Chapter 12
Defensive Roles of Polyphenol Oxidase in Plants

C. Peter Constabel and Raymond Barbehenn

Plant polyphenol oxidases (PPOs) are widely distributed and well-studied oxidative enzymes, and their effects on discoloration in damaged and diseased plant tissues have been known for many years. The discovery in C.A. Ryan's laboratory in the mid-1990s that tomato PPO is induced by the herbivore defense signals systemin and jasmonate, together with seminal work on PPO's possible effects on herbivorous insects by G. Felton and S. Duffey has motivated many studies of PPO in the context of plant-herbivore defense. The cloning and characterization of PPO cDNAs from multiple plant species now allows for direct testing of defensive functions of PPO using transgenic plants. These have shown that PPO can contribute to insect herbivore and pathogen resistance, although how this occurs is only now being investigated more closely. Here we review progress in the functional analysis of PPO in plant defense against pests, and describe recent results that address the mechanisms of PPO as an anti-herbivore protein. We suggest that assumptions of how PPO functions as an anti-nutritive defense against lepidopterans needs to be re-examined in light of the near anoxic conditions in lepidopteran midguts. Ultimately, the efficacy of PPO should be directly tested in a greater variety of plant-insect interactions. In addition, the identification of the endogenous PPO substrates will help to define defensive and potentially other roles for PPO in plants.

12.1 Introduction

Polyphenol oxidases (PPOs) are ubiquitous copper-containing enzymes which use molecular oxygen to oxidize common *ortho*-diphenolic compounds such as caffeic acid and catechol to their respective quinones. PPO-generated quinones are highly reactive and may cross-link or alkylate proteins, leading to the commonly observed brown pigments in damaged plant tissues and plant extracts. The conspicuous pigments are generally undesirable in food products, and the role of PPO in browning

C.P. Constabel
Centre for Forest Biology and Department of Biology, University of Victoria, Victoria, BC, Canada V8W 3N5,
e-mail: cpc@uvic.ca

A. Schaller (ed.), *Induced Plant Resistance to Herbivory*,

has prompted numerous studies on PPO in food and beverages. In parallel, the potential roles for PPO in plant defense against pests have motivated many studies on PPO in an ecological context, though few of these have used a transgenic approach. Functional and mechanistic studies on PPO in plant-insect interactions using PPO-modified transgenic plants have recently been reported, providing new insight into the biology of this versatile enzyme.

12.2 The Biochemistry of Plant Polyphenol Oxidase

Polyphenol oxidase (catechol oxidase; E.C. 1.10.3.2) has been purified and characterized from a wide range of plant species and a variety of tissues (Constabel et al. 1996; Mayer 2006), and activity levels using common substrates vary widely (Constabel and Ryan 1998). Key features of PPOs are two conserved copper-binding domains, and N-terminal chloroplast and thylakoid transit peptides (van Gelder et al. 1997; Marusek et al. 2006). The size of the predicted mature PPO proteins is typically 54–62 kD (Constabel et al. 1996; van Gelder et al. 1997). However, some PPOs are processed further, and in some cases only the processed form is fully active (Rathjen and Robinson 1992). Many PPOs are predicted to contain a proteolytic processing site near the C-terminus of the polypeptide (Marusek et al. 2006).

Based on its association with browning reactions in crop plants, PPO has been characterized in a wide variety of food plants including banana, wheat, quince, and avocado, and a number of chemical inhibitors have been identified (reviewed in Mayer 2006; Yoruk and Marshall 2003). Although PPO is found at significant levels in a variety of fruits, vegetables and grains, its biological function in these tissues has rarely been studied. PPO is expressed in many different tissues and organs, including roots, leaves, flowers, and vascular tissue (Constabel et al. 1996). Its presence in chloroplasts led to the proposal that PPO may function in pseudocyclic photophosphorylation or as a modulator of oxygen levels, yet to date little evidence supports these roles (Steffens et al. 1994). A complicating issue regarding PPO function has been the separate localization of its phenolic substrates in plant vacuoles, so that the cell would have to be broken in order for PPO to oxidize phenols; this is most likely to occur following pest or pathogen challenge. Roles of PPO in plant defense are thus commonly discussed, although direct evidence for such roles has become available only recently.

A puzzling feature of PPO has been a variable degree of latency, so that PPOs from some species need to be activated with detergents or proteases for full activity. For example, tomato leaf PPO is extracted in its fully active form (Constabel et al. 1995), but poplar leaf PPO requires activation with protease or detergent (Constabel et al. 2000). Recent experiments suggest that such activating treatments function by removal of a peptide from the active site, via proteolysis or partial unfolding of the polypeptide, respectively (Gandia-Herrero et al. 2005). Treatment with low pH can also activate latent PPOs, presumably via a conformational change of the active site, increasing its accessibility to substrates (Kanade et al. 2006).

PPO latency may be significant for its defensive function; we recently showed that latent poplar PPO is activated by its passage through caterpillar guts (Wang and Constabel 2004).

PPOs are known to have broad substrate specificities. Thus, an enzyme from any given source may be capable of oxidizing a variety of simple *ortho*-diphenolics, such as caffeic acid and its conjugates, catechol derivatives, or dihydroxyphenylalanine (DOPA). However, enzymes from different plant species exhibit distinct preference profiles (reviewed in Constabel et al. 1996). Flavonoids with *ortho*-dihydroxy phenolic rings have been found to be PPO substrates, for example, catechin, (−)-epicatechin, and myricetin (Guyot et al. 1996; Jimenez and Garcia-Carmona 1999). Some reports also describe the oxidation of trihydroxy phenolics, such as gallic acid, by PPO (Shin et al. 1997), but it is not clear how widespread this is. Furthermore, for some PPOs a monophenolase activity has been described (Wuyts et al. 2006). Such enzymes may hydroxylate a monophenol such as tyrosine, which can then undergo further oxidation by the polyphenol oxidase activity to the quinone. Tyrosine hydroxylation by PPO has been described in pokeweed, where this reaction constitutes part of the biosynthetic pathway leading to betalains (Gandia-Herrero et al. 2005).

While the range of substrates accepted by isolated PPOs can be readily defined in vitro, knowledge of the substrates that are utilized *in planta* or during defense reactions is much scarcer. Caffeic acid esters such as chlorogenic acid (caffeoylquinate) are excellent PPO substrates and are very common plant metabolites. In tomato and coffee, chlorogenic acid has been identified as the most likely in vivo PPO substrate (Melo et al. 2006; Li and Steffens 2002). Caffeic acid esters are ubiquitous as lignin precursors (Humphreys and Chapple 2002), but in most species these may not accumulate to sufficient levels to be considered likely PPO substrates. In *Populus tremuloides*, catechol is postulated to be released by the breakdown of the abundant phenolic glycosides (Clausen et al. 1989), and would therefore be available as a substrate in damaged tissues (Haruta et al. 2001). For most PPOs, however, the endogenous substrates are unknown. The importance of identifying the *in planta* PPO substrates and understanding the overall phytochemical context of PPO-containing plants is emphasized by recent reports of PPO-like enzymes with biosynthetic roles as hydroxylases of secondary metabolites (Cho et al. 2003; Nakayama et al. 2000).

