that plant MKs are localized to the cytosol while PMKs are in peroxisomes [33] [40]. The last step of IPP biosynthesis through the MVA pathways is an ATP-dependent decarboxylation of MVAP. The enzyme that catalyses this reaction is MVA diphosphate decarboxylase (MPDC; EC 4.1.1.33), which is encoded by a single gene in most plants. Over the years, there has been conflicting information on localization of this enzyme. It has been reported to have an N-terminal peroxisomal targeting sequence, PTS2 [32] [33] but recent mass spectrometry reports have suggested a cytosolic localization [31].

The MEP pathway, which starts with the condensation of thiamin and D-glyceraldehyde 3-phosphate (GAP) is catalyzed by an enzyme known as 1-deoxy-D-xylulose 5-phosphate synthase (DXS; EC 2.2.1.7). It is a committed irreversible step in the MEP pathway that commits carbon to the pathway and releases CO₂. In most plants, DXS is encoded by multiple gene paralogs. For example, in *Arabidopsis thaliana* three *DXS*-like genes have been discovered, one named *CLA1* (*AtDXS*) has been reported to encode a functional DXS enzyme and the functions of the other two genes (*AtDXL1* and *AtDXL2*) are unknown [41] [42]. The enzyme, DXP reductoisomerase (DXR; EC 1.1.1.267) cat-

alyzes the next reaction of an intermolecular rearrangement and reduction of DXP to MEP. This enzyme is encoded by a single gene (IspC) and is known to be inhibited by an antibiotic referred to as fosmidomycin that was originally isolated from culture broths of bacteria of the genus *Streptomyces*. The conversion of MEP to 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol (CDP-ME) in a CTP-dependent reaction is catalyzed by an enzyme, 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase (MCT; EC 2.7.7.60). Further, phosphorylation of the hydroxyl group in the Carbon2 of CDP-ME position by an enzyme 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase (CMK; EC 2.7.1.148) results in the synthesis of 2-phospho-4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol (CDP-ME2P). Subsequent conversion of CDP-ME2P to 2-C-methyl-D-erythritol 2, 4-cyclodiphosphate (MEcPP) is catalyzed by the enzyme 2-C-methyl-D-erythritol 2, 4-cyclodiphosphate synthase (MDS; EC 4.6.1.12). Reduction of MEcPP to 4-hydroxy-3-methylbut-2-enyldiphosphate (HMBPP) is achieved with the help of an enzyme 4-hydroxy-3-methylbut-2-enyldiphosphate synthase (HDS; EC 1.17.7.1). The need for NADPH in this reaction as a reducing agent cannot be understated. The last stage of IPP synthesis through the MEP pathway is the conversion of HMBPP into two compounds, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). This reaction is catalyzed by the enzyme HMBPP reductase (HDR; EC 1.17.1.2) and also requires NADPH. Over the years, studies in *Arabidopsis thaliana* and most other plants have shown that apart from the enzyme DXS that is encoded by multiple gene paralogs, all other enzymes in the MEP pathway are encoded by single copy genes [31] [42]. All activities of the MEP pathway enzymes in *Arabidopsis thaliana* has also been confirmed with all proteins residing in the plastid stroma [31] [43].

5. Physiology of VTC Action

5.1. Physiological Roles of Volatile Terpene Compounds in Plants

5.1.1. Defense against Insects and Herbivores

Volatile terpene compounds (VTCs) are toxins and feeding deterrents to a large number of plant-feeding insects and mammals thus, they play important defensive roles in the plant kingdom [6]. Many monoterpenes and their derivatives are important agents of insect toxicity, for example, pyrethroids, which are monoterpene esters produced in the leaves and flowers of *Chrysanthemum* species have shown very striking insecticidal activity, therefore, are often used in commercial insecticides [19]. Studies on conifers such as pine and fir, have commonly reported that VTCs such as α , β -pinene, limonene and myrcene accumulate in resin ducts found in needles, twigs, and trunk pose as toxic compounds to serious pests of conifer such as bark beetle and balsam woolly adelgid [17] [22] [44]. Bark beetle-associated blue-stain fungus is reported to induce nearly 100-fold increase in total mono- and sesquiterpene levels at the inoculation site of Norway spruce [45]. The blue-stain fungus *Grosmannia clavigerai* also known to induce the formation of monoterpenes and diterpenes in 2-year-old

lodgepole pine saplings [46] and monoterpene formation in mature (about 80 years old) lodgepole pines [47].

