Chapter 11
Ozone-Induced Changes in Plant Secondary Metabolism

Marcello Iriti and Franco Faoro

11.1 Introduction

In their ecosystem, plants have to cope with a plethora of potentially unfavourable conditions. Stress factors affecting plant's fitness not only derive from natural sources, such as adverse temperature fluctuations (heating, chilling and freezing), high irradiance (photoinhibition, photooxidation), osmotic imbalance (salinity and drought), hypoxia/anoxia (flooding), mineral (macro- and micronutrient) deficiency, wounding, phytophagy and pathogen attack, but also from anthropogenic activities. The latter include xenobiotics employed in agriculture (herbicide, pesticides and fungicides), environmental (air, soil and water) pollutants and increased UV radiations. Particularly, many atmospheric pollutants, belonging to greenhouse gases, may increase the greenhouse effect, a natural warming process that prevents heat from diffusing to the outer atmosphere, thus balancing Earth cooling processes. Without the natural greenhouse effect, temperature on Earth would be much lower than it is now, and the existence of life would have not been possible. However, the rising emissions of greenhouse gases due to anthropogenic activities, namely carbon dioxide (CO_2), chlorofluorocarbons (CFCs), nitrous oxide (N_2O), tropospheric ozone (O_3) and water vapour, may cause a short-term increase of the mean global temperature on the planet surface with consequent changes in precipitation patterns (Krupa and Kickert 1989). In this scenario, life on the earth depended from the co-evolution between atmosphere and biosphere, because the gradual and long-term climate changes enabled living organism adaptation to the new temperatures, precipitation patterns and other climate conditions (Voronin and Black 2007).

Regardless of natural or anthropogenic stress factors, plants have to cope with their stressors. From a pathophysiological point of view, a plant may avoid or adapt to a particular stress, with a dose-dependent mechanism (Fig. 11.1). Under a certain threshold, a mild stress may be compensated by the plant, whereas, at higher

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S.N. Singh (ed.), *Climate Change and Crops*, Environmental Science and Engineering,
DOI 10.1007/978-3-540-88246-6_11,

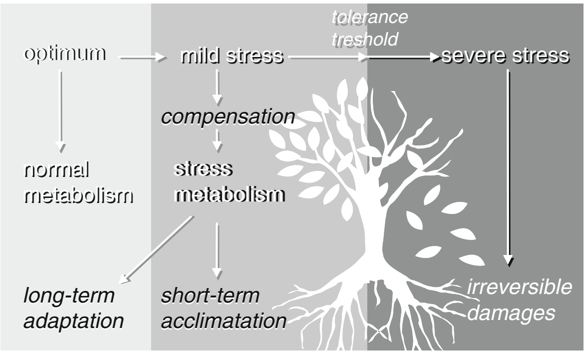


Fig. 11.1 Stressor dose-stress effect relationships in plants

concentration, the detrimental effects of a severe stress may cause irreversible damages, according to the stressor dose-stress effect relationship (Lichtenthaler 1998). Furthermore, the stress tolerance threshold depends not only on the type of stressor and exposure time, but also on the plant stress-coping capacity. Anyway, the shift between normal and stress metabolism represents a fundamental trait in plant acclimation (short-term) and adaptation (long-term) strategies, although it is almost impossible to define exactly the threshold between them (Heiser and Elstner 1998).

In this chapter, we deal with the phytotoxic potential of tropospheric ozone, due to partially reduced oxygen intermediates produced by the pollutant in biological systems. These intermediates are more reactive than molecular oxygen in its ground state and include both radical (superoxide anion, $\cdot O_2^-$ and hydroxyl radical, $OH\cdot$) and non-radical (hydrogen peroxide, H_2O_2) forms, collectively termed as reactive oxygen species (ROS) (Halliwell 2006). Plant tolerance mechanisms will also be discussed with emphasis on ozone-induced metabolic fluxes between primary (normal) and secondary (stress) metabolism, due to acute or chronic pollutant exposure. Besides, ozone is a greenhouse gas, although it plays a minor role in regulating the air temperature and in contributing to the warming effect (Wang et al. 1995).

11.2 The Ozone in the Atmosphere

Ozone is an important constituent of the atmosphere, although present in trace amounts. As a matter of fact, two different pools of O_3 exists, the beneficial and the detrimental one (Fig. 11.2). In the stratosphere (the higher atmosphere, ranging approximately from 15 to 40 km in altitude), the ozone layer absorbs the harmful UV-B and UV-C radiations, thus saving the living organisms (Dutsch 1978; Kerr and McElroy 1993). In the past decades, emission of ozone-depleting chemicals led to the reduction of the ozone shield (commonly referred to as 'ozone hole') against UV radiation, worsening its harmful effects on animals and plants (Platt and Hönninger 2003). Otherwise, in the troposphere (the lower part of the atmosphere, approximately from the earth surface to 10–12 km in altitude), that is to say the layer

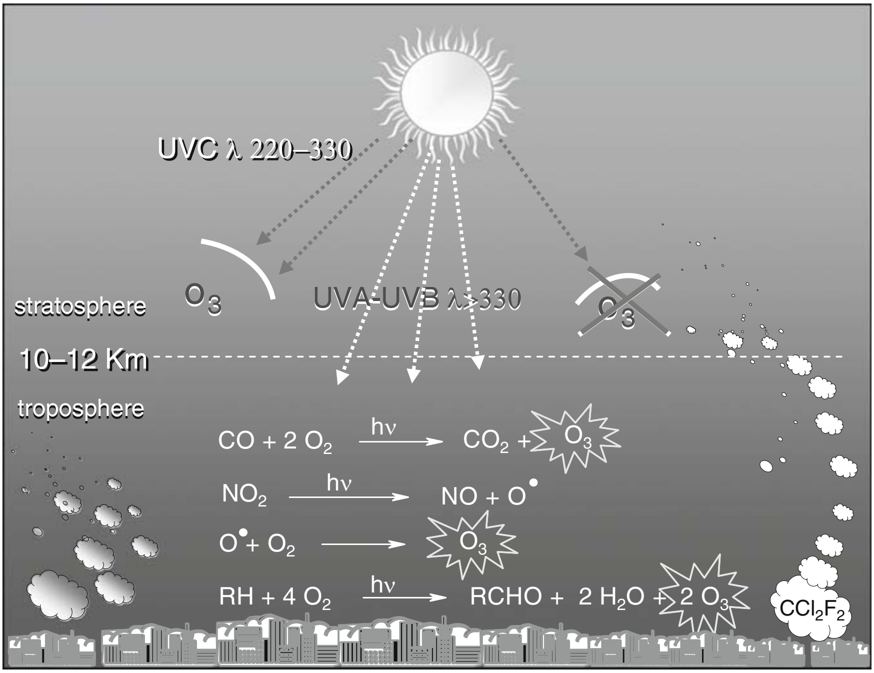


Fig. 11.2 Beneficial and detrimental pools of ozone in the stratosphere and troposphere, respectively. In the stratosphere, this pool is depleted by fluorochlorocarbons (CCl_2C_2) while in the troposphere, it is raised by photochemical smog

where the climatic conditions originate, and temperature decreases with elevation, ozone is regarded as a pollutant (Logan 1985).