PPO has been extensively studied by biologists, plant pathologists, and ecologists interested in mechanisms of defense against pests and pathogens. Based on the browning reactions resulting from the reactive PPO-generated quinones, PPO has often been suggested to function as a defense against pests and pathogens. A most dramatic illustration of the efficacy of PPO in this context comes from work on *Solanum berthaultii* in which extremely high PPO levels (45% of soluble protein) are found in glandular trichomes (Kowalski et al. 1992). Breakage of the trichomes by small-bodied insects such as aphids leads to rapid PPO-mediated oxidation and polymerization of phenolics, ultimately entrapping insects, or occluding their mouthparts with a sticky polymer (Kowalski et al. 1992). In most species, however, leaf PPO is found not in trichomes but in mesophyll cells. Here, the PPO-generated

quinones were proposed to alkylate dietary protein during insect feeding, and to degrade essential amino acids in insect guts (Felton et al. 1989, 1992). PPO-mediated protein alkylation has been demonstrated to operate against lepidopteran pests in the presence of oxygen and in artificial diets, and is now being investigated in more detail in midgut fluids (see Section 12.5 below).

12.3 PPO and Induced Herbivore Defense in Tomato and Other Plants

The idea that PPO may act as an anti-nutritive defense against leaf-eating insects was first suggested by G. Felton and S. Duffey, who showed an inverse correlation of *Heliothis zea* growth and PPO levels in tomato plants (Felton et al. 1989). Strong support for an anti-herbivore role of PPO came from the discovery that the herbivore defense-inducing signal molecules systemin and methyl jasmonate (MeJA) induce PPO activity and PPO mRNA levels in tomato leaves (Constabel et al. 1995). Systemin is a short peptide required for systemic wound signaling in tomato which strongly upregulates tomato herbivore defenses (Ryan 2000; Narváez-Vásquez and Orozco-Cárdenas, this volume). Though no longer considered to be the primary systemic signal, it is required for the generation of such a signal (Schilmiller and Howe 2005). PPO and other defenses were also found to be strongly induced by MeJA and oligogalacturonic acid, major plant defense signaling compounds (Constabel et al. 1995). Since PPO induction in tomato by multiple signals occurs in parallel with a suite of other anti-herbivore proteins including several types of protease inhibitors (PIs) and the anti-nutritive enzymes arginase and threonine deaminase (Bergey et al. 1996; Chen et al. 2005), PPO is thought to play a similar role in defense against insects.

In tobacco, PPO and PIs are upregulated by tobacco systemin as well as by MeJA (Constabel and Ryan 1998; Ren and Lu 2006). Likewise, strong herbivore-, wound-, and MeJA-induction of PPO was shown in leaves of several poplar species (Constabel et al. 2000; Haruta et al. 2001). Hybrid poplar (*Populus trichocarpa x P. deltoides*) has a strong systemic inducible defense response, which also includes trypsin inhibitors and chitinases, both with confirmed anti-insect activities (Parsons et al. 1989; Lawrence and Novak 2001, 2006). Recent large scale genomics experiments have underscored the complexity of the herbivore defense response in hybrid poplar, which involves upregulation of many additional putative defense genes (Christopher et al. 2004; Ralph et al. 2006; Major and Constabel 2006). Overall, the co-induction of PPO with other herbivore defense proteins in several plant species has provided support for its anti-herbivore function.

The induction of PPO in tomato by both insects and MeJA has been replicated in both laboratory and field studies (Stout et al. 1998; Thaler et al. 1996, 2002). Furthermore, the early work in tomato stimulated numerous studies on inducible PPO in diverse plant species. The inducibility of PPO by wounding or MeJA treatment has been confirmed in other plants, including both herbaceous crops and trees (Constabel and Ryan 1998). The induction of PPO by herbivory has now been shown

Table 12.1 Studies of PPO induction in plants by herbivores with unknown effects on herbivores

Plant taxa	Insect species (Order)[1]	Induction	Reference
Solanaceae			
Tomato	*Leptinotarsa decemlineata* (C)	~2X	Felton (1992)
Potato	*L. decemlineata* regurgitant	~3–7X	Kruzmane et al. (2002)
Salicaceae			
Poplar (hybrid)	*Malacosoma disstria* (L)	~12X	Constabel et al. (2000)
Betulaceae			
Black alder	*Agelastica alni* (C)	~3X	Tscharntke et al. (2001)
Poaceae			
Buffalograss	*Blissus occiduus* (H)	None	Heng-Moss et al. (2004)
Barley, wheat, oats	*Diuraphis noxia* (Ho)	None	Ni et al. (2001)
Fabaceae			
Common bean	*Melanoplus differentialis* (O)	~2X	Alba-Meraz and Choe (2002)
Soybean	*Helicoverpa zea* (L)	None	Bi and Felton (1995)
Soybean	*Ceratoma trifurcata* (C)	None	Felton et al. (1994)
Soybean	*Spissistilus festinus* (H)	1.6X	Felton et al. (1994)
Malvaceae			
Cotton	*Helicoverpa zea* (L)	Not detectable	Bi et al. (1997)
Theaceae			
Tea	*Helopeltis theivora* (H)	~2–3X	Chakraborty and Chakraborthy (2005)

[1] C = Coleoptera, L = Lepidoptera, H = Heteroptera, Ho = Homoptera, O = Orthoptera.

for a taxonomically diverse group of plants (Table 12.1), and this induction has commonly been interpreted as a direct response against the herbivore. By contrast, studies of the potential impact of induced PPO against the herbivores have largely been done with a more limited range of plants and herbivores, primarily noctuid caterpillars on tomato and other species in the Solanaceae (Table 12.2). The results of 11 of 16 experiments demonstrate PPO induction and are consistent with the hypothesis that induced PPO contributes to defense against herbivores. As with all correlative studies, however, it is not possible to determine the specific impact of induced PPO on herbivores due to the other biochemical changes that occur in damaged plants (e.g., Hermsmeier et al. 2001; Thaler et al. 2001; Chen et al. 2005; Major and Constabel 2006). The direct effects of PPO on insect herbivores can thus best be tested using transgenic plants, where PPO levels are manipulated independently of other traits (see Section 12.4).

