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Review article

Oxidative metabolism is associated with physiological disorders in fruits stored under multiple environmental stresses

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ABSTRACT

In combination with low temperature, controlled atmosphere storage and 1-methylcyclopropene (ethylene antagonist) application are used to delay senescence of many fruits and vegetables. Controlled atmosphere consists of low O_2 and elevated CO_2. When sub-optimal partial pressures are used, these practices represent multiple abiotic stresses that can promote the development of physiological disorders in pome fruit, including flesh browning and cavities, although there is some evidence for genetic differences in susceptibility. In the absence of surface disorders, fruit with flesh injuries are not easily distinguished from asymptomatic fruit until these are consumed. Oxidative stress metabolites tend to accumulate (*e.g.*, γ-aminobutyrate) or rapidly decline (*e.g.*, ascorbate and glutathione) in vegetative tissues exposed to hypoxic and/or elevated CO_2 environments. Moreover, these phenomena can be associated with altered energy and redox status. Biochemical investigations of *Arabidopsis* and tomato plants with genetically-altered levels of enzymes associated with the γ-aminobutyrate shunt and the ascorbate–glutathione pathway indicate that these metabolic processes are functionally related and critical for dampening the oxidative burst in vegetative and fruit tissues, respectively. Here, we hypothesize that γ-aminobutyrate accumulation, as well energy and antioxidant depletion are associated with the development of physiological injury in pome fruit under multiple environmental stresses. An improved understanding of this relationship could assist in maintaining the quality of stored fruit.

Contents

1. Introduction

Metabolic activity leading to senescence is often minimized to maintain quality and prolong the supply period for fleshy fruits. This is done in storage by using low temperature often in combination with controlled atmosphere (*i.e.*, reduced O_2 and elevated CO_2 partial pressures), which lowers respiration and delays ethylene production and the onset of senescence-related disorders. Low temperature controlled atmosphere storage in commercial operations may be combined with chemical treatments such as 1-methylcyclopropene (1-MCP) to further inhibit ethylene-mediated ripening [1].

Storage recommendations are generally specific to fruit species and their cultivars, as well as geographic location [2]. By contrast, storage under low temperatures and O_2 and CO_2 partial pressures which are not optimal for prolonged duration can lead to the devel-

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Table 1
A summary of select storage-related disorders in pome fruit and their cultivars.

Physiological Disorder	Species/cultivars	Symptoms	Storage parameters	References
Soft scald	*Malus* × *domestica* Borkh. cv. 'Honeycrisp'	Smooth, irregularly shaped soft brown patches with defined edges on peel	0.5–3 °C	[4,5]
Soggy breakdown	*M. domestica* cv. 'Honeycrisp'	Initiates as diffuse browning of cortex, surrounded by barrier of healthy tissue Later, tissue becomes moist and spongy; vasculature undergoes intense browning	0–0.5 °C	[5,6]
Vascular breakdown	*M. domestica* cv. 'Cortland' cv. 'McIntosh'	Browning of vascular bundles which can extend into surrounding cortical tissue	0 °C	[4,7]
Core browning	*M. domestica* cv. 'Cortland' cv. 'McIntosh'	Brown, necrotic flesh around the core	0–3 °C	[4,8]
Senescent breakdown	*M. domestica* cv. 'Cortland' cv. 'McIntosh' cv. 'Macoun'	Brown and soft flesh tissue that initiates underneath peel Largeportions of flesh tissue can become dry	1–3 °C	[4,8,9]
Superficial scald	*M. domestica* cv. 'Granny Smith' cv 'Cortland' cv 'Law Rome' cv. 'Delicious'	Irregular brown patches of peel with no effect on underlying flesh tissue	0–4 °C; some symptoms can occur up to 25 °C	[4,10 and references therein]
	*Pyrus communis*L. cv. 'Bartlett' cv. 'd'Anjou'	Associated with collapse of hypodermal cells culminating in skin browning		
Senescent scald	*P. communis* cv. 'Bartlett'	Dark brown discolouration of the peel, which is associated with aging Fruit remains firm	0–1 °C; can develop following the removal of fruit from storage	[4]
Flesh browning	*M. domestica*	Firm browning of cortical tissues	0.5 °C, 3 kPa O_2, 2 kPa CO_2	[11]
	cv. 'Empire'			
	M. domestica cv. 'Pink Lady'	Brown patches within flesh, which can develop into cavities	0.5 °C, 1.5 kPa O_2, 5 kPa CO_2	[12]
	M. domestica cv. 'Braeburn'	Cortical browning that can be accompanied by lens-shaped cavities In some cases, browning can extend to peel	0.5 °C, 2 kPa O_2, 2-5 kPa CO_2	[13]
Low-O_2 injury	*M. domestica* cv. 'Cox's Orange Pippin' cv. 'McIntosh' cv. 'Delicious'	Areas of peel turn purple or become water soaked and brown, regardless of blush Injury can extend into sub-epidermal region	<1.5 kPa O_2	[4]
Controlled atmosphere-related injury	*M. domestica* cv. 'Honeycrisp'	Irregular browning of cortex, which can be associated with lens-shaped cavities	3 °C, 1–4.5 kPa O_2, 0.5–3 kPa CO_2; can occur to a small extent at 21 kPa O_2	[14,15]
Internal CO_2 injury (also known as brown heart)	*M. domestica* cv. 'Fuji'	Dark brown flesh that can be accompanied by cavities	0.5 °C, 0.5 kPa O_2, 3 kPa CO_2	[16,17 and references therein]
	P. communis cv. 'Conference'	Brown cortical tissue and/or cortical cavities	−0.5 °C, 2 kPa O_2, 5 kPa CO_2	
Core break-down/internal break-down/internal browning	*P. communis* cv. 'Conference' cv. 'Bartlett'	Initiates as water soaked and brown core tissue, followed by browning and cavitation within surrounding tissues	0.5–3 kPa O_2 with or without 10 kPa CO_2	[4,17 and references therein,18]
External CO_2 injury	*M. domestica* cv. 'Empire' cv. 'McIntosh'	Rough, brown uneven sunken lesions of the peel	2–2.5 kPa O_2, 2–5 kPa CO_2; can be exacerbated by 1-MCP	[19,20]

opment of physiological disorders in temperate fruits ([3]; Table 1), including two of the most highly cultivated tree fruits in the world, apple (*Malus* × *domestica* Borkh.) and European pear (*Pyrus communis* L.) (Food and Agriculture Organization of the United Nations: Statistics, FAOSTAT 2012). Visible symptoms of a select number of physiological disorders associated with storage under these multiple environmental stresses are summarized in Table 1, including for new germplasm (*i.e.*, 'Honeycrisp' apples) for which no consistent controlled atmosphere storage recommendations exist. The predominant chilling injuries in apple fruit include core browning,