Tropospheric ozone is an oxidant constituent of the photochemical smog. It is a secondary pollutant produced through reactions among primary pollutants, emitted directly into the air (mainly nitric oxides, sulphur oxides, carbon oxides and hydrocarbons), catalyzed by sunlight (Crutzen and Lelieveld 2001) (Fig. 11.2). Hence, ozone is produced on bright sunny days over areas with intense primary pollution, mainly due to vehicle exhausts, fossil fuel burning and industrial processes, in the so-called photochemical cycle (Fowler et al. 1999; Kley et al. 1999). Meteorological conditions may exacerbate the rate of ozone formation too, particularly atmospheric inversion, a restricted air circulation associated to a warmer air layer above a cooler zone (Baumbach and Vogt 2003).

11.3 Chemistry of Ozone Injury

From the Greek *ozein* (to smell), ozone, the triatomic allotropic form of oxygen, is a colourless gas with a slightly sweet, water melon-like odour (odour threshold between 0.0076 and 0.036 ppm). Because of its strong oxidizing potential

(+ 2.07 eV), O_3 is a powerful oxidizing agent capable of reacting with virtually any biomacromolecule, including lipids, proteins, nucleic acids and carbohydrates, although it is neither a radical species nor a ROS (Mustafa 1990; Kelly et al. 1995). Ozone is considered too reactive to penetrate far into tissues, so that only a minor amount of the pollutant is believed to pass not reacted through a membrane, and nothing through a cell (Pryor 1992). Furthermore, its toxicity can be greatly enhanced by the spontaneous hydroxyl radical ($OH^{\cdot}$) generation in the aqueous solution, strongly accelerated by traces of Fe^{2+} and favoured at alkaline pH, though occurring even at physiological pH (Pryor 1994).

In cell membrane, polyunsaturated fatty acids represent the primary target for ozone, stimulating lipid peroxidation and impairing membrane fluidity. The chemistry of O_3-induced lipid peroxidation, known as Criegee ozonation pathway, involves ozonolysis of alkenes in polyunsaturated chains, i.e. the electrophilic O_3 addition across the carbon-carbon double bonds, to give the Criegee ozonide (Criegee 1957). Afterwards, ozonide decomposes, under suitable conditions, to form organic radicals, aldehydes and peroxides. In further steps, H_2O_2 can react with transition metals (Cu or Fe), according to Fenton or Haber-Weiss reactions, to form other ROS (Pryor et al. 1991; Pryor 1993).

As a result of the ozone-induced oxidation, modification of proteins also occurs, both in their structure and activity. The pollutant directly or through highly reactive free radical mediated reactions, oxidizes the amino acidic residues, mainly of tyrosine, tryptophan, cysteine, methionine and histidine (Mudd et al. 1969). In particular, it reacts with the exposed sulphydryl groups to form disulphides bridges, and with tryptophan to give protein ozonides, in turn generating protein hydroperoxides and hydrogen peroxide (Freeman and Mudd 1981). Tyrosine residues can be cross-linked too, after the oxidation of their HO- groups, to give O,O'-dityrosine (Ignatenko et al. 1984). DNA damage can be produced as well, as shown by the increased activity of poly (ADP-ribose) synthetase, a chromatin-bound enzyme promoting damaged DNA repair (Hussain et al. 1985).

11.4 Plant Secondary Metabolism

In living organisms, secondary metabolites are not essential for growth and development unlike the products of primary metabolism. However, this does not mean they are not necessary. Infact, secondary metabolites relate plants with the components of their ecosystem, that is to say the physic environment (biotope) and the living community (biocenosis), thus resulting indispensable for the survival of the species. Additionally, stress metabolism could be regarded as a particular expression of secondary metabolism, when stressful conditions, of both biotic and abiotic nature, change the dynamic equilibrium of the ecosystem. As an instance, phytoalexins are compounds synthesized ex novo or whose synthesis increases after pathogen challenging, raising their concentration in the tissues (Frank 1993; Paiva 2000).

In plants, chemical diversity has determined their evolutionary success. Because of their sessile habit, plants are unable to avoid the worsening environmental conditions as well as they cannot escape the plethora of the laying before biotic stresses. Consequently, unlike animals, plants have evolved an enormous number of secondary metabolites to overcome any danger. The functional role of these phytochemicals ranges from the ecology to defence, improving protection against both biotic and abiotic stresses, besides being involved in ecological roles as attractants or repellents for pollinators and phytophagy, respectively, and colours and scents of reproductive organs (flowers and fruits).

Generally, precursors of secondary metabolic pathways are products of the primary metabolism. Therefore, a severe or long-lasting stress factor could induce an excessive shift between primary and secondary metabolism and consequently, a diversion of essential available resources from growth to defence. To a large extent, secondary metabolites derive from three biosynthetic routes, namely the phenylpropanoid, isoprenoid and alkaloid pathways. Phytochemicals arising from these pathways include not only compounds with a broad-spectrum antibiotic activity, but also powerful antioxidants, able to efficiently scavenge ozone-induced ROS (Facchini 2001; Holstein and Hohl 2004; Iriti and Faoro 2004).

11.4.1 Phenylpropanoid Pathway

Phenylpropanoids are a class of phenylalanine derivatives with the basic skeletal C_6-C_3 (phenyl-propane) as depicted in Fig. 11.3.

Precursors of this pathway are phosphoenolpyruvate derived from glycolysis and erythrose 4-phosphate from pentose phosphate pathway, leading to two important intermediates, shikimic acid and chorismic acid. In further steps, after a branch point, phenylalanine and tyrosine are synthesized from prephenic and arogenic acid, whereas tryptophan comes from anthranilic acid (Weaver and Hermann 1997).

The removal of an amino group (deamination) from phenylalanine via the step catalyzed by the enzyme phenylalanine ammonia-lyase (PAL), leads to formation of cinnamic acid and, in turn, the precursor of hydroxycinnamates is produced after a series of hydroxylation of the benzene ring (Fig. 11.4). These compounds, including coumaric, ferulic and sinapic acids are reduced to the corresponding alcohol via aldehyde intermediates, namely coumaryl, coniferyl and sinapyl alcohol, collectively termed monolignols. Dimerization or polymerization of monolignols leads to formation of lignans and lignin, respectively (Fig. 11.4). Lignification is a complex reaction in which peroxidases catalyze the polymerization of lignin units consuming H_2O_2. Apposition of lignin in plant cell wall is a process occurring during the development of particular tissues, as well as in plant defence responses, in order to strengthen the cell wall and to protect the plasmalemma. Benzoic and hydroxybenzoic acids (C_6-C_1), such as salicylic acid (SA), a molecule involved in systemic acquired resistance (SAR), are another group of cinnamic acid derivatives,