Contrary to serving as deterrents to herbivores, some plants such as *C. solstitialis* and *C. cyanus* have also been reported to attract the weevil, *Ceratopion basicornis*, a candidate for biological control, this is a result of the ability of these trees to synthesize sesquiterpenes cyclosativene, R-ylangene, and *trans*-R-bergamotene [48]. It is also believed and reported that neighboring plants are able to communicate *via* VTCs emission when they are under attack [49] [50]. In some studies, partial defoliation of *Alnus glutinosa* results in induced resistance of neighboring trees against defoliation [51]. Exposure of *Phaseolus lunatus* to odors emitted from damaged leaves of conspecific plants as a result of spider-mite attack saw an induced expression of several defense related genes [52].

5.1.2. Stress, Senescence and the Role of VTCs

The role of VTCs in plant senescence has been a controversial discussion in the scientific community over the years. However, the physiological role of VTCs in senescence can be well understood if one takes a critical look at the effect of abiotic stress, a major contributing factor to plant senescence and how it influences the biosynthesis of VTCs.

Abiotic stress generally inhibits photosynthesis by way of reducing leaf CO₂ uptake and diffusion or altering the photochemical and biochemical reaction of photosynthesis. All these have been reported to be critical factors that trigger plant senescence since they regulate the fixation of carbohydrate in plants [9] [53]. One would expect that since photosynthesis is a major carbon source in plants, it would play a major role in VTC biosynthesis or emission, however it has been reported otherwise [9]. Although the disconnection between photosynthesis and VTC emission has been reported, the crucial requirement of photosynthetic carbon in the biosynthesis of VTCs has also been acknowledged [35], suggesting the possibility of alternative sources of carbon for VTC synthesis. Although not fully identified and understood, in recent years labelling studies have suggested that xylem-transported carbon and chloroplast starch may be alternative sources of carbon for VTCs biosynthesis [10] [11]. It is expected that during abiotic stress related senescence where there is colossal depletion of plant starch, VTC biosynthesis will cease, however the contrary has been reported and speculating that extra-chloroplastic sources of carbon may be activated and feed carbon to stimulate VTCs biosynthesis [11]. This suggests that a lot of research in this area is paramount in establishing the alternative sources of carbon feeding VTCs biosynthesis and emission.

Reports have indicated that monoterpenes have the ability to play a role in apoptosis-like cell death, an integral part of the process of plant senescence [14]. The immediate response of plant cells to citral, a known VTC has been investigated. It was discovered that microtubules of *Arabidopsis thaliana* were disrupted within minutes of citral exposure in the gaseous phase. In the same study, *in vitro* polymerization of microtubules was inhibited in the presence of citral,

suggesting the potential role of some VTCs in plant senescence. Volatile oils containing VTCs in plants have been found to suppress cell division, membrane disruption and oxidative stress, which are signs of plant senescence. When seedling of cucumber was introduced to increasing concentrations of essential oil with menthol, menthone, menthofuran, menthyl acetate, pulegone, neomenthol, 1,8-cineole and limonene as its constituent, there was an instant increase in membrane depolarization [13]. Other findings have shown that monoterpenes affect biological membranes by damaging their structure and changing their lipid packing density, resulting in increasing ion permeability and perturbs membrane-bound enzyme function [54]. This is similar to the effect of abscisic acid on *Arabidopsis* cells leading to abscission [55].

α -pinene also inhibits early root growth and causes oxidative damage in root tissue through enhanced generation of reactive oxygen species (ROS), increased lipid peroxidation, disruption of membrane integrity and elevated antioxidant enzyme levels [56] [57] [58]. Exposure of *Celtisoccidentalis* roots to α -pinene enhances solute leakage, and increases levels of malondialdehyde, proline and hydrogen peroxide, indicating lipid peroxidation and induction of oxidative stress. Activities of the antioxidant enzymes SOD, CAT, GPX, APX and GR have also been reported to be significantly elevated, indicating enhanced generation of ROS. An increase in the levels of scavenging enzymes also indicates VTCs induction of secondary defense mechanism in plants [56]. Analogs of volatile monoterpene, 1,4-cineole and 1,8-cineole, have been identified to severely inhibit or decrease the growth of roots and shoots, causing cork-screw shaped morphological distortion, germination rates of two weedy plants *Cassia obtusifolia* and *Echinochloa crus-galli*. Chlorophyll fluorescence data (Fv/Fm) from the same study also indicated a significant amount of physiological stress, resulting in decrease in photosynthetic yield and a severe decrease in mitosis in all stages upon the exposure of plants to the VTCs [12]. Another study investigated the effect of some volatile monoterpenes (1,8-cineole, β -pinene, α -pinene, and camphene) on *Brassica campestris* and showed the inhibition of both cell-nuclear and organelle DNA synthesis in the root apical meristem [59]. In recent studies, it has been demonstrated that balsam fir trees after harvest synthesize a minimum of twelve VTCs [15] (Figure 3).