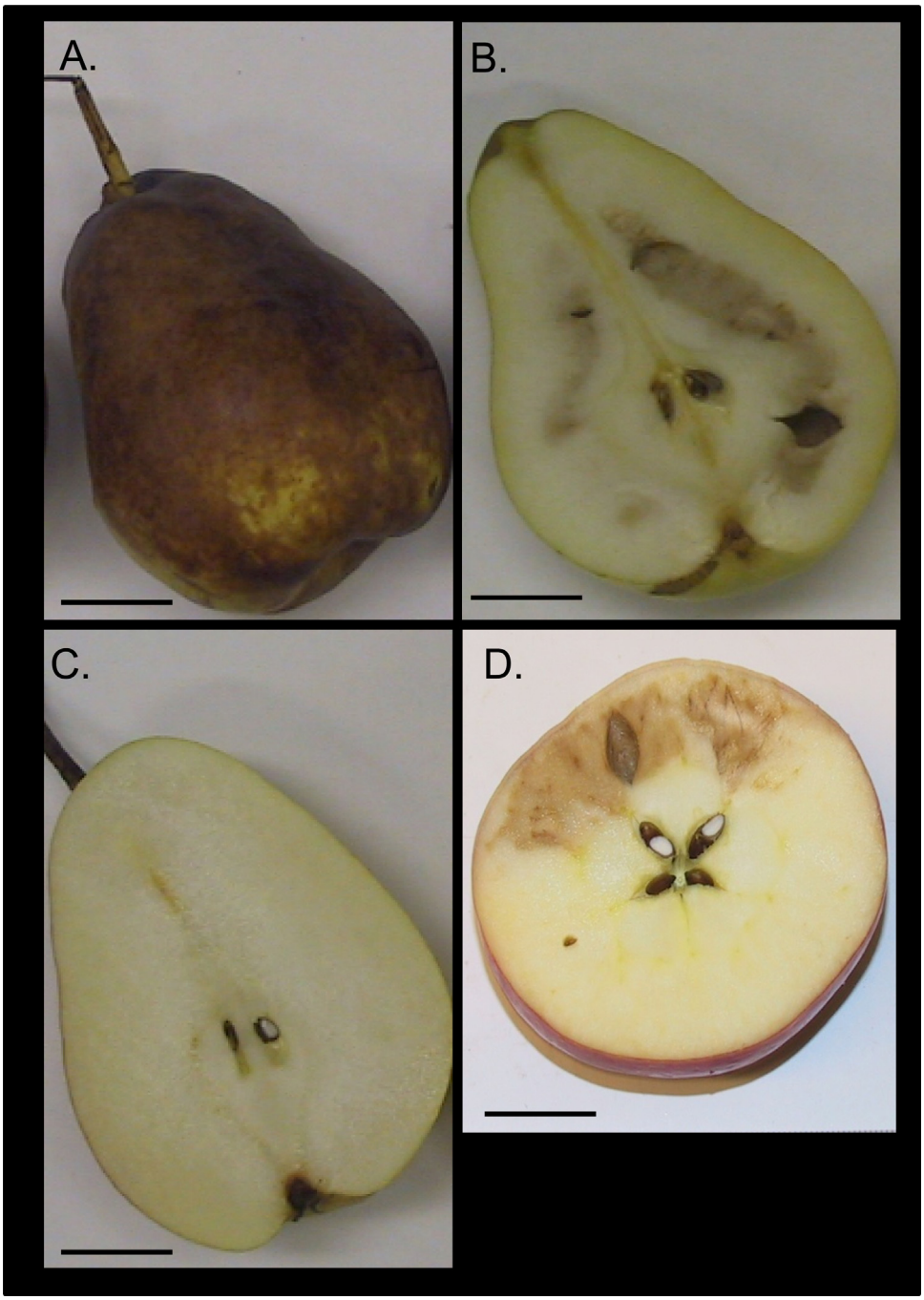

Fig. 1. Physiological injuries in apple and pear fruit associated with postharvest handling and storage.
(A) A representative cv. 'Harovin Sundown' pear with symptoms of senescent scald after 180 days at 0 °C and refrigerated air. (B) Cv. 'AC Harrow Crisp' and (C) cv. 'Harovin Sundown' pear fruit after 1-MCP treatment and prolonged storage (170 and 180 days, respectively) at 0 °C, 2.5 kPa O_2 and 2 kPa CO_2. Under these conditions; the cv. 'AC Harrow Crisp' pear has brown flesh and cavities symptomatic of internal breakdown and internal CO_2 injury, whereas the cv. 'Harovin Sundown' pear is free of visible symptoms for these disorders. (D) A representative cv. 'Honeycrisp' apple after pre-storage conditioning at 10 °C for 5 days and 35 weeks at 3 °C, 2.5 kPa O_2, and 2.5 kPa CO_2, displaying flesh browning and cavities typical of internal CO_2 injury (also known as controlled atmosphere-related injury). Scale bar at the bottom of each panel = 2 cm.

flesh/internal browning, soft scald, soggy breakdown, and vascular breakdown (Table 1). For some genotypes, multiple chilling injuries can occur simultaneously, especially in fruit of advanced maturity [6]. European pear fruits are susceptible to senescent scald during prolonged storage (Table 1; Fig. 1). Superficial scald can occur in both pome fruits.

Low O_2 disorders of apples are prevalent at partial pressures <1.5 kPa O_2, whereas pear injuries can occur up to 3 kPa O_2 (Table 1). Core breakdown of pear fruits is low O_2-related and symptoms are synonymous with those of internal breakdown and internal browning (Table 1); these injuries are proposed to develop as a consequence of a decreasing O_2 gas gradient towards the fruit center [17,21]. Most apple and pear cultivars are susceptible to some type of CO_2 injury, which is visible in one or all of the peel, cortex and core tissues (Table 1). High CO_2-inducible flesh disorders include browning of the vascular bundles, carpel interior

walls and core tissue and cavity formation (Fig. 1). Cavities may be evident with controlled atmosphere-related injury and internal CO_2 injury of apples, as well as pear fruit with symptoms of core breakdown, internal breakdown and internal browning (Table 1). Cultivars 'Gala', 'Golden Delicious', and 'Jonagold' tend to be less susceptible to CO_2 injury [2,21], suggesting that a genetic basis exists for sensitivity to this controlled atmosphere parameter.

Knowledge of the biochemical and molecular responses of apple and European pear fruits to controlled atmosphere storage, together with recent sequencing of their genomes [22,23], would establish these as model systems for studying physiological injuries and multiple environmental stresses during postharvest storage. Unravelling the genetic basis of storage disorders could provide key diagnostic information for genetically-related Rosaceous species, including cherry, peach, plum, and strawberry. In this review, apple and pear fruits are used, in combination with other model organisms such as *Arabidopsis* and tomato, to link physiological injury with oxidative stress metabolism. Knowledge about the temporal relationships among γ-aminobutyrate (GABA), γ-hydroxybutyrate (GHB), and NAD(P)H/NAD(P)$^+$, ascorbate/dehydroascorbate and glutathione/glutathione disulphide redox ratios could help in elucidating mechanisms responsible for storage-induced physiological injuries in apples and pears, and fleshy fruits in general.

A combination of low O_2 and elevated CO_2 partial pressure results in hypoxia within the core of the pear [17], which can lead to increased production of stress response compounds such as putrescine and trehalose in brown tissue [18]. Hypoxia in pears, as well as core and flesh disorders, have been linked with inhibition of respiratory processes [18], declines in whole fruit adenosine triphosphate (ATP) levels and ATP/adenosine diphosphate (ADP) ratios, and production of fermentation-derived ethanol and acetaldehyde [24], which may be considered as signatures of oxidative stress (Fig. 2).

2. Oxidative stress metabolism

Membrane damage and a subsequent loss of cellular and subcellular compartmentation often result in enzymatic oxidation of phenolic compounds to brown colored polymers [17,25]. Most plant polyphenol oxidases have broad substrate specificity; however, a polyphenol oxidase from cv. 'Fuji' apples prefers chlorogenic acid [25], which is present at substantial levels in apple fruit [26]. The level of chlorogenic acid is 60% less in 1-MCP-treated apples of cv. 'Delicious' stored at 1 °C for 4 months than in untreated fruit [26]. Also, cultivar-specific differences in controlled atmosphere-induced polyphenol oxidase-mediated browning are known [27]. Recently, a Canadian biotechnology company, Okanagan Specialty Fruits, used RNA interference technology to develop a new cultivar 'Arctic' that under-expresses two *POLYPHENOL OXIDASE* genes, resulting in fruits that do not brown upon slicing [28]. Similarly, traditional breeding approaches have identified a non-browning apple cultivar, 'Eden' [29]. It is unclear whether fruits of these cultivars are resistant to storage-related flesh browning. Polyphenol oxidase activity represents a biochemical mechanism coincident with physiological browning of damaged and/or stored fruit. By comparison, metabolic processes promoting the onset of physiological disorders during storage, including CO_2-related cavitation of fruit flesh, are little understood (Fig. 1).

2.1. Energy metabolism during controlled atmosphere storage

Susceptibility of pome fruit to controlled atmosphere disorders can be attributed, at least in part, to cultivar differences in fruit ultrastructure and tissue density, both of which appear to influence O_2 diffusion from peripheral to central tissues [21]. For example, measurements of internal gas gradients and gas flux from the outer environment to fruit center indicate that O_2 diffusion is unrestricted in cv. 'Jonagold' apples, which tend not to develop controlled atmosphere-induced flesh browning. By contrast, anoxic conditions are possible within cv. 'Braeburn' apples, which are susceptible to this disorder. Diffusion of O_2 through apple parenchyma tissue is confined to intercellular spaces, whereas CO_2 diffusion into cortical cells is unrestricted as it has higher solubility in aqueous solutions than O_2, resulting in higher CO_2 concentrations at the fruit center than the periphery [30]. Pears maintained under controlled atmosphere display an O_2 gradient that is approximately 90% lower at the center than the surface [17,and references therein], and flesh browning is associated with reduced ATP level and ATP/ADP ratio in the whole fruit [31]. More recently, it was proposed that localized ATP deficiency most likely occurs in apple fruit tissues susceptible to hypoxia during controlled atmosphere storage [32]. Similarly, a decreased adenylate energy charge, expressed as ([ATP] + ½])/([ATP] + [ADP] + [adenosine monophosphate]), is associated with a switch to fermentative metabolism in strawberry fruit exposed to elevated CO_2 for 3 days [33].