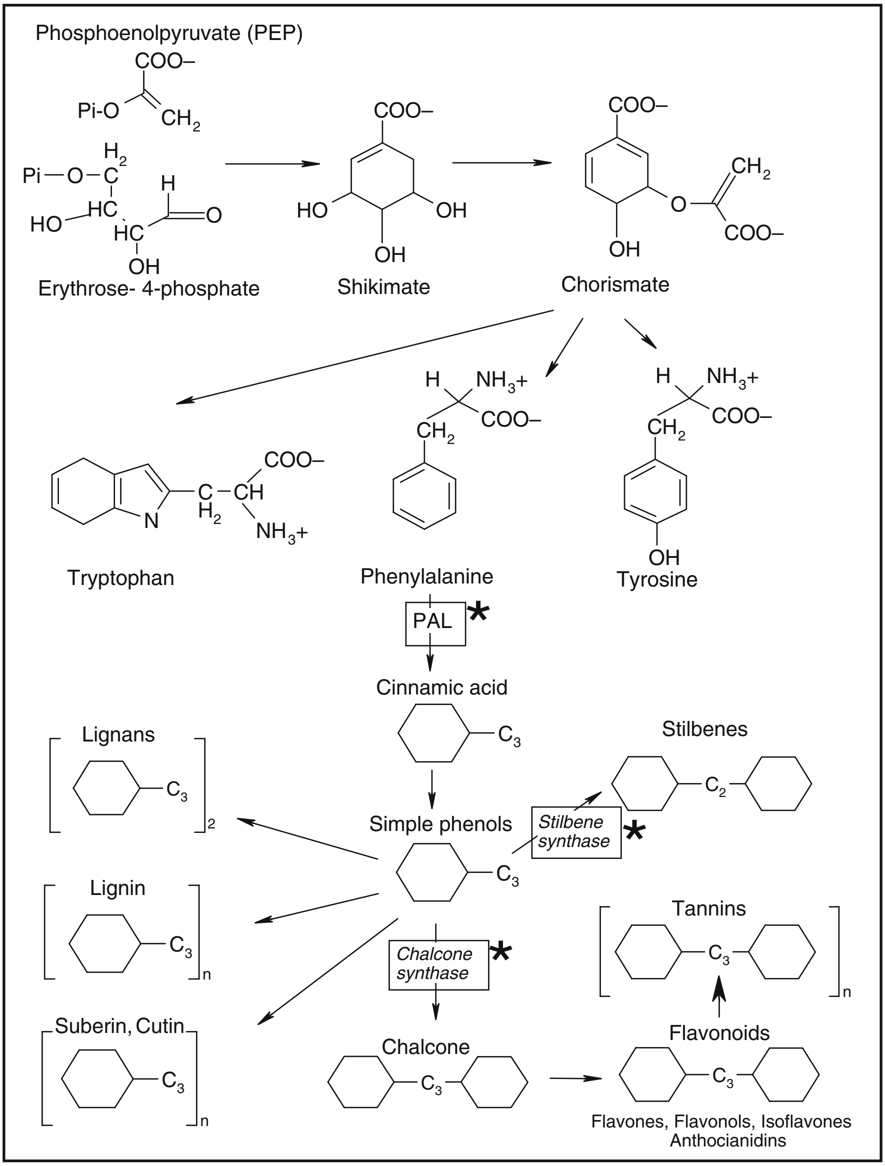


Fig. 11.3 Aromatic amino acid biosynthesis from shikimate and phenyl-alanine derivatives of the phenylpropanoid pathway. The enzymes with an asterisk are known to be influenced by ozone

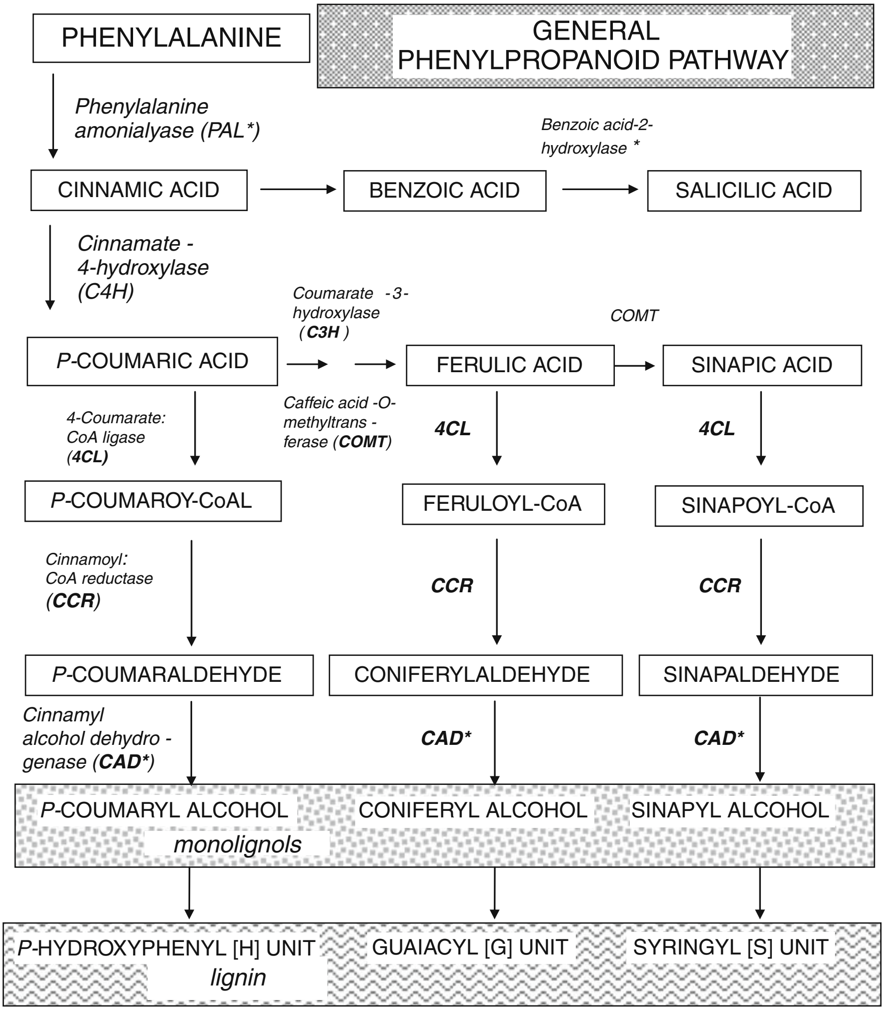


Fig. 11.4 Main steps of the phenylpropanoid pathway leading to formation of benzoic acids, hydroxycinnamates (coumaric, ferulic and sinapic acids) and lignin. The enzymes with an asterisk are known to be influenced by ozone

formed by cleavage of a C_2 fragment from the phenylpropane structure (Fig. 11.4) (Hahlbrock and Scheel 1989; Iriti and Faoro 2004).

The metabolites till now mentioned may be collectively named as simple phenols to differentiate them from polyphenolic compounds. The latter have an additional benzene ring, arising from the cyclization of three malonic acid (C_2) residues via stilbene sinthase (STS) or chalcone synthase (CHS), to give stilbenes (C_6-C_2-C_6) or flavonoids (C_6-C_3-C_6), respectively (Fig. 11.3). Finally, proanthocyanidins (PA), or condensed tannins include oligo- and polymeric flavonoid derivatives with polymerisation degree ranging from 2 to 17 and more. The numerous hydroxyl groups available in these molecules promote the formation of complexes with macromolecules, such as proteins, polysaccharides and metal ions (Hahlbrock and Scheel 1989; Iriti and Faoro 2004).

11.4.2 Isoprenoid Pathway

Isoprenoids, also named terpenoids, are the chemically and functionally most diversified class of low molecular mass lipids in plants, both primary and secondary metabolites (Iriti and Faoro 2006). They include electron carriers (quinones), membrane constituents (sterols), vitamins (A, D, E and K), plant hormones (side chain of cytokinins, abscisic acid, gibberellins and brassinosteroids), photosynthetic pigments (chlorophyll, phytol and carotenoids) and essential oils (Sacchettini and Poulter 1997).