Other studies of the association between PPO activity and insect herbivore performance using (1) plant genotypes that vary in resistance to herbivory, (2) ontogenetic variation in PPO activity within the plant, and (3) leaves treated with PPO, yielded mixed results. A potato genotype with high PPO activity had increased resistance to the Colorado potato beetle (*Leptinotarsa decemlineata;* Castanera et al. 1996), while resistance to the coffee leaf miner (*Leucoptera coffeella*; Diptera) was apparently unaffected by higher levels of PPO in coffee

Table 12.2 Induction of PPOs in plants with effects tested on herbivores

Plant taxa	Inducing agent (Order)[1]	Induction	Effect	Herbivore[1] and response[2]	Reference
Solanaceae					
Tomato	*Helicoverpa zea* (L)	~2X	(−)	*Spodoptera exigua* (L) GR	Stout et al. (1998)
Tomato	*Helicoverpa zea* (L)	~2X	(−)	Spider mite (A) numbers	Stout et al. (1998)
Tomato	Jasmonic acid	~3X	(0)	*Manduca sexta* (L) RGR	Thaler et al. (2002)
Tomato	Jasmonic acid	~3X	(−)	*Trichoplusia ni* (L) RGR	Thaler et al. (2002)
Tomato	Jasmonic acid	~3X	(−)	Thrips (T) damage	Thaler et al. (1999, 2002)
Tomato	Jasmonic acid	~3X	(−)	Spider mite (A) numbers	Thaler et al. (2002)
Tomato (wild)	Jasmonic acid	~3X	(0)	*Spodoptera exigua* (L) RGR	Thaler et al. (2002)
Tomato	Jasmonic acid	~5X	(−)	*Spodoptera exigua* (L) RGR	Thaler et al. (1999)
Tomato	*Macrosiphum euphorbiae* (H)	Decreased	(+)	*Spodoptera exigua* (L) RGR	Stout et al. (1998)
Tomato	Wind stress	Decreased	(0)	*Manduca sexta* (L) RGR	Cipollini and Redman (1999)
Tomato	Jasmonic acid	~2X	(−)	*Manduca sexta* (L) RGR	Redman et al. (2001)
Tobacco (wild)	clipped sagebrush	~4X	(−)	Field damage by (L) and (O)	Karban et al. (2000)
Potato (wild)	High constitutive PPO	~4–7X	(−)	*Leptinotarsa decemlineata* (C) RGR	Castanera et al. (1996)
Horsenettle	High N fertilizer	Decreased	(0)	*Manduca sexta* (L) herbivory	Cipollini et al. (2002)
Horsenettle	High N fertilizer	Decreased	(0)	*Epitrix* sp. (C) herbivory	Cipollini et al. (2002)
Betulaceae					
Montain birch	*Epirrita autumnata* (L)	~3X	(−)	*Epirrita autumnata* (L) RGR	Ruuhola and Yang (2006)

[1] C = Coleoptera, L = Lepidoptera, O = Orthoptera, T = Thysanoptera, A = Arachnida.
[2] GR = growth rate (mg/day), RGR = relative growth rate (mg/mg/day).

leaves (Melo et al. 2006). Relative growth rates of *Helicoverpa zea* caterpillars were negatively correlated with tomato leaf and tomato fruit PPO levels (Felton et al. 1989), while *Manduca quinquemaculata* caterpillars showed greater performance on younger tobacco leaves, which contain higher PPO levels (Kessler and Baldwin 2002). *Lymantria dispar* caterpillars were unaffected by an eight-fold increase in PPO levels, using mushroom PPO applied to the leaf surface (Barbehenn et al. 2007).

Correlations of PPO activity with defense may be confounded by the complexity of PPO gene families. For example, the poplar genome contains as many as 10–12 PPO genes (I.T. Major, L. Tran and C.P. Constabel, unpublished data), but to date, only three PPO genes have been studied. *PtdPPO1* is exclusively expressed in damaged leaves, while *PtdPPO2* and *PtdPPO3* are predominantly expressed in stems, petioles, or roots. Both *PtdPPO1* and *PtdPPO2* are inducible, but in different tissues (Wang and Constabel 2004). Tissue-specific PPO expression has been most carefully studied in tomato, where seven PPO genes were characterized by Steffens and co-workers (Hunt et al. 1993; Steffens et al. 1994). In elegant work using promoter-GUS fusions, in situ hybridization, and immunolocalization, a highly complex cell- and tissue-specific pattern of expression was established (Thipyapong 1997; Thipyapong and Steffens 1997). Each PPO showed a distinctive expression profile, but at least one PPO gene showed constitutive expression for any given tissue. Interestingly, only the PPO-F gene was found to be herbivore-inducible. In addition to systemin and MeJA, pathogen and abiotic stress signals such as salicylic acid and ethylene were also shown to regulate PPO-F, consistent with additional roles of PPO in pathogen or other stress resistance. In general, diverse expression patterns of PPO in tomato and poplar in response to both developmental and stress signals indicate that PPO may have additional stress-related functions in different systems or situations.

12.4 Some Defensive Functions of PPO have been Demonstrated in Transgenic Tomato and Poplar Plants

In tomato and poplar, the induction and regulation of PPO in the herbivore defense response, in parallel with many confirmed defense genes, has provided indirect evidence for its role in defense (‘guilt by association’). Nevertheless, it is possible that the herbivore-induced PPO contributes to wound healing and defense against opportunistic pathogens, rather than direct defense against insects. This question can best be addressed using transgenic plants. The availability of PPO cDNAs from a diversity of species has facilitated this approach in species susceptible to genetic transformation. Based on the commercial interest in the role of PPO in browning, several studies reported the successful anti-sense suppression of PPO in potato tuber and apple fruit (Bachem et al. 1994; Murata et al. 2001; Coetzer et al. 2001). Conversely, the overexpression of PPO in transgenic sugarcane resulted in darker juice (Vickers et al. 2005). However such PPO-modified plants were not used to address questions of biological functions or plant defense.

The defensive roles of PPO were first directly tested with PPO-overexpressing tomato plants, which showed fewer lesions and increased resistance to the bacterial pathogen *Pseudomonas syringae* pv *tomato* (Li and Steffens 2002), while antisense PPO-suppressed tomato showed greater susceptibility (more bacterial replication and more lesions; Thipyapong et al. 2004a). These plants also provide the strongest support to date for a defensive role of PPO against insect herbivores. Noctuid caterpillars *Heliothis armigera* and *Spodoptera litura* showed negative effects on growth when fed on PPO-overexpressing lines, and conversely, positive effects on growth when fed PPO-suppressed lines (P. Thipyapong, personal communication). In transgenic *Populus*, overexpression of the induced leaf PPO gene in low PPO poplar lines facilitated assays for roles of PPO against tree-feeding caterpillars. These studies have provided mixed results. First-instar caterpillars of *Malacosoma disstria* had decreased growth rates on elevated-PPO poplar (Wang and Constabel 2004), but only when experiments were performed in the fall, presumably a result of decreased caterpillar vigor. Similarly, fourth-instar caterpillars of *Lymantria dispar* had decreased growth rates on elevated-PPO poplar in the winter (Barbehenn et al. 2007). A second species of lymantriid caterpillar, *Orgyia leucostigma*, had decreased growth rates on elevated-PPO poplars in one experiment, but no negative effects were observed in another experiment (both in the winter). Here it appears that the varying and small effects of large increases in PPO activity in poplar (five to 40-fold increases) would make induced PPO alone ineffective as a defense against these tree-feeding caterpillars.