Conditioning of pears at 0 °C under ambient air for 21 days increases the ATP level and adenylate energy charge compared to fruit transferred directly to controlled atmosphere [31], suggesting that preservation of energy constituents can offset controlled atmosphere-related injuries. A decline in adenylate energy charge occurs simultaneously in O_2-deprived plant tissues with acidification and elevated Ca^{2+} levels within the cytosol, increased membrane disorganization, and over-production of reactive oxygen species (ROS) [34]. The impact of cytosolic acidification and increased Ca^{2+} concentration on controlled atmosphere-stored fruit might be expressed as altered levels of amino acid-derived GABA and GHB, as well as shifts in the ratio of reduced/oxidized form of nicotinamide adenine dinucleotide phosphate (NADPH/NADP$^+$) cofactors.

2.2. GABA, GHB and pyridine dinucleotides

The physiological roles of the non-proteinogenic amino acid, GABA, and its catabolite, GHB are subjects of much investigation [35–37]. Of particular interest is their accumulation in various plant tissues and parts exposed to abiotic stresses such chilling, low O_2, elevated CO_2 and mechanical stimulation [35,36]. GABA accumulation can involve cytosolic acidification-mediated stimulation of glutamate decarboxylase activity, Ca^{2+}/calmodulin activation of glutamate decarboxylase at neutral pH, or the differential up-regulation of *GLUTAMATE DECARBOXYLASE* expression (Fig. 2). Interestingly, the expression of GABA catabolism genes, *GABA TRANSAMINASE* and *GLYOXYLATE/SUCCINIC SEMIALDEHYDE REDUCTASE* (*AtGLYR*), are relatively unaffected in *Arabidopsis* by stress, suggesting that GHB accumulation is regulated by biochemical mechanisms [38]. GABA transaminase catalyzes pyruvate- or glyoxylate-dependent transamination of GABA to yield succinic semialdehyde, and can be located in the cytosolic, plastidial or mitochondrial compartment, depending on the plant species [38,39]. Succinic semialdehyde, a toxic aldehyde, is oxidized by succinic semialdehyde dehydrogenase to the tricarboxylic acid cycle intermediate, succinate, thereby completing a series of bypass reactions, known as the GABA shunt. As succinic semialdehyde dehydrogenase activity is dependent upon oxidized nicotinamide adenine dinucleotide (NAD$^+$), but inhibited by the reduced form NADH [38], abiotic stresses (*e.g.*, chilling, extreme heat and submergence) that result in elevated ratios of NADH/NAD$^+$ and NADPH/NADP$^+$ would divert the pool of succinic semialdehyde to an NADPH-regulated enzyme, succinic semialdehyde reductase, leading to the accumulation of GHB [35]. Notably, *succinic semialdehyde dehydrogenase* knockout mutants of *Arabidopsis* are dwarfed

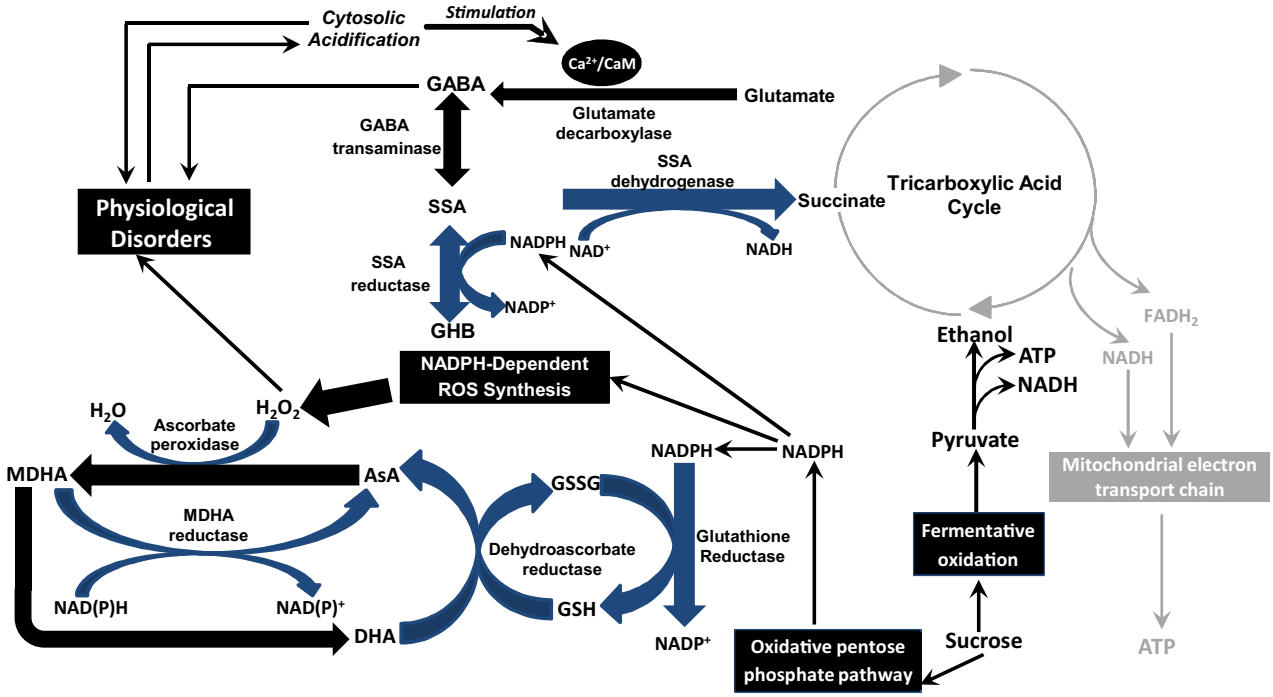

Fig. 2. Hypothetical relationship between the development of physiological disorders and oxidative stress metabolism in pome fruit.
γ-Aminobutyrate (GABA) formation *via* glutamate decarboxylase occurs in response to controlled atmosphere stress. GABA catabolism to γ-hydroxybutyrate (GHB), the production of reactive oxygen species (ROS; *e.g.*, H_2O_2) and the dissipation of the latter by the ascorbate (AsA)-glutathione (GSH) pathway are influenced by an increase in reduced nicotinamide adenine dinucleotide phosphate (NADPH). The ascorbate–glutathione pathway involves the cooperative functioning of ascorbate peroxidase, monodehydroascorbate (MDHA) reductase, dehydroascorbate reductase and glutathione reductase. For each turn of the cycle, two molecules of ascorbate are utilized by ascorbate peroxidase for H_2O_2 reduction to water and MDHA [65]. MDHA, a radical intermediate, is spontaneously converted to dehydroascorbate (DHA), which is in turn reduced to ascorbate *via* dehydroascorbate reductase. The conversion of MDHA to ascorbate can be promoted by NADPH-dependent MDHA reductase activity. GSH serves as a reductant for the conversion of DHA to ascorbate, resulting in the formation of oxidized GSH or glutathione disulphide (GSSG); GSSG is readily reduced by an NADPH-dependent glutathione reductase, yielding two molecules of GSH. It is proposed that GABA accumulation and declining ascorbate and GSH redox ratios may precede the onset of physiological disorders in controlled atmosphere-stored fruit, although cellular collapse associated with tissue browning may coincide with the aforementioned metabolic phenomena. Grey arrows and metabolites indicate multi-step pathways that are probably dysfunctional during controlled atmosphere storage, whereas black arrows indicate reactions induced during low temperature controlled atmosphere and blue arrows indicate limited operation of these reactions under prolonged low temperature controlled atmosphere conditions. Other abbreviations include: CaM, calmodulin; H_2O_2, hydrogen peroxide; NAD^+, oxidized nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; SSA, succinic semialdehyde.
This scheme was adapted from [38] and [67].

and possess elevated levels of ROS in response to UV-B light and heat stress, suggesting that the GABA shunt is essential for the control of these oxidative intermediates [40]. By comparison, *glyr1* and *glyr2* knockout mutants have a normal phenotype and reduced levels of GHB, suggesting that GHB can be derived from succinic semialdehyde *via* both GLYR isoforms *in vivo*, despite the preference of GLYRs for glyoxylate [36]. An alternative pathway for GABA formation involves the catabolism of the polyamines, especially putrescine. Putrescine accumulates in *Arabidopsis* in response to chilling and salinity and is catabolized to γ-aminobutyraldehyde by O_2-dependent amine oxidases, and then to GABA *via* NAD^+-dependent aminoaldehyde dehydrogenase activity [41–43].