Acetyl coenzyme A (CoA) represents the precursor for the isprenoid biosynthesis (Fig. 11.5). Firstly, two molecules of acetyl CoA react to give acetoacetyl CoA and then, with a further acetyl CoA to produce β-hydroxy-β-methylglutaryl-coenzime A (HMG-CoA). In plants, the same enzyme, HMG-CoA synthase, catalyses both reactions. The conversion of HMG-CoA into mevalonate, via HMG-CoA reductase, is the rate limiting enzyme of this pathway (Chappell 1995). Mevalonate kinase and mevalonate phosphate kinase phosphorylate, respectively, mevalonate and, then, mevalonate 5-phosphate, yielding mevalonate 5-diphosphate. Further, mevalonate diphosphate decarboxylation, via mevalonate diphosphate decarboxylase, produces isopentenyl diphosphate (IPP), the five-carbon building block for the formation of isoprenoid chains. The enzyme IPP:dimethylallyl-PP isomerase converts IPP into dimethylallyl diphosphate (DMAPP), the acceptor for successive transfers of isopentenyl residues (Bach 1987; Chappell 1995).

Hemiterpenes (C_5), such as isoprene, originate from dimethylallyl-PP, upon the release of diphosphate. Differently, dimethylallyl-PP can condense with IPP to form geranyl-PP via geranyl-PP synthase. In the same way, further chain elongation is attained by head to tail condensation of geranyl-PP to IPP, to produce farnesyl-PP via farnesyl-PP synthase. Analogously, geranylgeranyl-PP synthase catalyses the head to tail condensation of farnesyl-PP to IPP thus yielding geranylgeranyl-PP (Poulter and Rilling 1978).

Geranyl-PP is the precursor for the formation of monoterpenes (C_{10}) or essential oils, including highly volatile open chain and cyclic compounds, such as menthol, limonene, geraniol, linalool and pinene. They are active in plant-microbe, plant-pronubi, plant-phytophagous and plant-plant interactions due to their attractiveness and repulsiveness (Croteau 1987).

Farnesyl-PP is the precursor for the synthesis of open chain and cyclic sesquiterpenes (C_{15}), the largest group of isoprenoids, including essential oils and antibiotic compounds (phytoalexins) (Cane 1990). Diterpenes (C_{20}) derive from geranylgeranyl-PP consisting of phytoalexins, plant hormones, the phytol side chain of chlorophylls, tocopherols and phylloquinone (Dogbo and Camara 1987).

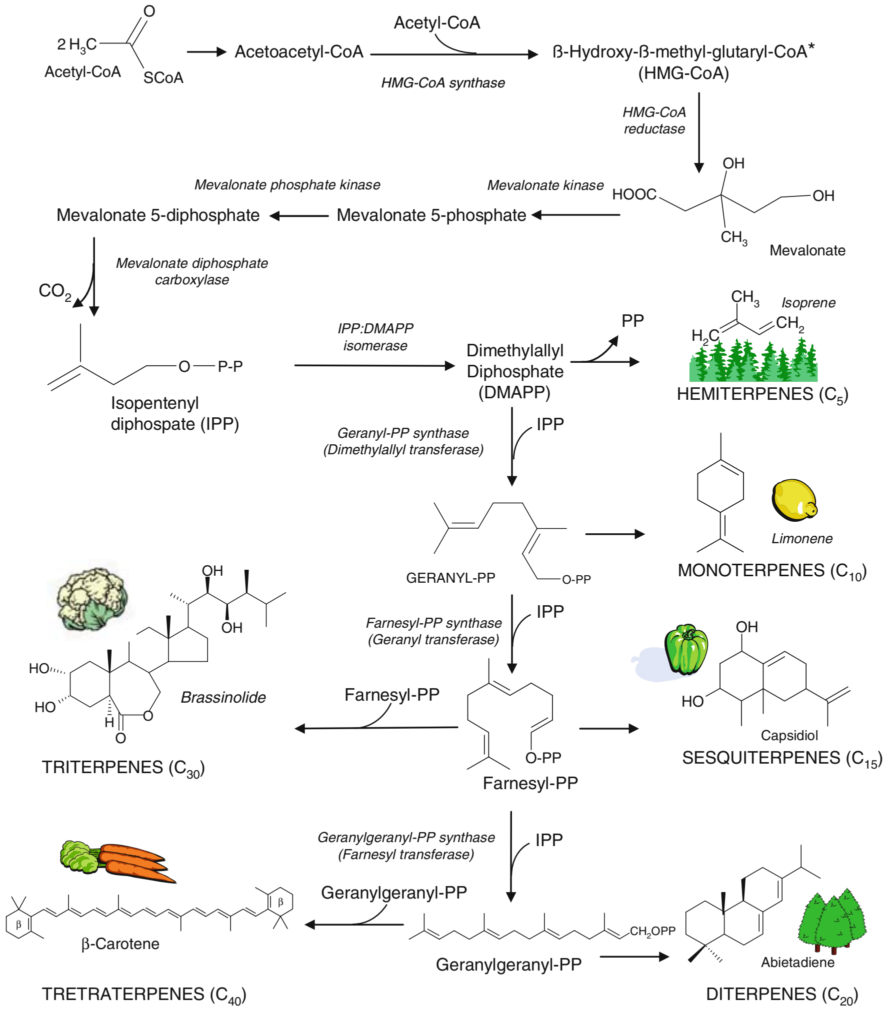


Fig. 11.5 Isoprenoid pathway from Acetyl-CoA. The enzyme with an asterisk is reported to be influenced by ozone

Furthermore, triterpenes (C_{30}) are synthesized from two molecules of farnesyl-PP (C_{15}), by a reductive head to head condensation. Squalene triterpene is the precursor for sterols, important membrane constituents, via squalene synthase (Abe et al. 1993). Analogously, head to head condensation of two molecules of geranylgeranyl-PP (C_{20}) leads to tetratepenes (C_{40}), such as carotenoids (carotene, lycopene) and xanthophylls (lutein, zeaxanthin, violaxanthin). Besides, isoprenoids are involved in protein prenylation, that is the synthesis of variously lengthened isoprenoid chains, anchoring proteins in membranes, such as G proteins, ubiquinone, plastoquinone and cytochrome-*a*. Finally, natural rubber is a polyterpen, composed

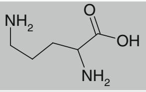
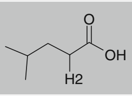
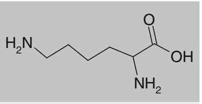
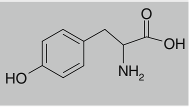
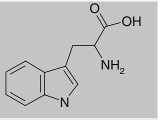
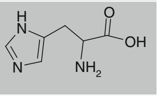
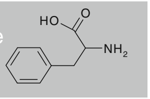
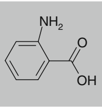
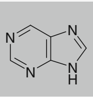
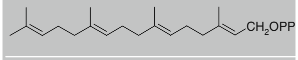
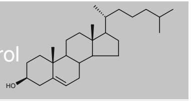
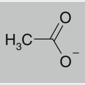
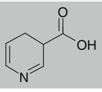
Precursors	Alkaloid classes	Examples
Ornithine 	Pyrrolidine[1], Tropane, Pyrrozidine, Polyamines[2]	1. Cocaine, Atropine 2. Putrescine, Spermine, Spermidine
Leucine 	Pyrrole	
Lysine 	Pyperidine, Quinolizidine, Indolizidine	
Tyrosine 	Isoquinoline Tetrahydroisoquinoline, Benzyltetrahydroisoquinoline[1], Catecholamines[2]	1. Morphine, Curarines, Papaverine 2. Noradrenaline, Adrenaline
Tryptofan 	Indole[1], Carbolines, Quinoline[2], Pyrrolindole, Indolamines[3]	1. Vindoline,Catharantine 2. Quinine, Capthotecin 3. Melatonin, Serotonin
Histidine 	Imidazole	
Phenylalanine 		Ephedrine, Capsaicin
Anthranilic acid 	Quinazoline, Qunoline,Acridine	
Purine 		Theobromine, Theophylline, Caffeine
Geranylgeranyldiphosphate 	Terpenoidic	
Cholesterol 	Steroidal	Solanin
Acetate 	Piperidine	
Nicotinic acid 	Pyridine	Nicotine

Fig. 11.6 Main classes of alkaloid precursors and derivatives (the example number refers to the corresponding alkaloid class)

of over 1000 isoprene units and derived from polymerisation of geranylgeranyl-PP units (Wendt and Schulz 1998).