Postharvest monitoring of these VTCs showed that five main VTCs (β -Pinene, β -Terpinene, Fenchyl acetate, Camphene and 3-Carene) are synthesized and emitted in significantly higher concentrations prior to needle abscission, suggesting a possible role of these VTCs in postharvest senescence or abscission of the trees [15] (Figure 4). All these studies have speculated that monoterpenes produced by plants play a significant role in senescence by suppressing cell division, membrane disruption, oxidative stress and eventually leading to abscission. In some cases, it influences even other plants in its vicinity through the inhibition of cell proliferation in the root apical meristem. Due to limited study in this area it is still unclear if VTCs serve as signal molecules at the initiation of the senescence process or a direct causal effect of senescence.

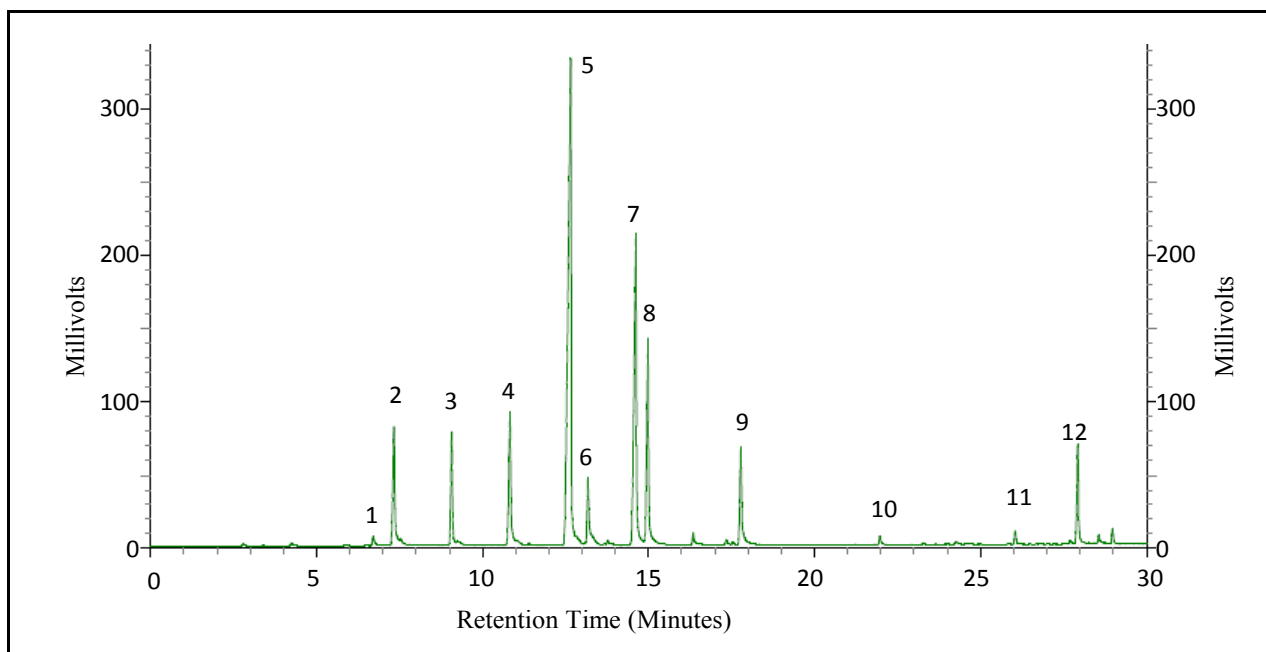


Figure 3. VTC profile for balsam fir trees using SPME with headspace sampling after 30 mins equilibration. Compounds determined were: (1) α -Pinene; (2) 3-Thujene; (3) Camphene; (4) β -Pinene; (5) 3-Carene; (6) β -Terpine; (7) D-Limonene; (8) β -Phellandrene; (9) γ -Terpinene; (10) Terpinolene; (11) Fenchyl acetate; (12) Bornyl acetate.