The occurrence of physiological injury in controlled atmosphere-stored or 1-MCP-treated fruit is associated with the accumulation of GABA. For example, tissues associated with chilling-related soggy breakdown in cv. 'Honeycrisp' apple flesh contain higher levels of GABA and membrane-derived triacylglycerides than asymptomatic tissues during storage at 1 °C for 12 weeks [44]. Here, GABA accumulation is most likely due to stimulation of glutamate decarboxylase activity by cytosolic acidification, which is expected to occur with cellular disintegration. Low-O_2-induced core breakdown in pear fruit is associated with higher relative levels of GABA and fumarate and a lower relative level of malate than sound tissue, indicating putative blocks in the GABA shunt and tricarboxylic acid cycle [18]. The level of GABA increases in cv. 'Empire' apple fruit following treatment with 1-MCP and/or controlled atmosphere storage [45], and declines rapidly upon transfer of the fruit to ambient conditions [39]. The increase in GABA level in controlled atmosphere-stored cv. 'Empire' apples is correlated with declining glutamate, but not well correlated with the levels of polyamines, including putrescine, over a 46 week storage period [45]. It has been argued that a polyamine catabolic route to GABA in pome fruit during controlled atmosphere would likely be limited by O_2 and NAD^+ availability [41–43]. In some cases, GABA levels seem to increase prior to flesh browning in 1-MCP-treated cv. 'Empire' apples [46] and in controlled atmosphere-stored cv. 'Braeburn' apples without or with diphenylamine treatment [47]. Diphenylamine reduces internal browning in cv. 'Braeburn' apple fruit [47]. Elevated GABA levels occur in tomato, cherimoya and melon fruits during the first few days of exposure to elevated CO_2 with no evidence of coincident disorders [48–51] and in broccoli florets prior to the onset of discoloration [52]. GABA levels are also reduced in cherimoya fruit and broccoli florets after removal from elevated CO_2 storage [48,52].

GABA hyperaccumulation and rapid catabolism are well known in tomato fruit [53]. GABA levels in tomato fruit are positively correlated with the expression of *SlGLUTAMATE DECARBOXYLASE2* and *SlGLUTAMATE DECARBOXYLASE3* before ripening [54] and negatively correlated with the expression of *SlGABA TRANSAMINASE1*

during ripening [55]. Also, overexpression of the mitochondrial *GABA TRANSAMINASE* in *Arabidopsis* reduces the GABA level by 30%, compared to wild-type plants, during cold shock (1 h exposure to 4 °C), whereas no effect is apparent in non-stressed plants, suggesting that GABA transaminase catabolizes stress-produced GABA [56]. Moreover, germinating seedlings of the *Atgaba transaminase* knockout mutant display a white bleached phenotype on minimal medium supplemented with GABA as the sole nitrogen source, and a shorter life cycle than when grown without nitrogen, implying that GABA transaminase is involved in a GABA signaling pathway [57]. Overall, these findings suggest that GABA transaminase plays an important role in the fate of GABA accumulated in controlled atmosphere-stored fruit.

GABA accumulation in high CO_2-treated vegetative and fruit tissues is proposed to counteract cytosolic acidification upon CO_2 dissolution [58]. Cytosolic acidification-mediated glutamate decarboxylase stimulation of GABA production may precede or coincide with CO_2-related physiological disorders in apples and pears. Since exposure of tomato fruit to elevated CO_2 increases the levels of both GABA and *GLUTAMATE DECARBOXYLASE* expression, GABA could serve as an early biochemical signature of controlled atmosphere stress [51,59]. Brief immersion of freshly harvested peach fruit in 5 mM GABA reduces chilling injury by 89% after a 5-week storage period and enhances the activities of the ROS-dissipating enzymes, ascorbate peroxidase and glutathione peroxidase [60], suggesting that GABA mediates an early antioxidant response to chilling. Moreover, a link between GABA and the dampening of the oxidative burst is apparent for salinity stress, as GABA treatment of *Caragana intermedia* roots appears to inhibit salt-induced changes in gene expression, including those associated with ROS formation [61]. Conversely, GABA-mediated inhibition of ROS production in hypoxic grapevine buds is not associated with widespread up-regulation of ROS-dissipating enzymes [62]. Other research has shown that GABA, GHB and ROS levels are markedly increased in *succinic semialdehyde dehydrogenase* mutants of *Arabidopsis* plants subjected to high light stress [63]. Tomato fruit from RNA*i* plants lacking L-galactono-1,4-lactone dehydrogenase, the terminal ascorbate biosynthesis enzyme, contain elevated levels of GABA and altered ascorbate/dehydroascorbate ratios [64]. Together, these findings suggest that GABA biosynthesis and catabolism are affiliated with shifts in redox balance and ROS metabolism in various plant tissues, but its precise influence on oxidative stress metabolism in response to controlled atmosphere and/or 1-MCP treatment of pome fruit remains unknown.

2.3. Antioxidants

Membrane lipid peroxidation, electrolyte leakage and adenylate depletion are hallmarks of O_2-deficient plant tissues [34]. Peroxidation of membrane lipids is initiated by elevated levels of ROS, including H_2O_2, superoxide (O_2^-) and hydroxyl radicals ($OH^\bullet$), which result from incomplete reduction of molecular O_2 [17]. Under ambient conditions, plant tissue ROS levels range from 0.05 to 5 μmol g^{-1} fresh mass, and their increase by abiotic and biotic stresses is accompanied by altered NADPH redox status within the cell [65,66]. ROS formed in stressed plants *via* a plasma membrane-bound NADPH oxidase can override ROS dissipation capacity of cells, resulting in protein oxidation and DNA damage, as well as peroxidation of membrane lipids and organelle disruption [67]. ROS in non-green tissues is predominantly mitochondrial in origin, whereas the generation of ROS from photosynthetic electron transport and photorespiratory glycolate oxidase is light-dependent [68]. Alternatively, the catabolism of polyamines could serve as a source of ROS in vegetative tissues, as both polyamine oxidases and amine oxidases are O_2-dependent steps that generate H_2O_2 as a by-product [41]. It is worth noting that polyamine and amine oxidases are presumably non-functional in low O_2-stored fruit [41]. Thus, polyamine catabolism-derived ROS is probably not associated with the hypoxic environment within controlled atmosphere-stored fruit; however, the involvement of a mitochondrial-derived or NADPH oxidase-mediated production of ROS seems plausible.

Regardless of their origin, ROS accumulation could promote the loss of membrane integrity which occurs with flesh browning in cv. 'Pink Lady' apples following controlled atmosphere storage [12]. There is 20% less ROS in 1-MCP-treated cv. 'Blanquilla' pear fruit than in untreated fruit after cold storage [69]. This is accompanied by greater activity of the ROS-dissipating enzymes catalase and ascorbate peroxidase [69], suggesting that antioxidant metabolism has a role in preventing chilling injury.