11.4.3 Alkaloid Pathways

A common pathway for alkaloid biosynthesis does not exist. The greater amounts of alkaloid compounds are amino acid derivatives, grouped on the base of their precursor and chemical structure. Therefore, the main groups include alkaloids arising from ornithine, leucine, lysine, tyrosine, tryptophan, histidine and phenylalanine, in addition to alkaloids arising from nicotinic (pyridine alkaloids) and anthranilic acid, acetate, isoprenoids and purine (Fig. 11.6). In turn, these classes include some minor divisions. Accordingly, pyrrole alkaloids arise from leucine; pyrrolidine, tropane and pyrrolizidin alkaloids from ornithine; piperidine, quinolizidine and indolizidine alkaloids derived from lysine; catecholamines, isoquinoline, tetrahydroisoquinoline and benzyltetrahydroisoquinoline alkaloids originated from tyrosine; indolamines, indole, carboline, quinoline, pyrrolindole, ergot alkaloids come from tryptophan and imidazole alkaloids from histidine. The same way, anthranilic acid is the precursor of quinazoline, quinoline and acridine alkaloids, whereas isoprenoid alkaloids include mono- (geraniol), di- (geranylgeranyl-PP) and triterpenes (cholesterol) derivatives (Fig. 11.6) (Cordell et al. 2001; Facchini 2001; Hughes and Shanks 2002).

Alkaloids consist of an enormous number of phytochemicals of toxicological, pharmacological, nutritional and cosmetic interest, and also of ecological importance for plants. For instance, tropane alkaloids include cocaine and atropine, nicotine is a pyridine alkaloid, noradrenaline (or norepinephrine), adrenaline (epinephrine), papaverine, curarines and morphine arise from tyrosine. Melatonin and serotonin are indolamines, vindoline, catharantine (and their derivatives vincristine and vinblastine) are indole alkaloids, quinine and capthotecin are quinoline alkaloids and lysergic acid diethylamide (LSD) is an ergot alkaloid, all these arising from tryptophan. Histamine is an imidazole alkaloid, ephedrine and capsaicin derive from phenylalanine, solanin is a steroid glycoalkaloid from cholesterol. Finally, purine alkaloids include theophylline, theobromine and caffeine which are found in tea, cacao and coffee, respectively (Cordell et al. 2001; Facchni 2001; Hughes and Shanks 2002).

11.5 Ozone and Phenylpropanoids

In plants, phenylpropanoid metabolism is induced as a general response to stress. Therefore, enhancement of key enzyme activities and accumulation of secondary metabolites are events that occur in order to improve the resistance against pathogen attack and/or tolerance to adverse environmental conditions and pollutants. PAL is an extremely sensitive indicator of stress conditions, and commonly considered as a biochemical marker indicating the activation of plant defences which include the synthesis of both structural and protective compounds (Fig. 11.7). In particular, ozone exposure elevates the level of flux through the phenylpropanoid pathway, thereby supplying carbon skeletons for secondary metabolites (Toumainen et al. 1996).

The enhancement of phenylpropanoid biosynthesis by ozone is well documented. A very early report, dated more than 30 years ago, demonstrated the accumulation of isoflavonoid in soybean following ozone exposure (Keen and Taylor 1975). Ever since, ozone-stimulated induction of transcripts for defence-related genes, the

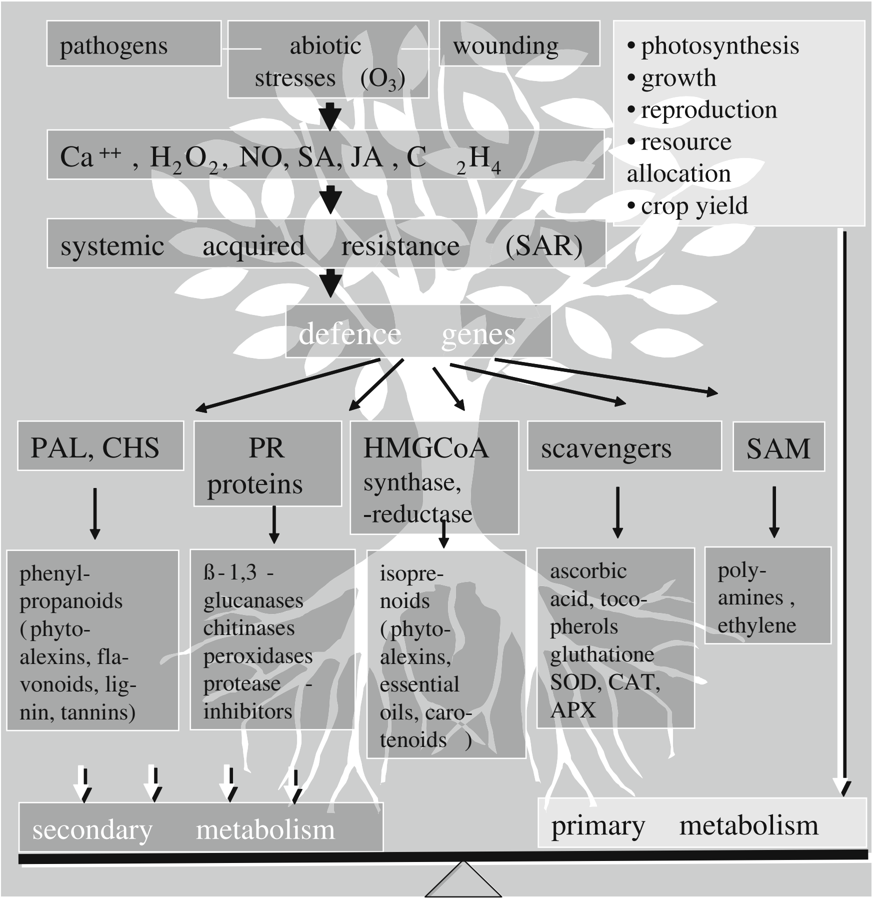


Fig. 11.7 Influence of different stresses on plant metabolism. The activation of systemic acquired resistance results in the accumulation of numerous defence compounds that may imbalance the equilibrium between primary and secondary metabolism, thus resulting in fitness costs for the plant. On the other end, the synthesis of secondary metabolites due to a stress may protect plant from other different stresses