The differences in the apparent effectiveness of overexpressing PPO in tomato vs. poplar may be due to several factors, including the differential susceptibilities of test insects. The cell-specific localization of PPO may also be important; in tomato a significant proportion of overexpressed PPO is found in glandular trichomes (P. Thipyapong, personal communication). This may favor pre-ingestive PPO oxidation of phenolics and avoid the anoxic environment of the gut (see below). Similarly, a rapid oxidation of phenolics in tomato may be favored by the lack of latency for the tomato, but not the poplar enzyme (Constabel et al. 1995, 2000).

PPO-overexpressing transgenic poplars have been useful tools for probing other aspects of PPO, such as its stability after ingestion. Defense proteins are predicted to be relatively stable in the harsh conditions found in insect digestive systems, and the recovery of significant amounts of PPO in frass of forest tent caterpillar feeding on transgenic foliage is consistent with this expectation (Wang and Constabel 2004). Furthermore, PPO was activated by its passage through the insect gut, since unlike PPO extracted from leaves, PPO in frass extracts was fully active. Western blot analysis using PPO-specific antibodies showed that PPO in frass migrated at a lower molecular weight, indicating that proteolytic processing at a discrete site occurred in the gut (Wang and Constabel 2004). As mentioned above, PPOs are frequently observed to have a C-terminal proteolytic processing site (Marusek et al. 2006). The biological significance of PPO activation by gut enzymes is not clear, but it may impact its effectiveness.

12.5 PPO Activity Against Insects: Mechanisms of Action and Limitations

The many studies of PPO induction by herbivores attest to the general belief that PPOs play a key role in defense against herbivores. However, since the work of Felton et al. (1989, 1992), studies have rarely examined the mode of action of ingested PPOs. At least three mechanisms have been proposed by which PPO might affect insect herbivores: (1) PPO-generated quinones could alkylate essential amino acids, decreasing plant nutritional quality, (2) redox cycling of quinones may produce oxidative stress in the gut lumen, and (3) phenolic oxidation products, such as quinones and reactive oxygen species (hydrogen peroxide) generated by quinone redox cycling, could be absorbed and have toxic effects on herbivores. The work that has addressed these mechanisms in insect herbivores is summarized below.

As originally shown by Felton et al. (1989, 1992), PPO can directly reduce protein quality in vitro, when incubated with dietary protein and chlorogenic acid at ambient oxygen at pH 7.0. The alkylation of essential amino acids with quinones under these conditions significantly decreased noctuid caterpillar performance, and up to 50% of radiolabeled chlorogenic acid was found bound to protein in frass of noctuid caterpillars fed on tomato foliage (Felton et al. 1989). High pH environments, such as the lepidopteran midgut, favor protein alkylation, and several essential amino acids (lysine, histidine, cysteine, methionine) are particularly susceptible to quinone alkylation (Felton et al. 1992). Under optimal conditions for PPO activity, PPO clearly has an effect on protein nutritional quality. However, limiting factors in insect digestive systems may be low oxygen levels and the presence of antioxidants such as ascorbate or glutathione (see below). Another potential effect of PPO is elevated oxidative stress in the gut lumen, which we examined recently using PPO-overexpressing poplar foliage (Barbehenn et al. 2007). High PPO levels had little effect on observed levels of oxidized proteins or semiquinone radical production in two tree-feeding caterpillar species. Coating leaf disks with a commercial PPO (fungal tyrosinase) likewise produced no increase in semiquinone radical levels. These data suggest that there is little increase in quinone formation following ingestion of high levels of PPO in poplar, contrary to expectations based on the model of Felton et al. (1989, 1992) and previous results with forest tent caterpillars (Wang and Constabel 2004). By contrast, Thipyapong and coworkers have recently shown decreased growth rates and decreased nutritional indices of some noctuid caterpillars on PPO-overexpressing tomato lines (P. Thipyapong, personal communication), consistent with post-ingestive mechanism(s) of PPO activity. We are unaware of any work that has examined the potential effect of PPO on oxidative stress or toxicity at the tissue level in insects.

The activity of ingested PPO is dependant on the chemical environment of the insect gut, such as oxygen and phenolic substrate levels, reductants, inhibitors, and pH. Surprisingly little work has been done to determine how the physiological conditions present in insect gut fluids influence PPO and other defensive reactions. Phenolic substrates must be present for PPO to be effective, but unfortunately these are typically not analyzed and are assumed to be present at sufficient levels.

Likewise, molecular oxygen is an absolute requirement for PPO, and its activity is halted by purging oxygen from the reaction mixture (Duckworth and Coleman 1970; Fig. 12.1). Significantly, the gut contents of caterpillars and grasshoppers contain low steady-state concentrations of oxygen, and are sometimes anaerobic (Johnson and Barbehenn 2000). Oxygen drops from ambient levels of 150 mm Hg (21.0%) to less than 10 mm Hg (1.4%) over a distance of several mm into the foreguts of some caterpillars. The midgut oxygen levels of 0.1–0.5 mm Hg seen in many species would be expected to decrease PPO activities to less than 1% of maximal PPO rates at ambient oxygen levels (Fig. 12.1). Some caterpillar species have enlarged foreguts with much higher oxygen levels than the midgut. Nevertheless, in our work with one such species, *Lymantria dispar*, we found little evidence for a strong effect of PPO (Barbehenn et al. 2007). The limited oxygen availability in midguts of many insects should be kept in mind when using purified proteins or macerated leaf tissues to model chemical processes within herbivores. Where an impact of PPO on herbivores is found, limiting factors in the gut argue for a preingestive mode of action of PPO.

Although pH optima of PPOs are commonly broad, the reactivity of quinones with amino acids in an acidic medium is greatly reduced. Thus, Felton et al. (1992) concluded that PPOs would likely be ineffective against the Colorado potato beetle due to the low pH (5.5–6.5) of the beetle's midgut. Consistent with this conclusion, no significant decreases in the levels of four essential amino acids were found in the feces of *L. decemlineata* that fed on potato leaves from varieties with higher PPO levels (Castanera et al. 1996). Grasshopper gut pH is also acidic, but we are unaware of work on the effects of PPO in the Orthoptera. The high pH found in lepidopteran midguts (ca. pH 9–10) would be expected to decrease the activities of ingested PPO, but the basic conditions favor protein alkylation (Felton et al. 1989).