In situ detection of ROS in plant tissues is performed by histochemical staining with diaminobenzidine or dichlorodihydrofluorescein diacetate based dyes [66,70,71]. For example, 2′,7′-dichlorodihydrofluorescein diacetate fluorescence staining detects ROS in the peel of cv. 'Granny Smith' affected by superficial scald [70]. Chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester stains for ROS in onion leaves and bulb scales, as well as *Arabidopsis* tissues and protoplasts [71]. Alternatively, H_2O_2 in plant tissue extracts can be quantified *in vitro* by chemiluminescence with a peroxidase-dependent assay [66]. Accurate quantification of ROS in plant extracts has been difficult because of the short life span for ROS, ascorbate inhibition of enzyme-mediated assays for determining ROS, and non-specificity and low sensitivity of ROS assays [66]. Therefore, identification of alternative and more reliable indicators of oxidative stress such as antioxidants (*e.g.*, ascorbate, glutathione, and total antioxidant capacity) or ROS-scavenging enzymes such as superoxide dismutase, ascorbate peroxidase and catalase would be desirable. Cv. 'Delicious' apples have a higher total antioxidant activity than cv. 'Empire' fruit [72], possibly explaining why cv. 'Delicious' is less susceptible to storage injuries than cv. 'Empire. However, assays for total antioxidant potential have the potential to be inhibited by sample-associated interfering molecules such as sugars, organic acids, carotenoids, aromatic amines and some amino acids [73]. The differential sensitivity and specificity for individual metabolites (*i.e.*, glutathione) [73] may lead to an underestimation of plant tissue antioxidant species. Diphenylamine, a synthetic antioxidant, has the capacity to control internal browning disorders in controlled atmosphere-stored apples, while inhibiting the accumulation of amino acids, acetaldehydes, ethanol and ethyl-ester volatile compounds [47]. The beneficial effect of diphenylamine suggests that controlled atmosphere-related stress can be controlled by maintaining redox balance.

2.4. Ascorbate and glutathione

Ascorbate and glutathione are directly oxidized by ROS, or indirectly *via* coupling reactions of the ascorbate–glutathione recycling pathway (Fig. 2) [65,67]. The net result of the ascorbate–glutathione cycle is the regeneration of reduced forms of ascorbate and glutathione, and the reduction of ROS to a less volatile form, usually in the form of water or an alcohol compound [67,and references therein]. The coordinated interaction of glutathione reductase and ascorbate peroxidase ensures that the endogenous glutathione and ascorbate pools are maintained at the high levels required for efficient operation of the antioxidant system within the plant cell [34]. This recycling is primarily dependent upon the cellular availability of NADPH (Fig. 2). For fruit stored under low O_2, NADPH could be formed from the oxidative pentose phosphate pathway. This pathway is the primary source of reductant in non-photosynthetic tissues and is dependent upon the conversion of sucrose to glucose 6-phosphate [74]. Under low O_2, dehydroascorbate and glutathione disulphide tend to accumulate, most likely due to the decline in

interconversion to their reduced forms [34]. This shift in redox balance could be due in part to NADPH utilization by NADPH oxidase to generate ROS [67].

The sum total concentration of reduced and oxidized ascorbate in photosynthetic tissues is 1–10 μmol g^{-1} fresh mass, the highest of all redox pairs, whereas the concentration of the glutathione redox pair is 0.1–1 μmol g^{-1} fresh mass [65]. By contrast, ascorbate concentrations in fruit are on the order of 0.11–73 μmol g^{-1} fresh mass [75]. At harvest, apples contain approximately 0.65–2 μmol ascorbate g^{-1} fresh mass, whereas the concentration in pear fruit is approximately 0.43–1.7 μmol ascorbate g^{-1} fresh mass [76]; cultivar differences have been reported [76,77]. The higher levels of this reductant in illuminated tissues relative to pome fruit could be due to greater availability of NADPH relative to the hypoxic conditions in bulky fruit; it is tempting to speculate that NADPH is required in photosynthetic tissues to drive the ascorbate–glutathione cycle as ROS production could be exacerbated by photo-oxidative processes. Typically, ascorbate levels of apple peels from freshly harvested fruit are approximately 80 to 180% greater than those in flesh and core tissues [78]. A silver staining procedure suggested that ascorbate is confined to seeds and surrounding cortical tissue, as well as periphery of brown tissue in controlled atmosphere-stored apples of cv. "Braeburn" [79], but this procedure may suffer from uneven penetration of the stain through cortical and vascular tissues. While evidence exists for multiple anabolic pathways for ascorbate in plants, including apple fruit, there is little information on the relative importance of anabolic, catabolic and transport processes on the cellular ascorbate level [78].

The utility of cold storage for different apple cultivars is correlated with the maintenance of total ascorbate and glutathione levels [77]. The ascorbate levels of peel and cortex of cv. 'Delicious', cv. 'Golden Delicious' and cv. 'Fuji' apple fruit in particular, decline within 1 month of cold storage [80]. Furthermore, reduced and oxidized ascorbate are 95% lower and 140% higher, respectively, in brown tissue of controlled atmosphere-stored cv. 'Pink Lady' apple fruit than in healthy tissue [12]. The ascorbate level in controlled atmosphere-stored pears declines within weeks and this has been associated with internal/core browning [81]. Under low O_2, the ascorbate level is greater in the non-damaged tissue bordering the brown flesh tissue of pears displaying core breakdown, which is consistent with anoxia at the fruit core [17,81]. Metabolome analysis has revealed that the ascorbate catabolite threonate accumulates in this brown tissue, suggesting that the ascorbate–glutathione recycling pathway is dysfunctional in controlled atmosphere-stressed fruit; this is most likely the result of a depletion of cellular reducing equivalents (*i.e.*, NADPH) required to regenerate glutathione for the dehydroascorbate reductase-mediated conversion of dehydroascorbate to ascorbate [18]. However, the threonate detected in the methanolic extracts could be due in part to spontaneous breakdown of dehydroascorbate *in vitro*, a phenomenon that is not without precedent [82], and it is unclear whether the extraction efficiency for ascorbate and/or dehydroascorbate was established. Metabolomics-driven approaches can suffer from many issues related to sample preparation and storage (*e.g.*, mechanical handling, absence of rapid freezing, storage at −20 rather than −80 °C), replication (*e.g.*, use of technical or biological replication rather than experimental replication), and quantification (*e.g.*, inconsistent instrument performance, analysis of complex mixtures in a non-linear range of detection, incomplete extraction of metabolites, instability of metabolites) [38,83]. Moreover, these high throughput technologies do not provide reliable quantitative data, and can only be used to determine relative changes as a function of storage period and/or treatment.

Like ascorbate, the pathway for glutathione biosynthesis in plants is well described [84]. Glutathione biosynthesis involves conjugation of glutamate to cysteine in the presence of a plastid glutamate-cysteine ligase, and subsequent addition of glycine by an ATP-dependent glutathione synthase. Approximately 30 and 50% of the total cellular glutathione in *Arabidopsis* is distributed in the vacuole and cytosol, respectively [85], and the glutathione level in plant tissues is markedly influenced by the activity of glutamate-cysteine ligase and availability of cysteine [86]. Glutathione interacts directly with ROS, or indirectly *via* the ascorbate–glutathione cycle (Fig. 2).

Intracellular glutathione pools represent 95% and 50% of the total glutathione pool in the absence and presence of abiotic stress, respectively [67,87]. In the absence of ROS-dissipating enzymes, the antioxidative capacity of the cell undergoes adjustment. For example, glutathione disulphide levels are more pronounced in a catalase-deficient *Arabidopsis* mutant (*catalase2*) than the wild type [88], and the glutathione reduction state is dramatically lower in a glutathione reductase-deficient *glutathione reductase1*/*catalase2* *Arabidopsis* double mutant than the *catalse2* mutant [88]. Elevated glutathione disulphide is also associated with enhanced activities of enzymes associated with the ascorbate–glutathione recycling pathway, such as ascorbate peroxidase and monodehydroascorbate reductase [89]. These findings suggest that glutathione oxidation is a biochemical signature of abiotic stress, and its regeneration by glutathione reductase activity is important in ROS scavenging [67]. Apart from the fact that biosynthesis of GABA and glutathione is dependent upon glutamate availability, no other information exists on the metabolic relationship between these two molecules. It is expected that under low O_2 or other abiotic stresses, the accumulation of GABA does not occur at the expense of glutathione as concentrations of these in vegetative tissues are in the micromolar and millimolar range, respectively [35,84].