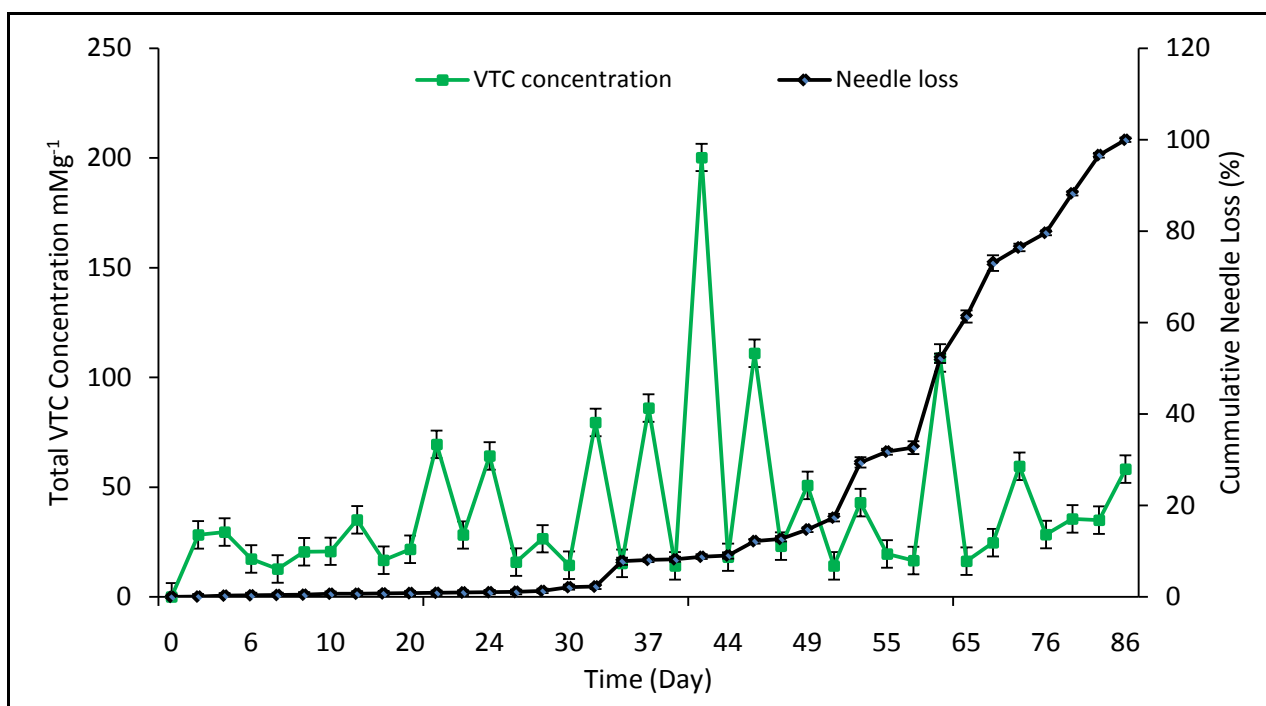


Figure 4. The progression of needle loss (as a percentage of needle fresh weight) and total VTC evolution in balsam fir trees with a peak VTC evolution of 42 days and NRD of 86 days (n = 10).

Although the underlying molecular mechanisms and genes involved in the induction of cell cycle arrest and apoptosis/senescence of plants in the presence of volatile terpene compounds are also not well understood, it has been shown

that plants respond to VTCs by substantial changes in transcriptome [60] [61] [62] and in most cases, result in plant senescence [63]. Using suppression subtractive hybridization (SSH) approach, several geraniol-responsive proteins encoding genes for signal transduction, cellular metabolism, reactive oxygen species (ROS), ethylene signaling, apoptosis and DNA damage response have been reported in tomato [63]. Treatment of plants with geraniol saw early senescence process and upregulation of NADPH oxidase and antioxidant gene and also increased ROS level, suggesting the role of ROS in geraniol-mediated senescence. Expression analysis at the ripening stage of the tomato confirmed the role of VTC-responsive genes in plant senescence. Other studies have also reported that geraniol binds and inhibits the activity of 3-hydroxy-3-methylglutaryl-CoA reductase, the enzyme that converts HMG-CoA to mevalonic acid and subsequently reduces the cell growth [64]. These reports suggest that monoterpenes, including geraniol have the ability to induce physiological changes in plants especially during senescence. In spite of the amount of molecular work done, there are a lot of VTCs synthesized by plants with limited study and therefore, it is still not clear the exact number of VTCs synthesized and the molecular mechanism adopted by plants during senescence.