Extensive work by food scientists has identified many PPO inhibitors that reduce browning of processed foods. Among these inhibitors, ascorbate is ubiquitous in leaves and present at high levels, and would be co-ingested with PPO. When present in midgut fluid or in vitro reaction mixtures at 0.2–0.5 mM, ascorbate can chemically reduce quinones and semiquinone radicals, thereby limiting the effectiveness of PPO as an oxidative defense (Martinez-Cayuela et al. 1988; Janovitz-Klapp et al. 1990; Felton and Duffey 1992; Barbehenn et al. 2007). Levels of ascorbate

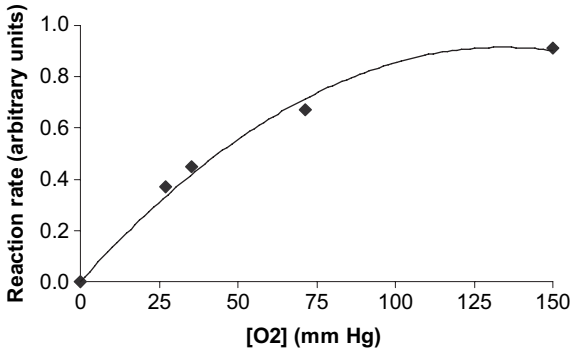

Fig. 12.1 Effect of molecular oxygen concentration on PPO reaction rate [modified from Duckworth and Coleman (1970)]. Reaction mixtures contained PPO (125 μg/ml) and pyrocatechol (0.25 mM) in 0.1 M sodium phosphate buffer (pH 7.0)

in the midgut fluids of tree-feeding caterpillars feeding on young trembling aspen, red oak, and hybrid poplar foliage are sufficiently high to prevent the net production of quinones by PPO (Barbehenn et al. 2003, 2007). Furthermore, an ascorbate recycling system is considered to be central to the antioxidant defenses of tree-feeding caterpillars (Barbehenn et al. 2001). However, it is not known whether the ascorbate ingested by tomato-feeding caterpillars, such as noctuids, is maintained in their gut fluids at sufficiently high levels to inhibit PPO.

Thiols such as glutathione and cysteine also decrease the net production of quinones by PPO and may inhibit the enzyme directly (Negishi and Ozawa 2000). In leaves, glutathione concentrations are roughly 5% as high as ascorbate levels, but can be equal to ascorbate levels in caterpillar midgut fluids (Barbehenn et al. 2001, 2003). Midgut glutathione at 50–100 μM in tree-feeding caterpillars is thus theoretically high enough to inhibit PPO activity (Barbehenn et al. 2003; Nagai and Suzuki 2003; Aydemir and Akkanh 2006), although apparently higher levels of cysteine are necessary to inhibit PPO from some plants (e.g., Janovitz-Klapp et al. 1990). Other potential PPO inhibitors reported include simple phenolics and quercetin (Walker and McCallion 1980; Le Bourvellec et al. 2004; Nerya et al. 2004).

12.6 PPO and Pathogen Defense

A role for PPO in defense against pathogens has been postulated from the earliest days of PPO research. This hypothesis has been supported by many correlative studies, such as the upregulation of PPO in pathogen-challenged plants (reviewed in Constabel et al. 1996; Mayer 2006). Pathogen-induced PPO activity continues to be reported for a variety of plant taxa, including monocots and dicots (e.g., Chen et al. 2000; Deborah et al. 2001). Similarly, studies describing correlations of high PPO levels in cultivars or lines with high pathogen resistance continue to provide support for a pathogen defense role of PPO (Raj et al. 2006). Several groups have also attempted to correlate the protective effects of rhizosphere bacteria with an induction of defense enzymes including PPO, with mixed success (Chen et al. 2000; Ramamoorthy et al. 2002).

Direct evidence for a role of PPO in inhibiting pathogen ingress or growth comes from transgenic tomato plants with enhanced or suppressed PPO levels. When challenged by the bacterial pathogen *Pseudomonas syringae* pv tomato, PPO-overexpressing plants showed reduced bacterial growth, whereas PPO antisense-suppressed lines supported greater bacterial numbers (Li and Steffens 2002; Thipyapong et al. 2004a). These studies are the only direct demonstrations to date of PPO's importance in pathogen defense. Whether such a function extends to other types of pathogens such as fungi remains to be tested. In poplar, infection with *Melampsora medusae* (a foliar rust pathogen) does not induce PPO, but represses its expression together with many other herbivore defense genes (Miranda et al. 2007).

The demonstration that PPO can inhibit bacterial plant pathogens suggests that, in addition to effects on some herbivores, PPO is important for inhibiting microbes introduced into damaged leaves via the mouthparts of feeding insects. These might be opportunistic bacteria or pathogens that are vectored by specific insects, although little is known about the microflora of caterpillar mouthparts and digestive systems. Thus, the distinction between pest and pathogen defenses may be artificial, and these may be seen as synergistic and complementary responses. This contrasts with the view that pest and pathogen defense responses are mutually exclusive. This hypothesis was based on early work on tomato showing that salicylic acid, a potent inducer of pathogen defense and systemic acquired resistance, inhibits the jasmonate-regulated herbivore defense response (Doares et al. 1995; Thaler et al. 2002). Current models of defense signaling outline a complex and overlapping set of responses, regulated by jasmonate, ethylene, and salicylic acid-based signals, leading to several possible outcomes (Devoto and Turner 2005). Functionally, crosstalk is supported by the observation of herbivore-induced resistance of *Arabidopsis* against microbial pathogens, including *P. syringae* (De Vos et al. 2006). In tomato, the PPO-F gene is induced during both the wound response and infection with the pathogen *P. syringae* (Thipyapong 1997, 2004a).

To date, no clear mechanism for the potential anti-pathogen effects of PPO has been demonstrated. Li and Steffens (2002) suggest several possibilities, including (1) general toxicity of PPO-generated quinones to pathogens and plant cells, accelerating cell death, (2) alkylation and reduced bioavailability of cellular proteins to the pathogen, (3) cross-linking of quinones with protein or other phenolics, forming a physical barrier to pathogens in the cell wall, and (4) quinone redox cycling leading to H_2O_2 and other reactive oxygen species (Jiang and Miles 1993). While reactive oxygen species are known to be important factors in plant pathogen interactions and defense signaling, and PPO is implicated in the formation of melanin-like polymers in potato blackspot lesions (Stevens et al. 1998), none of these hypotheses of how PPO might affect pathogens has been tested rigorously so far.