same induced by pathogens, as well as the increased activity of key enzymes of the phenylpropanoid pathway have been reported in several plant systems (Figs. 11.3, 11.4, and 11.7). In Arabidopsis (*Arabidopsis thaliana*), PAL mRNA is rapidly and transiently induced within 3h of ozone treatment (300 nL L^{-1} daily for 6 h), reaching a 3-fold higher levels than control plants (Sharma and Davis 1994). A similar trend has been reported in parsley (*Petroselinum crispum*) plants, in which ozone treatment (200 nL L^{-1} for 10 h) induced an early 3-fold and 1.2-fold increase of PAL and CHS activity, respectively, followed by a 2-fold increase of total leaf furanocoumarins and flavone glycosides (Eckey-Kaltenbach et al. 1994). The content of psoralen, bergapten and other furanocoumarins in celery (*Apium graveolens*) dropped 24 h after ozone (0.2 nL L^{-1} for 2 h) fumigation, but levels of these chemicals, in treated leaves, increased rapidly at 120 h (Dercks et al. 1990). Further studies have shown an overlap between patterns of genes induced by ozone exposure and pathogen infection, probably due to the role of ROS as effector molecules involved in transduction pathways activated either by pathogens and ozone. The enhancement of SA content in plant tissues due to ozone treatment is well documented (Rao and Davis 2001). Intriguingly, in tobacco (*Nicotiana tabacum*) plants, a pulse ozone treatment (120–170 nL L^{-1} for 5 h) enhanced the emission of methyl salicylate, a volatile SA derivative, to a greater extent in sensitive cv. Bel W3 compared to tolerant cv. Bel B (Heiden et al. 1999). In Arabidopsis, SA accumulation, necessary for the expression of hypersensitive response to pathogens (HR) and SAR, is also required for the accumulation of some ozone-induced mRNAs, particularly PAL and pathogenesis related protein 1 (PR1) transcripts, although a SA-independent signal transduction pathway is activated by the pollutant (Sharma et al. 1996). In fact, in transformed *Arabidopsis* plants unable to accumulate these transcripts, ozone exposure induces resistance against the bacterial pathogen *Pseudomonas syringe*, a phenomenon known as cross induction (Sharma et al. 1996). HR is a type of programmed cell death (PCD) triggered at the attempted pathogen penetration site, frequently at the onset of systemic immunity (SAR) (Langebartels et al. 2002; Iriti and Faoro 2007), whereas PR proteins are enzymes induced in plants by pathogen infection as well as by abiotic and environmental stresses including ozone (Schraudner et al. 1992; Thalmair et al. 1996; Pääkkönen et al. 1998). In sensitive bean (*Phaseolus vulgaris*) cv. Pinto, ozone exposure (120 nL L^{-1} for 4 h) causes a stimulation of phenylpropanoid route and flavonoid branch, as shown by the increased mRNA accumulation of PAL, CHS and chalcone isomerase (CHI), the latter involved in isoflavonoid biosynthesis (Paolacci et al. 2001). In grapevine (*Vitis vinifera*), STS, the first enzyme of the stilbene branch involved in the synthesis of resveratrol and other stilbenic compounds, is considered the most sensitive ozone-induced biomarker (Schubert et al. 1997). In grapevine callus, either PAL or STS activity increase, after ozone fumigation (0.3 μmol mol^{-1} for 2 h) unlike CHS (Sgarbi et al. 2003). This could be due to the STS and CHS competition for the same substrate (Fig. 11.3), during the véraison (the berry ripening), when a metabolic switch occurs between the two branches of the same pathways. At this phenological stage, declined STS activity and resveratrol concentration in berries may cause grey mould (*Botrytis cinerea*) infection, whereas enhanced CHS activity and anthocyanin accumulation are required for berry colouring (Jeandet et al. 1995). Furthermore, a general drop in the amount of some assayed phenylpropanoids (coumaric acid, ferulic acid, gallic acid and catechin) has been reported unlike caffeic acid, whose level raised only in one cell line (Sgarbi et al. 2003). In 20 soybean (*Glyicine max*) cultivars, ozone tolerance was associated with the presence of kaempferol glycosides, a powerful antioxidant flavonol, as well as tolerance to manganese (Mn) toxicity in one soybean line (Foy et al. 1995). In European silver birch (*Betula pendula*) chronically exposed to ozone, it has been reported a 16.2% increase in total phenylpropanoids and a corresponding 9.9% increase of 10 compounds, among simple phenols and flavonoids, such as chlorogenic acid and catechin, respectively (Saleem et al. 2001). Interestingly, the combined action of CO_2 and ozone greatly enhances the synthesis of total and polymeric PA, in *Betula* sp. leaf tissues, suggesting an additive effect of these environmental pollutants on phenylpropanoid biosynthesis (Karonen et al. 2006).

Shikimate dehydrogenase (SKDH), a key enzyme of shikimate pathway, PAL and cinnamyl alcohol dehydrogenase (CAD), a key enzyme of lignin biosynthesis which forms monolignols (Fig. 11.4), have been investigated in poplar (*Populus tremula x alba*) leaves. Under ozone exposure (60–120 $\mathrm{nL\,L^{-1}}$, during the 14 h light period, for 1 month), either CAD activity and transcript levels were rapidly and strongly stimulated, increasing up to 15-fold and 23-fold the control values, respectively. In contrast, SKDH and PAL activities raised only in old and middle-aged leaves, but not in the youngest ones. Interestingly, the increased activity of these enzymes was associated with a higher lignin content in ozone-exposed leaves and additionally, the newly synthesized lignin structurally differed from the control lignin. Particularly, stress lignin appeared more condensed, i.e. enriched in carbon-carbon interunit linkages, in *p*-hydroxyphenyl (H) units and in terminal units with free phenolic groups (Fig. 11.4) (Cabané et al. 2004). The same enzymes have been studied in two genotypes of ozone-treated (150 $\mathrm{nL\,L^{-1}}$ for 3 h) tomato (*Lycopersicon esculentum*) plants (Guidi et al. 2005). However, while SKDH and PAL activity augmented significantly only in one line, CAD activity diminished in both the genotypes in contrast to the results reported by other authors (Galliano et al. 1993; Cabané et al. 2004). An explanation could reside in the different response of herbaceous and woody plants and in the different acute or chronic ozone dose employed in tomato and poplar, respectively.

To conclude, the importance of phenylpropanoids in plant tolerance against ozone injury is related to their different properties (Iriti and Faoro 2004). These compounds include an array of molecules with a plethora of biological activities, besides being precursors of structural biopolymer, such as lignin. Particularly, their protective role is mainly inherent to their antioxidant power, i.e. the ability to trap free radicals (ROS) functioning as electron donors. Nonetheless, increased synthesis of lignin, as well as structural modifications of lignin itself, represent another important defence mechanism in order to protect plasmalemma from the ROS injury, thus preventing membrane damages due to lipid peroxidation.

11.6 Ozone and Isoprenoids

Strictly speaking, not all the isoprenoids are secondary metabolites, as some primary metabolites, such as sterols, arise from isoprenoid pathway as well. The effects of the pollutant on either the sterol concentration and composition have been early reported in several plants (Tomlinson and Rich 1971; Trevathan et al. 1979; Grunwald and Endress 1985). Sterols are important component of cell membranes, involved in their stabilization. Generally, exposure to high ozone concentrations ($>12\,\mu L\,L^{-1}$) results in a decrease of free sterols (FS) and an increase in bound sterols (BS). Conversely, at low ozone concentration ($<12\,\mu L\,L^{-1}$), an accumulation of FS occurs, resulting in a decrease of the FS:BS ratio. In this case, the enhancement of FS synthesis is followed by a shift towards sterols with a more bulky C-17 side chain, i.e. sitosterol and stigmasterol vs. campesterol. As FS have a much greater capacity to stabilize membranes than BS, the FS:BS ratio has a more determinant effect on membrane permeability than the composition of FS fraction itself. Accordingly, a decrease in FS:BS ratio with high ozone concentration results in cell injury and visible damages, whereas modification of FS composition, occurring at lower ozone levels, results neither in permanent membrane injury nor in visible damages (Grunwald 1971; Evans and Ting 1973; Grunwald 1974). In tobacco leaves, ozone fumigation enhances total lipid concentrations, but it decreases levels of FS and triglycerides (Trevathan et al. 1979), whereas a higher amount of total phytosterols was reported in fumigated plants by other researchers (Menser et al. 1977).