5.2. The Proposed Link between VTCs, Ethylene, Jasmonic Acid and Plant Senescence

Discussing the crosstalk between VTCs and phytohormones, and their interactive roles in plant senescence, several postulations have been made. Ethylene and jasmonic acid (JA) have long been considered as endogenous regulators of plant organ abscission, as exogenous applications of ethylene and JA promote foliage abscission [65] [66]. In a study by [16], exogenous application of JA promoted ethylene and VTCs emission in numerous excised-leaf bioassays. Signaling interactions between JA and ethylene have also been demonstrated to result in either synergistic [67] or antagonistic interactions [68] in the expression of plant defense responses to pathogens, insects and mechanical wounds. However, in the case of VTCs emission in plants, the role of ethylene and/or JA remains unclear. In other studies, exposure of cultured soybean cells and shoot primordia of *Matricaria chamomilla* to VTC (geraniol) as a chemical stress resulted in an up-regulation of glutathione S-transferase (GST) and transcription factors of ethylene response element binding protein (EREBP) and WRKY families [69] [70]. Delayed onset of seedling senescence in tomato and induced expression of geraniol-responsive genes in geraniol-treated ethylene receptor mutant have suggested that geraniol-mediated senescence involves both ethylene dependent and independent pathways [63]. Using detached lima bean leaves (*Phaseolus lunatus* L), [71] demonstrated that exogenous applications of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid, enhance JA-induced VTC emission. However, in tobacco (*Nicotiana attenuata*), [72] did not detect any significant interactions between exogenous methyl jasmonate and ethylene upon in-

duced emission of sesquiterpenes. This suggests a promising trend that jasmonic acid may induce ethylene and VTCs during stress such as mechanical wounding, and it is possible that plant senescence may be triggered by VTCs through ethylene or JA dependent or independent pathways.

Current study contradicts reports of VTC-dependent ethylene senescence or *vice versa*. In a study to determine the effect of ethylene on VTC synthesis and how their interaction affect postharvest balsam fir needle abscission through the inhibition of ethylene synthesis using aminoethoxyvinylglycine (AVG), it was discovered that although AVG was effective in inhibiting ethylene synthesis and delaying postharvest needle abscission there was no changes in the type and amount of VTCs synthesized compared to the control [15]. Although these studies have helped to throw more light on the interrelationship between VTCs and phytohormones, since most of these works were conducted under pre-harvest conditions, there is no clear understanding of the interrelationship between these phytohormones, VTC and postharvest senescence or abscission which is more predominant in most horticultural industries.

6. Future Research Directions

Focusing on future research to address the knowledge gap in relation to the role of VTCs in plant senescence, it is important to establish the various factors that initiate biosynthesis of these VTCs, their interaction with plant hormones and the pathway by which this complex interaction triggers the process of senescence and abscission. The proposed pathway for plant senescence and abscission via VTCs is shown in **Figure 5**. Few areas of discussion and research needed to further develop the theory are as discussed.

Apart from plant age, mechanical stress through wounding, insect feeding and mechanical injury leads to a decrease in stomatal conductance and xylem pressure potential [73] [74], and eventual decrease in plant water uptake. Plant response to low water uptake and dehydration is known to induce synthesis and or emission of VTCs prior to abscission [15]. This suggests the possibility that VTC biosynthesis and emission is triggered by mechanical stress in plants, however the specific pathway through which this action happens has to be explored. The key question however is, whether VTC biosynthesis directly linked to mechanical stress or through other plant hormones.

Plants respond to mechanical injury and dehydration by initiating the biosynthesis of various phytohormones such as ethylene, abscisic acid, auxin and jasmonic acid. These phytohormones have been speculated to trigger plant senescence and abscission with or without VTCs [15] [65] [66]. A number of researches have to be conducted to establish the possible interrelationship between phytohormones and VTCs biosynthesis.