12.7 Conclusions and Future Directions

Although the transgenic approach has led to the direct demonstration of the efficacy of inducible PPO as a defense against some lepidopteran herbivores in some cases, the mechanisms of action against these attackers are still unclear. More detailed analyses of midgut chemistry in a greater variety of insects feeding on high-PPO foliage will begin to address this question. In particular, the extent of quinone binding to protein and amino acids in the gut contents of herbivores needs to be established. The interpretation of feeding studies would be enhanced by testing not only PPO levels and insect performance, but consumption rate and plant nutritional and chemical quality, so that deterrence or compensatory feeding can be detected. In addition, our knowledge of PPO effects would benefit from greater attention to endogenous PPO substrates, rather than simply extrapolating from substrate speci-

ficities observed in vitro. In few cases have the probable substrates been identified, yet the biochemical reactivities of PPO-produced quinones are dependent on the specific structure (Jiang and Miles 1993).

Much recent work on PPO has emphasized potential roles in defense. Nevertheless, alternate roles for this enzyme are likely and have been demonstrated. For example, antisense PPO tomato plants have enhanced pathogen resistance and drought tolerance (Thipyapong et al. 2004b), and PPO-like enzymes can act as hydroxylases in secondary metabolism (Steiner et al. 1999; Nakayama et al. 2000; Cho et al. 2003). Given the tremendous variation in PPO expression patterns, activity levels, and potential substrates in different species, similar variation in the adaptive roles played by PPO in defense and other processes may be anticipated.

Acknowledgments The authors would like to thank Piyada Thipyapong for providing unpublished data. Grant support for work in the authors' laboratories is from the Natural Sciences and Engineering Research Council (NSERC) of Canada (CPC), the University of Victoria's Centre for Forest Biology (CPC), and the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service, grant number 2004-35302-14840 to RVB and CPC.

References

Alba-Meraz A, Choe HT (2002) Systemic effects on oxidative enzymes in *Phaseolus vulgaris* leaves that have been wounded by the grasshopper *Melanoplus differentialis* (Thomas) or have had a foliar application of jasmonic acid. Int J Plant Sci 163:317–328

Aydemir T, Akkanh G (2006) Partial purification and characterization of polyphenol oxidase from celery root (*Apium graveolens* L.) and the investigation of the effects on the enzyme activity of some inhibitors. Int J Food Sci Tech 41:1090–1098

Bachem CWB, Speckmann GJ, Vanderlinde PCG, Verheggen FTM, Hunt MD, Steffens JC, Zabeau M (1994) Antisense expression of polyphenol oxidase genes inhibits enzymatic browning in potato tubers. Biotechnology 12:1101–1105

Barbehenn RV, Bumgarner SL, Roosen E, Martin MM (2001) Antioxidant defenses in caterpillars: role of the ascorbate recycling system in the midgut lumen. J Insect Physiol 47:349–357

Barbehenn RV, Walker AC, Uddin F (2003) Antioxidants in the midgut fluids of a tannin-tolerant and a tannin-sensitive caterpillar: effects of seasonal changes in tree leaves. J Chem Ecol 29:1099–1116

Barbehenn RV, Jones CP, Yip L, Tran L, Constabel CP (2007) Does the induction of polyphenol oxidase defend trees against caterpillars? Assessing defenses one at a time with transgenic poplar. Oecologia 154:129–400

Bergey DR, Howe GA, Ryan CA (1996) Polypeptide signaling for plant defensive genes exhibits analogies to defense signaling in animals. Proc Natl Acad Sci USA 93:12053–12058

Bi JL, Felton GW (1995) Foliar oxidative stress and insect herbivory: primary compounds, secondary metabolites, and reactive oxygen species as components of induced resistance. J Chem Ecol 21:1511–1530

Bi JL, Murphy JB, Felton GW (1997) Antinutritive and oxidative components as mechanisms of induced resistance in cotton to *Helicoverpa zea*. J Chem Ecol 23:97–117

Castanera P, Steffens JC, Tingey WM (1996) Biological performance of Colorado potato beetle larvae on potato genotypes with differing levels of polyphenol oxidase. J Chem Ecol 22:91–101

Chakraborty U, Chakraborty N (2005) Impact of environmental factors on infestation of tea leaves by *Helopeltis theivora*, and associated changes in flavonoid flavor components and enzyme activities. Phytoparasitica 33:88–96

Chen C, Belanger RR, Benhamou N, Paulitz TC (2000) Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiol Mol Plant Path 56:13–23

Chen H, Wilkerson CG, Kuchar JA, Phinney BS, Howe GA (2005) Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. Proc Natl Acad Sci USA 102:19237–19242

Cho MH, Moinuddin SGA, Helms GL, Hishiyama S, Eichinger D, Davin LB, Lewis NG (2003) (+)-Larreatricin hydroxylase, an enantio-specific polyphenol oxidase from the creosote bush (*Larrea tridentata*). Proc Natl Acad Sci USA 100:10641–10646

Christopher ME, Miranda M, Major IT, Constabel CP (2004) Gene expression profiling of systemically wound-induced defenses in hybrid poplar. Planta 219:936–947

Cipollini DF, Redman AM (1999) Age-dependent effects of jasmonic acid treatment and wind exposure on foliar oxidase activity and insect resistance in tomato. J Chem Ecol 25:271–281

Cipollini ML, Paulk E, Cipollini DF (2002) Effect of nitrogen and water treatment on leaf chemistry in horsenettle (*Solanum carolinense*), and relationship to herbivory by flea beetles (*Epitrix* spp.) and tobacco hornworm (*Manduca sexta*). J Chem Ecol 28:2377–2398

Clausen TP, Reichardt PB, Bryant JP, Werner RA, Post K, Frisby K (1989) Chemical model for short-term induction in quaking aspen *(Populus tremuloides*) foliage against herbivores. J Chem Ecol 15:2335–2346

Coetzer C, Corsini D, Love S, Pavek J, Tumer N (2001) Control of enzymatic browning in potato (*Solanum tuberosum* L.) by sense and antisense RNA from tomato polyphenol oxidase. J Agric Food Chem 49:652–657

Constabel CP, Ryan CA (1998) A survey of wound- and methyl jasmonate-induced leaf polyphenol oxidase in crop plants. Phytochemistry 47:507–511

Constabel CP, Bergey DR, Ryan CA (1996) Polyphenol oxidase as a component of the inducible defense response in tomato against herbivores. In: Romeo JT, Saunders JA, Barbosa P (eds) Phytochemical diversity and redundancy in ecological interactions. Plenum Press, New York, pp 231–252

Constabel CP, Bergey DR, Ryan CA (1995) Systemin activates synthesis of wound-inducible tomato leaf polyphenol oxidase via the octadecanoid defense signaling pathway. Proc Natl Acad Sci USA 92:407–411

Constabel CP, Yip L, Patton JJ, Christopher ME (2000) Polyphenol oxidase from hybrid poplar. Cloning and expression in response to wounding and herbivory. Plant Physiol 124:285–295

De Vos M, Van Zaanen W, Koornneef A, Korzelius JP, Dicke M, Van Loon LC, Pieterse CMJ (2006) Herbivore-induced resistance against microbial pathogens in *Arabidopsis*. Plant Physiol 142:352–363