Information on the impact of controlled atmosphere on glutathione metabolism in pome fruit is scarce and ambiguous. In one study, total glutathione was reduced by 50% in cv. 'Conference' pears within 5 days of storage, regardless of the controlled atmosphere regime utilized, but only those fruit maintained at −1 °C/2 kPa O_2/5 kPa CO_2 had a transient decline in glutathione disulphide/glutathione ratios and a spike in glutathione reductase activity [89]. In a second study, internal browning in cv. 'Rocha' pears was not associated with any change in total glutathione content or glutathione/glutathione disulphide ratio [90], whereas in a third study, core browning in CO_2-treated cv. 'Blanquilla' pear fruit was associated with an increase in glutathione reductase activity [91]. These variations in glutathione metabolism in response to controlled atmosphere could be due to genotypic differences, as three different cultivars were used in these studies. Notably, the glutathione level in spinach leaves decreases by ~56% within 35 days of storage at 10 °C with 10 kPa CO_2 and 0.8 kPa O_2, and a transient decline in glutathione/glutathione disulphide ratio is apparent [92]. To date, fruit studies have lacked a comprehensive comparison of the development of disorders and changes in redox pair ratios as a function of storage time in response to controlled atmosphere and/or 1-MCP. For example, although it is well known that the formation of ethylene from 1-aminocyclopropane-1-carboxylate *via* 1-aminocyclopropane-1-carboxylate oxidase requires O_2 and ascorbate, no information exists on how whole fruit concentrations of ascorbate and dehydroascorbate are affected by the brief exposure to 1-MCP.

3. Concluding remarks and significance

The cellular response of pome fruit to low temperature controlled atmosphere storage appears to involve a network of redox reactions and induction of amino acid stress metabolism, each of which may influence ROS concentration and processes affecting

membrane and organelle/cell integrity. Limited information exists for early biochemical mechanisms that promote the development of physiological injury. Previous studies have shown that exposure of vegetative plant tissues to chilling, low O_2 and elevated CO_2 partial pressures rapidly causes the accumulation of GABA, suggesting that low temperature controlled atmosphere-stored pome fruit undergoes one or both of two well-known mechanisms for GABA production from glutamate: cytosolic acidification-mediated stimulation of glutamate decarboxylase or its activation by calcium and calmodulin. It is also well recognized that GABA is catabolized to GHB during oxidative stress (*e.g.*, submergence, [35]), and accumulation of GHB is linked by altered NADH and NADPH ratios. Elevated NADPH/$NADP^+$ ratios are often associated with shifts in antioxidants such as ascorbate and glutathione, where NADPH maintains the antioxidant status in a reduced state. These reduced antioxidants play an important role in quenching ROS, and as a result can lead to decreasing ascorbate/dehydroascorbate and glutathione/glutathione disulphide ratios during oxidative stress. Further study is required to establish whether the accumulation of GABA and alterations in redox ratios during oxidative stress are temporally associated with the development of physiological disorders in apples and pears during low temperature controlled atmosphere storage. The recent release of the transgenic apple cultivar 'Arctic' provides an example of how biotechnological advances based upon decades of biochemical and molecular research can address issues of scientific and economic importance. It seems plausible that in the post-genomics era, vast improvements to the postharvest quality of pome fruit *via* transgenic approaches (*e.g.*, RNA-interference) are possible following a better understanding of oxidative stress metabolism, including the biochemical mechanisms associated with GABA biosynthesis and catabolism, and the degradation of antioxidants, such as ascorbate and glutathione. The recent release of genomic sequence information for *M. domestica* [22] and *P. communis* [23] affords the possibility to identify genetic differences between disorder-susceptible and disorder-resistant genotypes (*e.g.*, 'Eden'), and the opportunity to conduct state-of-the-art transcript analyses (*i.e.*, RNA sequencing) to better understand the impact of controlled atmosphere parameters on the expression of genes associated with oxidative stress metabolism. In fact, a transcriptomics approach using RNA sequencing has identified several genes, including one involved in oxidative fermentation (*i.e.*, pyruvate dehydrogenase), which are up-regulated under low temperature controlled atmosphere conditions, thereby leading to flesh browning in cv. 'Braeburn' apples [93]. Future biotechnological approaches could target the expression of one or more steps in the oxidative pentose phosphate pathway (*i.e.*, *GLUCOSE 6-PHOSPHATE DEHYDROGENASE*) to boost NADPH availability and preserve ascorbate and glutathione levels, or in the GABA shunt (*i.e.*, *GLUTAMATE DECARBOXYLASE*, *GABA TRANSAMINASE*) to alter GABA levels, as a means of minimizing oxidative stress and enhancing stress tolerance under controlled atmosphere conditions.

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References

[1] C.B. Watkins, The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables, Biotechnol. Adv. 24 (2006) 389–409.
[2] E. Kupferman, Controlled atmosphere storage of apples and pears, Acta Hortic. 600 (2003) 729–735.
[3] A.K. Thompson, Controlled atmosphere storage, in: R.W. Hartel (Ed.), Fruit and Vegetable Storage. Hypobaric, Hyperbaric and Controlled Atmosphere, Springer International Publishing, New York, 2016, pp. 21–36.
[4] M. Meheriuk, R.K. Prange, P.D. Lidster, S.W. Porritt, Postharvest Disorders of Apples and Pears, Agriculture and Agri-Food Canada Publication, Ottawa, Ontario, 1994, pp. 1–61, 1737/E. http://publications.gc.ca/collections/collection_2015/aac-aafc/A53-1737-1994-eng.pdf.
[5] J.R. DeEll, B. Ehsani-Moghaddam, Preharvest 1-methylcyclopropene treatment reduces soft scald in 'Honeycrisp' apples during storage, HortScience 45 (2010) 414–417.
[6] C.B. Watkins, J.F. Nock, S.A. Weis, S. Jayanty, R.M. Beaudry, Storage temperature, diphenylamine, and pre-storage delay effects on soft scald, soggy breakdown and bitter pit of 'Honeycrisp' apples, Postharvest Biol. Technol. 32 (2004) 213–221.
[7] J.R. DeEll, R.K. Prange, Disorders in 'Cortland' apple fruit are induced by storage at 0 °C in controlled atmosphere, HortScience 33 (1998) 121–122.
[8] J.M. DeLong, R.K. Prange, P.A. Harrison, The influence of 1-methylcyclopropene on 'Cortland' and 'McIntosh' apple quality following long-term storage, HortScience 39 (2004) 1062–1065.
[9] R.E. Moran, P. McManus, Firmness retention, and prevention of coreline browning and senescence in 'Macoun' apples with 1-methylcyclopropene, HortScience 40 (2005) 161–163.
[10] S. Lurie, C.B. Watkins, Superficial scald, its etiology and control, Postharvest Biol. Technol. 65 (2012) 44–60.
[11] S.K. Jung, C.B. Watkins, Involvement of ethylene in browning development of controlled atmosphere-stored 'Empire' apple fruit, Postharvest Biol. Technol. 59 (2011) 219–226.
[12] E. de Castro, D.M. Barrett, J. Jobling, E.J. Mitcham, Biochemical factors associated with a CO_2-induced flesh browning disorder of Pink Lady apples, Postharvest Biol. Technol. 48 (2008) 182–191.
[13] H.J. Elgar, D.M. Burmeister, C.B. Watkins, Storage and handling effects on a CO_2-related internal browning disorder of 'Braeburn' apples, HortScience 33 (1998) 719–722.
[14] C.B. Watkins, J.F. Nock, Controlled-atmosphere storage of 'Honeycrisp' apples, HortScience 47 (2012) 886–892.
[15] C. Contreras, N. Alsmairat, R. Beaudry, Prestorage conditioning and diphenylamine improve resistance to controlled-atmosphere-related injury in 'Honeycrisp' apples, HortScience 49 (2014) 76–81.
[16] L. Argenta, X. Fan, J. Mattheis, Delaying establishment of controlled atmosphere or CO_2 exposure reduces 'Fuji' apple CO_2 injury without excessive fruit quality loss, Postharvest Biol. Technol. 20 (2000) 221–229.
[17] C. Franck, et al., Browning disorders in pear fruit, Postharvest Biol. Technol. 43 (2007) 1–13.
[18] R. Pedreschi, et al., Metabolic profiling of 'Conference' pears under low oxygen stress, Postharvest Biol. Technol. 51 (2009) 123–130.
[19] C.B. Watkins, K.J. Silsby, M.C. Goffinet, Controlled atmosphere and antioxidant effects on external CO_2 injury of 'Empire' apples, HortScience 32 (1997) 1242–1246.
[20] K.L. Deyman, et al., Effects of elevated CO_2 and 1-methylcyclopropene on storage-related disorders of Ontario-grown Empire apples, Can. J. Plant Sci. 94 (2014) 857–865.
[21] Q.T. Ho, et al., Genotype effects on internal gas gradients in apple fruit, J. Exp. Bot. 61 (2010) 2745–2755.
[22] R. Velasco, et al., The genome of the domesticated apple (*Malus* × *domestica* Borkh), Nat. Genet. 42 (2010) 833–839.
[23] D. Chagne, et al., The draft genome sequence of European pear (*Pyrus communis* L. 'Bartlett'), PLoS One 9 (2014) e92644.
[24] C. Chervin, J.K. Truett, J. Spiers, Alcohol dehydrogenase expression and alcohol production during pear ripening, J. Am. Soc. Hortic. Sci. 124 (1999) 71–75.
[25] A.M. Mayer, Polyphenol oxidases in plants and fungi: going places? A review, Phytochemistry 67 (2006) 2318–2331.
[26] D.D. MacLean, D.P. Murr, J.R. DeEll, C.R. Horvath, Postharvest variation in apple (*Malus* × *domestica* Borkh.) flavonoids following harvest storage, and 1-MCP treatment, J. Agric. Food Chem. 54 (2006) 870–878.
[27] M. Leja, A. Mareczek, J. Ben, Antioxidant properties of two apple cultivars during long term storage, Food Chem. 80 (2003) 303–307.
[28] N. Carter, Petition for Determination of Nonregulated Status Arctic™ Apple (*Malu* × *domestica*) Events GD743 and GS784, United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS), 2012 (accessed 03.05.14) http://www.aphis.usda.gov/brs/aphisdocs/10_16101p.pdf.
[29] S. Khanizadeh, et al., Eden™, a non-browning apple suitable for fresh cut slice and fruit salad, Acta Hortic. 814 (2009) 273–274.
[30] Q.T. Ho, et al., A three-dimensional multiscale model for gas exchange in fruit, Plant Physiol. 155 (2011) 1158–1168.
[31] A.A. Saquet, J. Streif, F. Bangerth, Energy metabolism and membrane lipid alterations in relation to brown heart development in 'Conference' pears during delayed controlled atmosphere storage, Postharvest Biol. Technol. 30 (2003) 123–132.
[32] Q.T. Ho, P. Verboven, B.E. Verlinden, A. Schenk, B.M. Nicolaï, Controlled atmosphere storage may lead to local ATP deficiency in apple, Postharvest Biol. Technol. 78 (2013) 103–112.
[33] M. Blanch, et al., CO_2-driven changes in energy and fermentative metabolism in harvested strawberries, Postharvest Biol. Technol. 110 (2015) 33–39.
[34] O. Blokhina, E. Virolainen, K.V. Fagerstedt, Antioxidants, oxidative damage and the oxygen deprivation stress: a review, Ann. Bot. 91 (2003) 179–194.