Another question that one would also ask is whether the role of VTCs in plant senescence and abscission is a signal transduction or direct causal compound. Studies by [15] hypothesized the direct effect of VTCs on postharvest

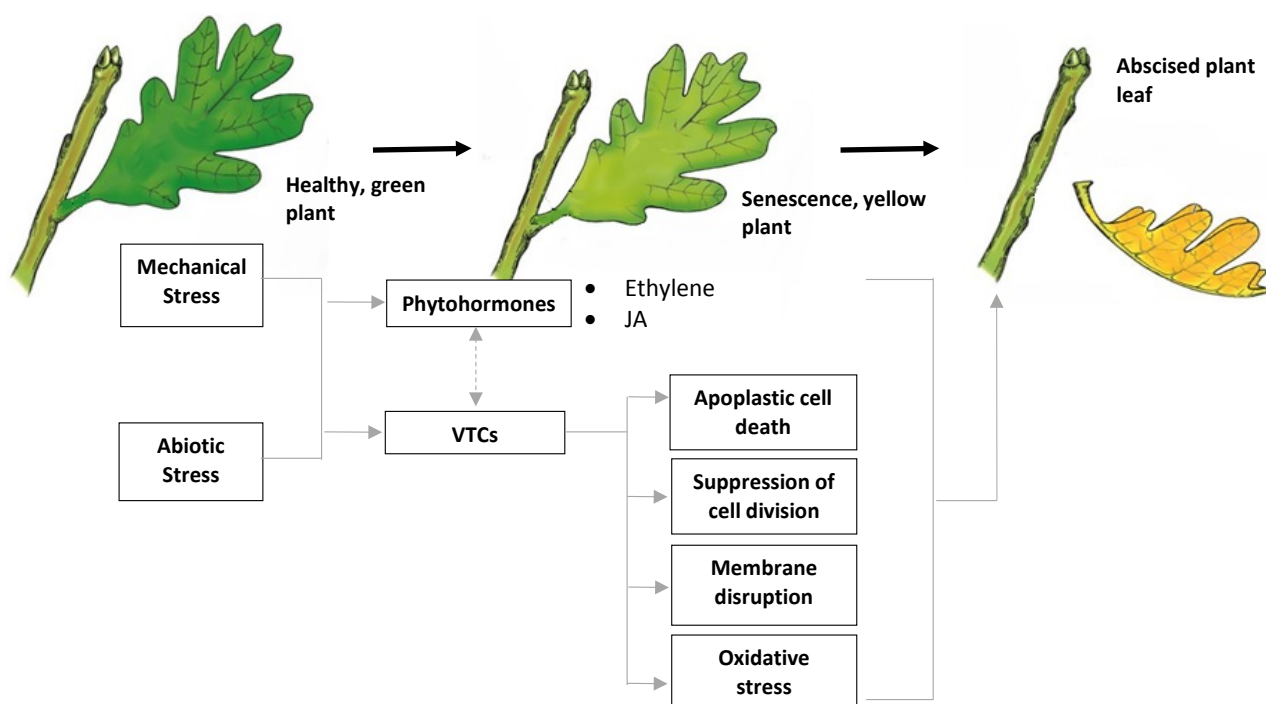


Figure 5. Proposed pathways for VTCs-induced senescence and abscission in plants. Elements in boxes represent events that are known/proposed to occur during VTCs-induced senescence, abscission. Solid arrows indicate portions of the pathway that are known to occur. Dashed arrow is used to represent speculative portions of the partway.

balsam fir needle abscission and concluded that the increase in VTCs prior to needle abscission observation was evident that VTCs have a direct effect on abscission. Apart from that, VTCs such as monoterpenes are known to cause senescence and abscission through cell death and suppress cell division [14], membrane disruption [13], and oxidative stress [56]. However, the argument is still not clear as to whether VTCs trigger cellulase, weakening cell wall of the abscission zone to cause abscission. On the other hand, do ROS cause membrane damage and then induce VTC biosynthesis? Or dose the membrane damage a direct effect of the VTCs increase? Studies to establish plant response to exogenous application of VTCs as well as inhibiting the biosynthesis of these VTCs might give us such an insight. In that light, one will recommend studies that adopt the use of either mevastatin that inhibits IPP from the MVA pathway or fosmidomycin, the inhibitor of IPP from MEP pathway in accessing the role of VTCs in plant senescence.

A holistic understanding of the theory behind the role of VTCs in plant senescence and abscission is imperative to the development of future methods and tools to curb the process of senescence and abscission by either delaying or halting it completely. However, that cannot be said without a clear and better understanding of VTCs, its biosynthesis and regulation, especially during senescence, and reference to the interrelationship between VTCs and phytohormones. Future molecular studies to profile expressed genes in plants during senescence and abscission will help indicate the induced transcripts that encode for VTCs

and phytohormones.

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Author Contributions Statement

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