Deborah SD, Palaniswami A, Vidhyasekaran P, Velazhahan R (2001) Time-course study of the induction of defense enzymes, phenolics and lignin in rice in response to infection by pathogen and non-pathogen. J Plant Dis Prot 108:204–216

Devoto A, Turner JG (2005) Jasmonate-regulated *Arabidopsis* stress signalling network. Physiol Plant 123:161–172

Doares SH, Narváez-Vásquez J, Conconi A, Ryan CA (1995) Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. Plant Physiol 108:1741–1746

Duckworth HW, Coleman JE (1970) Physicochemical and kinetic properties of mushroom tyrosinase. J Biol Chem 245:1613–1625

Felton GW, Donato K, Delvecchio RJ, Duffey SS (1989) Activation of plant foliar oxidases by insect feeding reduces nutritive quality of foliage for noctuid herbivores. J Chem Ecol 15: 2667–2694

Felton GW, Duffey SS (1992) Avoidance of antinutritive plant defense: role of midgut pH in Colorado potato beetle. J Chem Ecol 18:571–583

Felton GW, Donato KK, Broadway RM, Duffey SS (1992) Impact of oxidized plant phenolics on the nutritional quality of dietary protein to a noctuid herbivore, *Spodoptera exigua*. J Insect Physiol 38:277–285

Felton GW, Summers CB, Mueller AJ (1994) Oxidative responses in soybean foliage to herbivory by bean leaf beetle and three-cornered alfalfa hopper. J Chem Ecol 20:639–650

Gandia-Herrero F, Jimenez-Atienzar M, Cabanes J, Garcia-Carmona F, Escribano J (2005) Evidence for a common regulation in the activation of a polyphenol oxidase by trypsin and sodium dodecyl sulfate. Biol Chem 386:601–607

Guyot S, Vercauteren J, Cheynier V (1996) Structural determination of colourless and yellow dimers resulting from (+)-catechin coupling catalysed by grape polyphenoloxidase. Phytochemistry 42:1279–1288

Haruta M, Pedersen JA, Constabel CP (2001) Polyphenol oxidase and herbivore defense in trembling aspen (*Populus tremuloides*): cDNA cloning, expression, and potential substrates. Physiol Plant 112:552–558

Heng-Moss T, Sarath G, Baxendale F, Novak D, Bose S, Ni XH, Quisenberry S (2004) Characterization of oxidative enzyme changes in buffalograsses challenged by *Blissus occiduus*. J Econ Entomol 97:1086–1095

Hermsmeier D, Schittko U, Baldwin IT (2001) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. I. Large-scale changes in the accumulation of growth- and defense-related plant mRNAs. Plant Physiol 125:683–700

Humphreys JM, Chapple C (2002) Rewriting the lignin roadmap. Curr Op Plant Biol 5:224–229

Hunt MD, Eannetta NT, Yu HF, Newman SM, Steffens JC (1993) cDNA cloning and expression of potato polyphenol oxidase. Plant Mol Biol 21:59–68

Janovitz-Klapp AH, Richard FC, Goupy PM, Nicolas JJ (1990) Inhibition studies on apple polyphenol oxidase. J Agric Food Chem 38:926–931

Jiang Y, Miles PW (1993) Generation of H_2O_2 during enzymatic oxidation of catechin. Phytochemistry 33:29–34

Jimenez M, Garcia-Carmona F (1999) Myricetin, an antioxidant flavonol, is a substrate of polyphenol oxidase. J Sci Food Agric 79:1993–2000

Johnson KS, Barbehenn RV (2000) Oxygen levels in the gut lumens of herbivorous insects. J Insect Physiol 46:897–903

Kanade SR, Paul B, Rao AGA, Gowda LR (2006) The conformational state of polyphenol oxidase from field bean (*Dolichlos lablah*) upon SDS and acid-pH activation. Biochem J 395:551–562

Karban R, Baldwin IT, Baxter KJ, Laue G, Felton GW (2000) Communication between plants: induced resistance in wild tobacco plants following clipping of neighboring sagebrush. Oecologia 125:66–71

Kessler A, Baldwin IT (2002) *Manduca quinquemaculata*'s optimization of intra-plant oviposition to predation, food quality, and thermal constraints. Ecology 83:2346–2354

Kowalski SP, Eannetta NT, Hirzel AT, Steffens JC (1992) Purification and characterization of polyphenol oxidase from glandular trichomes of *Solanum berthaultii*. Plant Physiol 100: 677–684

Kruzmane D, Jankevica L, Ievinsh G (2002) Effect of regurgitant from *Leptinotarsa decemlineata* on wound responses in *Solanum tuberosum* and *Phaseolus vulgaris*. Physiol Plant 115:577–584

Lawrence SD, Novak N (2001) A rapid method for the production and characterization of recombinant insecticidal proteins in plants. Molec Breeding 8:139–146

Lawrence SD, Novak NG (2006) Expression of poplar chitinase in tomato leads to inhibition of development in colorado potato beetle. Biotech Lett 28:593–599

Le Bourvellec C, Le Quere J-M, Sanoner P, Drilleau J-F, Guyot S (2004) Inhibition of apple polyphenol oxidase by procyanidins and polyphenol oxidation products. J Agric Food Chem 52:122–130

Li L, Steffens JC (2002) Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. Planta 215:239–247

Major IT, Constabel CP (2006) Molecular analysis of poplar defense against herbivory. Comparison of wound- and insect elicitor-induced gene expression. New Phytol 172:617–635

Martinez-Cayuela M, Faus MJ, Gil A (1988) Effects of some reductants on the activity of cherimoya polyphenol oxidase. Phytochemistry 27:1589–1592

Marusek CM, Trobaugh NM, Flurkey WH, Inlow JK (2006) Comparative analysis of polyphenol oxidase from plant and fungal species. J Inorg Biochem 100:108–123

Mayer AM (2006) Polyphenol oxidases in plants and fungi: going places? A review. Phytochemistry 67:2318–2331

Melo GA, Shimizu MM, Mazzafera P (2006) Polyphenoloxidase activity in coffee leaves and its role in resistance against coffee leaf miner and coffee leaf rust. Phytochemistry 67:277–285

Miranda M, Ralph SG, Mellway R, White R, Heath MC, Bohlmann J, Constabel CP (2007) The transcriptional response of hybrid poplar (*Populus trichocarpa* x *P. deltoides*) to infection by *Melampsora medusae* leaf rust involves induction of flavonoid pathway genes leading to the accumulation of proanthocyanidins. Molec Plant Microbe Interact 20:816–831

Murata M, Nishimura M, Murai N, Haruta M, Homma S, Itoh Y (2001) A transgenic apple callus showing reduced polyphenol oxidase activity and lower browning potential. Biosc Biotech Biochem 65:383–388

Nagai T, Suzuki N (2003) Polyphenol oxidase from bean sprouts (*Glycine max* L.). J Food Sci 68:16–20