[35] W.L. Allan, et al., Detoxification of succinate semialdehyde in *Arabidopsis* glyoxylate reductase and NAD kinase mutants subjected to submergence stress, Botany 90 (2012) 51–61.
[36] B.J. Shelp, et al., Strategies and tools for studying the metabolism and function of γ-aminobutyrate in plants. II. Integrated analysis, Botany 90 (2012) 781–793.
[37] S.A. Ramesh, et al., GABA signalling modulates plant growth by directly regulating the activity of plant-specific anion transporters, Nat. Commun. (2015), http://dx.doi.org/10.1038/ncomms8879.
[38] B.J. Shelp, G.G. Bozzo, C.P. Trobacher, G. Chiu, V.S. Bajwa, Strategies and tools for studying the metabolism and function of γ-aminobutyrate in plants. I. Pathway structure, Botany 90 (2012) 651–668.
[39] C.P. Trobacher, et al., Catabolism of GABA in apple fruit: subcellular localization and biochemical characterization of multiple γ-aminobutyrate transaminases, Postharvest Biol. Technol. 75 (2013) 106–113.
[40] N. Bouché, A. Fait, D. Bouchez, S.G. Møller, H. Fromm, Mitochondrial succinic-semialdehyde dehydrogenase of the γ-aminobutyrate shunt is required to restrict levels of reactive oxygen intermediates in plants, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 6843–6848.
[41] B.J. Shelp, et al., Hypothesis/review: contribution of putrescine to 4-aminobutyrate (GABA) production in response to abiotic stress, Plant Sci. 193–194 (2012) 130–135.
[42] A. Zarei, C.P. Trobacher, B.J. Shelp, NAD^+-aminoaldehyde dehydrogenase candidates for 4-aminobutyrate (GABA) and β-alanine production during terminal oxidation of polyamines in apple fruit, FEBS Lett. 589 (2015) 2695–2700.
[43] A. Zarei, et al., Apple fruit copper mine oxidase isoforms: peroxisomal MdAO1 prefers diamines as substrates whereas extracellular MdAO2 exclusively utilizes monoamines, Plant Cell Physiol. 56 (2015) 137–147.
[44] R.S. Leisso, et al., Chilling-related cell damage of apple (*Malus domestica* Borkh.) fruit cortical tissue impacts antioxidants, lipid, and phenolic metabolism, Physiol. Plant 153 (2015) 204–220.
[45] K.L. Deyman, C.J. Brikis, G.G. Bozzo, B.J. Shelp, Impact of 1-methylcyclopropene and controlled atmosphere storage on polyamine and 4-aminobutyrate levels in 'Empire' apple fruit, Front. Plant Sci. 5 (2014) 144.
[46] J. Lee, D.R. Rudell, P.J. Davies, C.B. Watkins, Metabolic changes in 1-methylcyclopropene (1-MCP)-treated 'Empire' apple fruit during storage, Metabolomics 8 (2012) 742–753.
[47] J. Lee, J.P. Mattheis, D.R. Rudell, Antioxidant treatment alters metabolism associated with internal browning in 'Braeburn' apples during controlled atmosphere storage, Postharvest Biol. Technol. 68 (2012) 32–42.
[48] C. Merodio, M.T. Munoz, B. Del Cura, M.D. Buitrago, M.I. Escribano, Effects of high CO_2 levels on the titres of γ-aminobutyric acid, total polyamines and some pathogenesis-related proteins in cherimoya fruit stored at low temperature, J. Exp. Bot. 49 (1998) 1339–1347.
[49] Y. Makino, N. Soga, S. Oshita, Y. Kawagoe, A. Tanaka, Stimulation of γ-aminobutyric acid production in vine-ripe tomato (*Lycopersicon esculentum* Mill.) fruits under modified atmospheres, J. Agric. Food Chem. 56 (2008) 7189–7193.
[50] B. Biais, et al., Metabolic accumulation to hypoxia revealed by metabolite gradients in melon fruit, J. Plant Physiol. 167 (2010) 242–245.
[51] R. Deewatthanawong, P. Rowell, C.B. Watkins, γ-Aminobutyric acid (GABA) metabolism in CO_2 treated tomatoes, Postharvest Biol. Technol. 57 (2010) 97–105.
[52] M.E. Hansen, H. Sørensen, M. Cantwell, Changes in acetaldehyde, ethanol and amino acid concentrations in broccoli florets during air and controlled atmosphere storage, Postharvest Biol. Technol. 22 (2001) 227–237.
[53] T. Akihiro, et al., Biochemical mechanism on GABA accumulation during fruit development in tomato, Plant Cell Physiol. 49 (2008) 1378–1389.
[54] M. Takayama, et al., Tomato glutamate decarboxylase genes SlGAD2 and SlGAD3 play key roles in regulating γ-aminobutyric acid levels in tomato (*Solanum lycopersicum*), Plant Cell Physiol. 56 (2015) 1533–1545.
[55] S. Koike, C. Matsukura, M. Takayama, E. Asamizu, H. Ezura, Suppression of γ-aminobutyric acid (GABA) transaminases induces prominent GABA accumulation, dwarfism and infertility in the tomato (*Solanum lycopersicum* L.), Plant Cell Physiol. 54 (2013) 793–807.
[56] J.P. Simpson, et al., γ-Aminobutyrate transaminase limits the catabolism of γ-aminobutyrate in cold-stressed *Arabidopsis* plants: insights from an overexpression mutant, Botany 88 (2010) 522–527.
[57] S.M. Clark, et al., Biochemical characterization, mitochondrial localization, expression and potential functions for an *Arabidopsis* γ-aminobutyrate transaminase that utilizes both pyruvate and glyoxylate, J. Exp. Bot. 60 (2009) 1743–1757.
[58] C.P. Trobacher, et al., Calmodulin-dependent and calmodulin-independent glutamate decarboxylases in apple fruit, BMC Plant Biol. 13 (2013) 144.
[59] R. Deewatthanawong, J.F. Nock, C.B. Watkins, γ-Aminobutyric acid (GABA) accumulation in four strawberry cultivars in response to elevated CO_2 storage, Postharvest Biol. Technol. 57 (2010) 92–96.
[60] A. Yang, S. Cao, Z. Yang, Y. Cai, Y. Zheng, γ-Aminobutyric acid treatment reduces chilling injury and activates the defence response of peach fruit, Food Chem. 129 (2011) 1619–1622.
[61] S.Q. Shi, et al., Effects of exogenous GABA on gene expression of *Caragana intermedia* roots under NaCl stress: regulatory roles for H_2O_2 and ethylene production, Plant Cell Environ. 33 (2010) 149–162.
[62] R. Vergara, F. Parada, S. Rubio, F.J. Pérez, Hypoxia induces H_2O_2 production and activates antioxidant defence system in grapevine buds through mediation of H_2O_2 and ethylene, J. Exp. Bot. 63 (2012) 4123–4131.
[63] A. Fait, A. Yellin, H. Fromm, GABA shunt deficiencies and accumulation of reactive oxygen intermediates: insight from *Arabidopsis* mutants, FEBS Lett. 579 (2005) 415–420.