In ozone treated ($300\,nL\,L^{-1}$ for 8 h) Scots pine (*Pinus sylvestris*), it was reported a transient increase of a transcript corresponding to the cytosolic/endoplasmic ß-Hydroxy-ß-methyl-glutaryl-CoA (HMG-CoA) synthase, a key enzyme of isoprenoid biosynthesis (Wegener et al. 1997). The C_5 precursor of isoprenoids, IPP, arises either from mevalonate pathway, in the cytosol, as previously described, or from plastidial precursors (Cheng et al. 2007). In ozone-treated pine seedlings ($250\,nL\,L^{-1}$, 12 h day^{-1} for 4 days), the biosynthesis of plastidial IPP was inhibited, differently from the mevalonate synthesis in the cytosol, due to the resource allocation between the two IPP synthesis pathways (Shamay et al. 2001).

Biogenic Volatile Organic Compounds (BVOC) comprise mainly isoprenoids (particularly hemi-, mono- and sesquiterpenes) emitted from plants during cell growth and in response to several kinds of stresses, they are having various ecophysiological functions and mediating plant-arthropod interactions (Fig. 11.8) (Kesselmeier and Staudt 1999). BVOC can act as attractants for pollinators or repellents for noxious insects, besides being involved in tritrophic signalling, i.e. the relationship between plant, herbivorous and carnivorous arthropods. In particular, phytophagus feeding can induce BVOC emission from plants, which can function as foraging cues for the recruitment of the natural enemies of herbivores (Dicke 2000). Besides wounding and trithrophic interactions, several environmental factors can affect the BVOC emission from plants, such as light intensity, temperature, water supply and pollutants (Fig. 11.8) (Peñuelas and Llusià 2001).

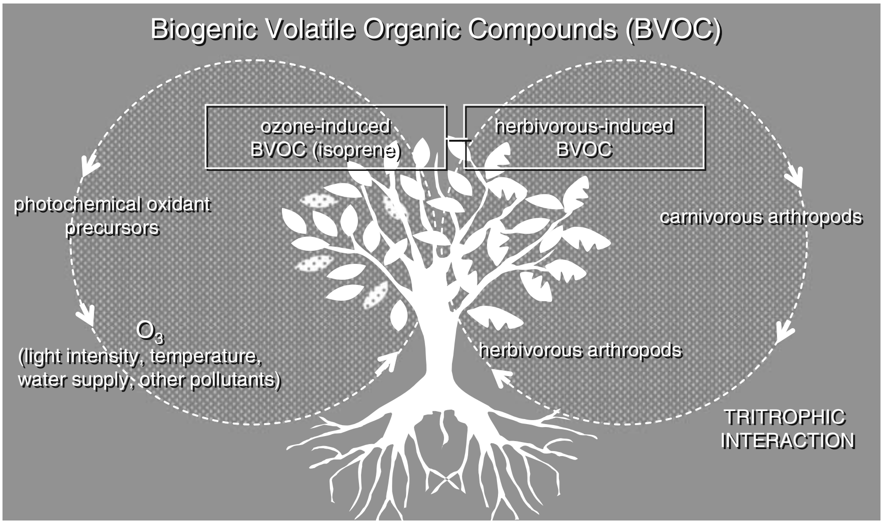


Fig. 11.8 Relationships between ozone and herbivorous-induced BVOCs and their influence on photochemical ozone precursors and on tritrophic interactions, respectively

The relationships between ozone and BVOC is somewhat complex, both exerting a mutual influence (Llusià et al. 2002). On one hand, the release of either BVOC or volatile organic compounds (VOC) from anthropogenic origin into the atmosphere can constitute a significant input of photochemical oxidant precursors, thus contributing to the regional-scale air pollution, on the other, BVOC emission can be triggered by the exposure to high ozone concentration (Roselle 1994; Heiden et al. 1999). Additionally, chronic ozone exposure can modify the composition of the plant BVOC emissions, thus not only affecting the tritrophic interactions, but also directly weakening plant defence responses against arthropods (Alstad et al. 1982). Isoprenoids can be synthesized and emitted in order to tolerate the ozone injury. Isoprene, a hemiterpene (Fig. 11.5), has been reported to reduce ozone damages in leaves, because of its antioxidant activity. This gas protects the photosynthetic apparatus, quenches ozone byproducts and radical species responsible for lipid peroxidation of cell membranes and cell death (Loreto and Velikova 2001; Loreto et al. 2001; Velikova et al. 2005). Besides, monoterpenes may exert an isoprene-like antioxidant activity, too (Loreto et al. 2004).

11.7 Ozone and Alkaloids

The effect of ozone on alkaloid biosynthetic pathways has not been extensively investigated, although a generalized influence of the pollutant on the nitrogen metabolism has been ascertained (Menser and Chaplin 1975; Aycock 1975; Jackson et al. 2000). Generally, ozone exposure reduces the amount of total alkaloids in tobacco plants (Menser and Chaplin 1969; Aycock 1975), and lower levels of nicotine, a pyridine alkaloid, in ozone exposed plants were related to increased survival, growth and development of hornworm (*Manduca sexta*) larvae (Jackson et al. 2000). Several studies reported increased preferences or enhanced survival and fitness of different insect species on ozonated plants, thus pointing out again the detrimental effect of the pollutant on the plant chemical defences (Jeffords and Endress 1984; Endress and Post 1985; Trumble et al. 1987; Chappelka et al. 1988; Heagle et al. 1994).

The role of polyamines, important alkaloid precursors (Fig. 11.9), has been correlated with ozone tolerance. Polyamines are polycationic nitrogenous compounds of low molecular weight ubiquitous in all living organisms. In plants, they function as growth regulators involved in an array of physiological processes, being involved in embryogenesis, cell division, morphogenesis, development, flowering and senescence (Martin-Tanguy 2001). In addition, they serve as an integral component of plant response to both biotic and abiotic stresses (Walters 2000;

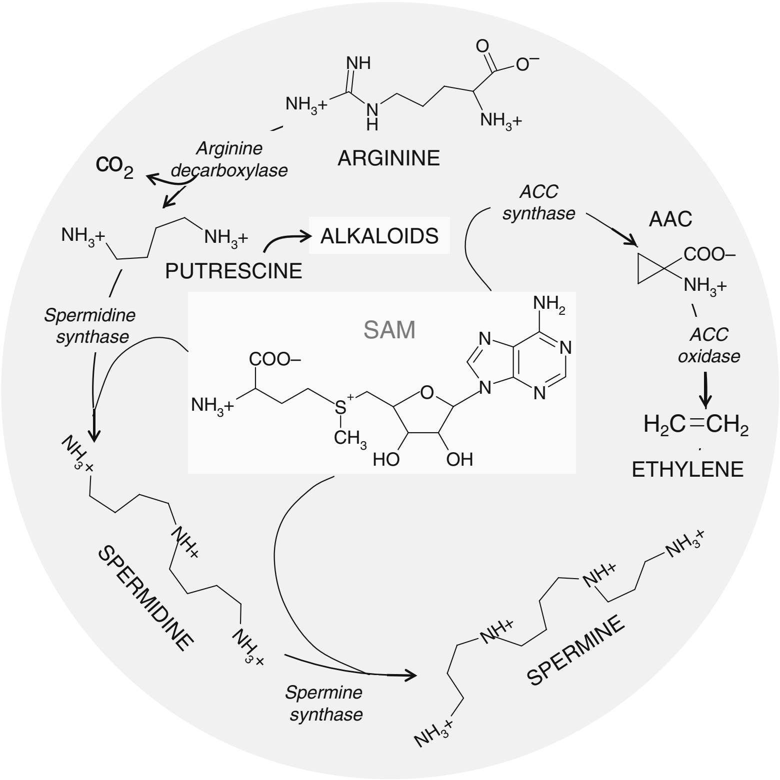


Fig. 11.9 Involvement of S-adenosylmethionine (SAM) in polyamine and ethylene biosynthesis. The metabolic shift to ethylene or polyamine biosynthesis can enhance ozone susceptibility or tolerance, respectively