Nakayama T, Yonekura-Sakakibara K, Sato T, Kikuchi S, Fukui Y, Fukuchi-Mizutani M, Ueda T, Nakao M, Tanaka Y, Kusumi T, Nishino T (2000) Aureusidin synthase: a polyphenol oxidase homolog responsible for flower coloration. Science 290:1163–1166

Negishi O, Ozawa T (2000). Inhibition of enzymatic browning and protection of sulfhydryl enzymes by thiol compounds. Phytochemistry 54:481–487

Nerya O, Musa R, Khatib S, Tamir S, Vaya J (2004) Chalcones as potent tyrosinase inhibitors: the effect of hydroxyl positions and numbers. Phytochemistry 65:1389–1395

Ni X, Quisenberry SS, Heng-Moss T, Markwell J, Sarath G, Klucas R, Baxendale F (2001) Oxidative responses of resistant and susceptible cereal leaves to symptomatic and nonsymptomatic cereal aphid (Hemiptera: Aphididae) feeding. J Econ Entomol 94:743–751

Parsons TJ, Bradshaw HD, Gordon MP (1989). Systemic accumulation of specific mRNAs in response to wounding in poplar trees. Proc Natl Acad Sci USA 86:7895–7899

Raj SN, Sarosh BR, Shetty HS (2006) Induction and accumulation of polyphenol oxidase activities as implicated in development of resistance against pearl millet downy mildew disease. Funct Plant Biol 33:563–571

Ralph S, Oddy C, Cooper D, et al. (2006) Genomics of hybrid poplar (*Populus trichocarpa* x *deltoides*) interacting with forest tent caterpillars (*Malacosoma disstria*): normalized and full-length cDNA libraries, expressed sequence tags, and cDNA microarray for the study of insect-induced defences in poplar. Mol Ecol 15:1275–1297

Ramamoorthy V, Raguchander T, Samiyappan R (2002) Induction of defense-related proteins in tomato roots treated with *Pseudomonas fluorescens* Pf1 and *Fusarium oxysporum* f. sp lycopersici. Plant Soil 239:55–68

Rathjen AH, Robinson SP (1992) Aberrant processing of polyphenol oxidase in a variegated grapevine mutant. Plant Physiol 99:1619–1625

Redman AM, Cipollini DF, Schultz JC (2001) Fitness costs of jasmonic acid-induced defense in tomato, *Lycopersicon esculentum*. Oecologia 126:380–385

Ren F, Lu YT (2006) Overexpression of tobacco hydroxyproline-rich glycopeptide systemin precursor A gene in transgenic tobacco enhances resistance against *Helicoverpa armigera* larvae. Plant Sci 171:286–292

Ruuhola T, Yang S (2006) Wound-induced oxidative responses in mountain birch leaves. Ann Bot 97:29–37

Ryan CA (2000) The systemin signaling pathway: differential activation of plant defensive genes. Biochim Biophys Acta 1477:112–121

Schilmiller AL, Howe GA (2005) Systemic signaling in the wound response. Curr Opin Plant Biol 8:369–377

Shin R, Froderman T, Flurkey WH (1997) Isolation and characterization of a mung bean leaf polyphenol oxidase. Phytochemistry 45:15–21

Steffens JC, Harel E, Hunt MD (1994) Polyphenol oxidase. In: Ellis BE, Kuroki GW, Stafford HA (eds) Genetic engineering of plant secondary metabolism. Plenum Press, New York, pp 276–304

Steiner U, Schliemann W, Bohm H, Strack D (1999) Tyrosinase involved in betalain biosynthesis of higher plants. Planta 208:114–124

Stevens LH, Davelaar E, Kolb RM, Pennings EJM, Smit NPM (1998) Tyrosine and cysteine are substrates for blackspot synthesis in potato. Phytochemistry 49:703–707

Stout MJ, Workman KV, Bostock RM, Duffey SS (1998) Specificity of induced resistance in the tomato, *Lycopersicon esculentum*. Oecologia 113:74–81

Thaler JS, Stout MJ, Karban R, Duffey SS (1996) Exogenous jasmonates simulate insect wounding in tomato plants (*Lycopersicon esculentum*) in the laboratory and field. J Chem Ecol 22: 1767–1781

Thaler JS, Fidantsef AL, Duffey SS, Bostock RM (1999) Trade-offs in plant defense against pathogens and herbivores: a field demonstration of chemical elicitors of induced resistance. J Chem Ecol 25:1597–1609

Thaler JS, Stout MJ, Karban R, Duffey SS (2001) Jasmonate-mediated induced plant resistance affects a community of herbivores. Ecol Entomol 26:312–324

Thaler JS, Karban R, Ullman DE, Boege K, Bostock RM (2002) Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. Oecologia 131:227–235

Thipyapong P, Steffens JC (1997) Tomato polyphenol oxidase – Differential response of the polyphenol oxidase F promoter to injuries and wound signals. Plant Physiol 115:409–418

Thipyapong P, Joel DM, Steffens JC (1997) Differential expression and turnover of the tomato polyphenol oxidase gene family during vegetative and reproductive development. Plant Physiol 113:707–718

Thipyapong P, Hunt MD, Steffens JC (2004a) Antisense downregulation of polyphenol oxidase results in enhanced disease susceptibility. Planta 220:105–117

Thipyapong P, Melkonian J, Wolfe DW, Steffens JC (2004b) Suppression of polyphenol oxidases increases stress tolerance in tomato. Plant Sci 167:693–703

Tscharntke T, Thiessen S, Dolch R, Boland W (2001) Herbivory, induced resistance, and interplant signal transfer in *Alnus glutinosa*. Biochem Syst Ecol 29:1025–1047

van Gelder CWG, Flurkey WH, Wichers HJ (1997) Sequence and structural features of plant and fungal tyrosinases. Phytochemistry 45:1309–1323

Vickers JE, Grof CPL, Bonnett GD, Jackson PA, Knight DP, Roberts SE, Robinson SP (2005) Overexpression of polyphenol oxidase in transgenic sugarcane results in darker juice and raw sugar. Crop Sci 45:354–362

Walker JRL, McCallion RF (1980) Selective inhibition of ortho-diphenol and para-diphenol oxidases. Phytochemistry 19:373–377

Wang JH, Constabel CP (2004) Polyphenol oxidase overexpression in transgenic *Populus* enhances resistance to herbivory by forest tent caterpillar (*Malacosoma disstria*). Planta 220:87–96

Wuyts N, De Waele D, Swennen R (2006) Extraction and partial characterization of polyphenol oxidase from banana (*Musa acuminata* Grande naine) roots. Plant Physiol Biochem 44: 308–314

Yoruk R, Marshall MR (2003) Physicochemical properties and function of plant polyphenol oxidase: a review. J Food Biochem 27:361–422