[64] M. Alhagdow, et al., Silencing of the mitochondrial ascorbate synthesizing enzyme L-galactono-1. dehydrogenase affects plant and fruit development in tomato, Plant Physiol. 145 (2007) 1408–1422.
[65] G. Noctor, Metabolic signaling in defense and stress: the central roles of soluble redox couples, Plant Cell Environ. 29 (2006) 409–425.
[66] G. Queval, J. Hager, B. Gakière, G. Noctor, Why are literature data for H_2O_2 contents so variable? A discussion of potential difficulties in the quantitative assay of leaf extracts, J. Exp. Bot. 59 (2008) 135–146.
[67] C.H. Foyer, G. Noctor, Ascorbate and glutathione: the heart of the redox hub, Plant Physiol. 155 (2011) 2–18.
[68] D.M. Rhoads, A.L. Umbach, C.C. Subbaiah, J.N. Siedow, Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling, Plant Physiol. 141 (2006) 357–366.
[69] C. Larrigaudière, R. Vilaplana, Y. Soria, I. Recasens, Oxidative behaviour of Blanquilla pears treated with 1-methylcyclopropene during cold storage, J. Sci. Food Agric. 84 (2004) 1871–1877.
[70] R. Sabban-Amin, O. Feygenberg, E. Belausov, E. Pesis, Low oxygen and 1-MCP pretreatments delay superficial scald development by reducing reactive oxygen species (ROS) accumulation in stored 'Granny Smith' apples, Postharvest Biol. Technol. 62 (2011) 295–304.
[71] K.A. Kristiansen, P.E. Jensen, I.M. Møller, A. Schulz, Monitoring reactive oxygen species formation and localisation in living cells by use of the fluorescent probe CM-H_2DCFDA and confocal laser microscopy, Plant Physiol. 136 (2009) 369–383.
[72] D.D. MacLean, D.P. Murr, J.R. DeEll, A modified total oxyradical scavenging capacity assay for antioxidants in plant tissues, Postharvest Biol. Technol. 29 (2003) 183–194.
[73] R.L. Prior, X. Wu, K. Schaich, Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements, J. Agric. Food Chem. 53 (2005) 4290–4302.
[74] N.J. Kruger, A. von Schaewen, The oxidative pentose phosphate pathway: structure and organisation, Curr. Opin. Plant Biol. 6 (2003) 236–246.
[75] M.W. Davey, et al., Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing, J. Sci. Food Agric. 80 (2000) 825–860.
[76] C. Kevers, J. Pincemail, J. Tabart, J.O. Defraigne, J. Dommes, Influence of cultivar harvest time, storage conditions, and peeling on the antioxidant capacity and phenolic and ascorbic acid contents of apples and pears, J. Agric. Food Chem. 59 (2011) 6165–6171.
[77] M.W. Davey, A. Auwerkerken, J. Keulemans, Relationship of apple vitamin C and antioxidant contents to harvest date and postharvest pathogen infection, J. Sci. Food Agric. 87 (2007) 802–813.
[78] M.J. Li, F.W. Ma, M. Zhang, F. Pu, Distribution and metabolism of ascorbic acid in apple fruits (*Malus domestica* Borkh cv. Gala), Plant Sci. 174 (2008) 606–612.
[79] E. Felicetti, J.P. Mattheis, Y. Zhu, J.K. Fellman, Dynamics of ascorbic acid in 'Braeburn' and 'Gala' apples during on-tree development and storage in atmospheres conducive to internal browning development, Postharvest Biol. Technol. 61 (2011) 95–102.
[80] E. Felicetti, J.P. Mattheis, Quantification and histochemical localization of ascorbic acid in 'Delicious', 'Golden Delicious' and 'Fuji' apple fruit during on-tree development and cold storage, Postharvest Biol. Technol. 56 (2010) 56–63.
[81] C. Franck, et al., Ascorbic acid mapping to study core breakdown development in 'Conference' pears, Postharvest Biol. Technol. 30 (2003) 133–142.
[82] H.T. Parsons, T. Yasmin, S.C. Fry, Alternative pathways of dehydroascorbic acid degradation *in vitro* and in plant cell cultures: novel insights into vitamin C catabolism, Biochem. J. 440 (2011) 375–383.
[83] A.R. Fernie, et al., Recommendations for reporting metabolite data, Plant Cell 23 (2011) 2477–2482.
[84] G. Noctor, et al., Glutathione in plants: an integrated overview, Plant Cell Environ. 35 (2012) 454–484.
[85] G. Queval, D. Jaillard, B. Zechmann, G. Noctor, Increased intracellular H_2O_2 availability preferentially drives glutathione accumulation in vacuole and chloroplasts, Plant Cell Environ. 34 (2011) 21–32.
[86] P.D. Vivancos, et al., Recruitment of glutathione into the nucleus during cell proliferation adjusts whole-cell redox homeostasis in *Arabidopsis thaliana* and lowers the oxidative defense shield, Plant J. 64 (2010) 825–838.
[87] A. Mhamdi, et al., Arabidopsis GLUTATHIONE REDUCTASE 1 plays a crucial role in leaf responses to intracellular H_2O_2 and in ensuring appropriate gene expression through both salicylic acid and jasmonic acid signaling pathways, Plant Physiol. 153 (2010) 1144–1160.
[88] V.P. Roxas, S.A. Lodhi, D.K. Garrett, J.R. Mahan, R.D. Allen, Stress tolerance in transgenic tobacco seedlings that overexpress glutathione *S*-transferase/glutathione peroxidase, Plant Cell Physiol. 41 (2000) 1229–1234.
[89] C. Larrigaudiere, I. Lentheric, E. Pintó, M. Vendrell, Short-term effects of air and controlled atmosphere storage on antioxidant metabolism in conference pears, J. Plant Physiol. 158 (2001) 1015–1022.
[90] F.J.P. Silva, M.H. Gomes, F. Fidalgo, J.A. Rodrigues, D.P.F. Almeida, Antioxidant properties and fruit quality during long-term storage of Rocha pear: effects of maturity and storage conditions, J. Food Qual. 33 (2010) 1–20.

[91] E. Pintó, I. Lentheric, M. Vendrell, C. Larrigaudière, Role of fermentative and antioxidant metabolisms in the induction of core browning in controlled-atmosphere stored pears, J. Sci. Food Agric. 81 (2001) 364–370.
[92] D.M. Hodges, C.F. Forney, The effects of ethylene, depressed oxygen and elevated carbon dioxide on antioxidant profiles of senescing spinach leaves, J. Exp. Bot. 51 (2000) 645–655.
[93] I. Mellidou, et al., Transcriptomic events associated with internal browning of apple during postharvest storage, BMC Plant Biol. 14 (2014) 328.