Navakoudis et al. 2003; Liu et al. 2007). The most important polyamines are the diamine putrescine (Put), the triamine spermidine (Spd) and the tetraamine spermine (Spm), arising directly from the free non-proteinogenic amino acid ornithine, by ornithine decarboxylase (ODC), or indirectly from arginine by arginine decarboxylase (ADC). Further steps include Put conversion into Spd, via spermidine synthase (SPDS), and the similar synthesis of Spm from Spd via spermine synthase (SPMS). Either of two enzymes employed the aminopropyl moiety provided by S-adenosylmethionine (SAM) (Bagni and Tassoni 2001). In plants, ADC activity is enhanced by ozone exposure, whereas ODC remains unchanged (Rowland-Bamford et al. 1989; Langebartels et al. 1991). Free and conjugated polyamines improve ozone tolerance with two different mechanisms: (i) by inhibiting the ethylene biosynthesis and (ii) by direct ROS scavenging (Ormrod and Beckerson 1986; Bors et al. 1989). Ethylene and polyamines share the same biosynthetic precursor, SAM and thereby, they mutually inhibit their own biosynthesis. In particular, aminocyclopropane carboxylic acid (ACC), a precursor of ethylene, arises from SAM via ACC synthase, a rate-limiting step in ethylene production. Therefore, this metabolic shift to ethylene or polyamine biosynthesis can enhance ozone susceptibility or tolerance, respectively, due to the correlation between the stress ethylene production and visible ozone injury (Langebartels et al. 1991).

Additionally, apoplastic polyamines can form conjugates with hydroxycinnamates and phenolic acid derivatives, effective in ROS detoxification (Bouchereau et al. 1999). As reported above, polyamine are precursors of alkaloids and apart from it, they play a role in ozone tolerance. Higher amounts of these compounds, as a consequence of ozone exposure, could induce an increase of pyrrolidine, tropane and pyrrolizidin alkaloids, deriving from ornithine via Put.

11.8 Conclusion

In the global climate change scenario, the relation between the anthropogenic emissions of greenhouse gases, responsible for climate warming and rainfall alteration, and troposheric ozone is quite complex, specially in the agroecosystem context. Crop potential yield depends on defining factors (CO_2, radiation, temperature and crop traits), limiting factors (water, nitrogen and phosphorous) and reducing factors (pests, pathogens, weeds and pollutants) (Goudrian and Zadoks 1995). A deep knowledge of the role played by these factors under altered climatic conditions is necessary to evaluate the responses at crop and agroecosystem level (Fuhrer 2003).

It is well known that increasing troposheric ozone concentration at ambient CO_2, causes a decline in the yield of many crop species, and this negative effect is reduced in a CO_2-enriched atmosphere, probably due to the decrease of stomatal conductance and ozone flux, or to the increase in the activity of anti-oxidant enzymes (Heagle et al. 1998, 1999, 2000). Thus, the detrimental effect of enhanced CO_2 concentration on Heart temperature is in some way, compensate by its protective role in plants under ozone stress, as assessed by biomass and yield stimulation studies in these conditions (McKee et al. 1995, 1997).

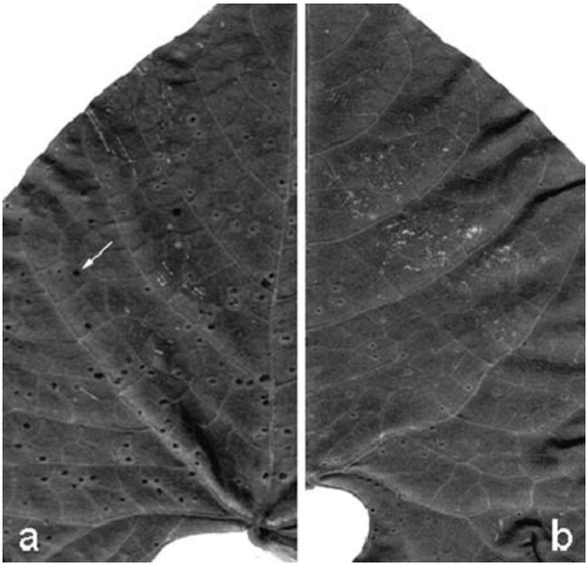


Fig. 11.10 Protective effect of ozone against *Uromyces appendiculatus*, causal agent of bean rust (Iriti and Faoro, unpublished). The leaf in (**b**) has been fumigated with ozone, 48 h before inoculation, and does not show any disease symptoms, while the control leaf in (**a**), only inoculated with the fungus, has developed typical rust pustules (*arrow*)

The influence of global climate change in plant-pathogen interactions is quite complex too (Violini 1995; Manning and von Tiedemann 1995). On one hand, ozone may induce the same sequel of events involved in plant immunity (Iriti and Faoro 2007) i.e. oxidative burst and hypersensitive response at the onset of systemic acquired resistance (SAR) (Fig. 11.10) (Langebartels et al. 2002), on the other, plants weakened by ozone stress may be particularly susceptible to infections (Manning and von Tiedemann 1995). Interestingly, wheat rust (*Puccinia recondita* f. sp. *tritici*) is strongly inhibited by ozone, but unaffected by elevated CO_2, both in presence or absence of ozone stress (von Tiedemann and Firsching 2000). Vice versa, a protective effect of rust (*Uromyces fabae*) infection in broad bean (*Vicia faba*) was reported against ozone, sulphur dioxide either alone or combined (Lorenzini et al. 1994).

Finally, regardless of the specific ecophysiological meaning, plants cope with the plethora of stressful abiotic and biotic conditions by modifying their secondary metabolic pathways. In this view, during the evolution, the chemical diversity improved the fitness of plant organisms, thus ensuring their evolutionary radiation. Phytochemicals, with their broad spectrum activities, are primarily involved in plant tolerance against environmental pollutants and worsening climatic conditions, as well as in resistance against pests and pathogens. The metabolic processes activated in these defence responses may be tightly separated or overlapping, according to the stress factor and the plant cultivar, as a result of negative (trade off) or positive (cross resistance) cross talk, that is to say the communication between molecular signals and transduction pathways involved in different plant defence responses. The most important consequence of this cross talk is the alert of defence mechanisms against an abiotic stress in consequence of priming with a biotic elicitor and vice versa.

Acknowledgments This work was funded by the Regione Lombardia, Piano per la ricerca e lo sviluppo 2003, d.g.r. 13077/2003 and ERSAF Lombardia.

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