Available online at www.sciencedirect.com
ScienceDirect
Postharvest Biology and Technology 46 (2007) 222–229
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Aromatic- and di-carboxylates inhibit wound-induced phenolic accumulation in excised lettuce (*Lactuca sativa* L.) leaf tissue

Mikal E. Saltveit*, Young-Jun Choi

Mann Laboratory, Department of Plant Sciences, University of California, One Shields Ave., Davis, CA 95616-8631, USA

Received 31 August 2006; accepted 13 May 2007

Abstract

Excision of de-bladed 5-mm mid-rib segments from Romaine lettuce leaf tissue induced a five-fold rise in phenolic concentration when held at 10 °C for 48 h. Immersion in aqueous solutions of various di-carboxylates and aromatic carboxylates for 1 h reduced this wound-induced increase. The decrease was linear for di-carboxylates with increasing length from 3 to 14 carbons, with the phenolic concentration becoming less than the control for compounds with more than 6 carbons. Aromatic carboxylates with a hydroxyl at the 2 position and another at an even position produced an average reduction of 46%, while aromatic carboxylates with no hydroxyl groups, groups at odd positions, or adjacent groups produced an average 12% reduction. The decrease in wound-induced phenolic accumulation produced by 10 mM solutions of aromatic- and di-carboxylates was linearly correlated ($R^2 = 0.91$) with the rate of carbon dioxide production, indicating possible tissue damage. This possibility was supported by the fact that most effective concentrations were highly correlated with increased ion leakage, another measure of tissue damage. While delaying the application of inhibitors of wound signal synthesis (e.g., *n*-alcohols) decreases their effectiveness, delaying the 1 h immersion in aromatic and di-carboxylate solutions for 4 h did not significantly reduce their effectiveness. Like mono-carboxylates, aromatic and di-carboxylates do not appear to be interfering with the synthesis or propagation of a wound signal, but appear to be inhibiting phenolic synthesis and/or accumulation at some subsequent step in the wound response.

Keywords: Abiotic stress; Hydroxylated benzoates; Carboxylic acids; Fresh-cut; Phenolic metabolism; Wound signal; Wounding

1. Introduction

Mechanical wounds induce a number of physiological changes in plant tissue including the production of a wound signal that stimulates phenolic metabolism through the *de novo* synthesis of phenylalanine ammonia lyase (PAL, EC 4.3.1.5), and the synthesis and accumulation of soluble phenolic compounds (e.g., chlorogenic acid) in adjacent non-wounded tissue (Tomás-Barberán et al., 1997; Brecht et al., 2004; Campos-Vargas et al., 2004). This signal is produced at the site of injury and moves or propagates into adjacent non-injured lettuce leaf tissue at 5 mm h^{-1} (Ke and Saltveit, 1989). The wound signal in lettuce leaf tissue does not appear to be a volatile or an easily extracted water-soluble compound (Kang and Saltveit, 2003). A number of chemicals with putative wound signaling capability in other plant tissues were surveyed, but none induced the same three- to five-fold increase in phenolic metabolism as did excision of lettuce mid-rib segments (Campos-Vargas and Saltveit, 2002).

Studies with inhibitors of specific enzymes have implicated the phospholipid signaling pathway as a source of wound signals in lettuce (Choi et al., 2005; Saltveit et al., 2005). Phospholipase D (PLD, EC 3.1.4.4), an enzyme involved in the phospholipid signaling pathway is specifically inhibited by *n*-butanol (Munnik et al., 1995; Lee et al., 2001; Gardiner et al., 2003). Lipoxygenase (LOX) converts linolenic acid released by reactions subsequent to the action of PLD into compounds that are either phyto-active in themselves, or serve as substrates for the synthesis of other phyto-active compounds (Porta and Rocha-Sosa, 2002). Salicylic acid (SA) inhibits these progressive changes by interfering with the activity of allene oxide synthase (AOS) (Creelman and Mullet, 1997; Harms et al., 1998). Each of these enzymes (PLD, LOX, and AOS) increased upon wounding in various tissues and their inhibition was overcome by supplying

* Corresponding author.
E-mail address: mesaltveit@ucdavis.edu (M.E. Saltveit).

0925-5214/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2007.05.004

treated tissue with the catalyzed product. The production and accumulation of wound-induced phenolic compounds was significantly suppressed by treating excised mid-rib lettuce tissue with inhibitors of these enzymes (Choi et al., 2005; Saltveit et al., 2005).

Salicylic acid (SA) appears to have dual functions in wounding. It can either elicit responses similar to those induced by certain stresses (Klessig and Malamy, 1994; Senaratna et al., 2000), or it can inhibit AOS activity and thereby reduce the activity of the oxylipin pathway (Peña-Cortés et al., 1993; Doares et al., 1995; Harms et al., 1998). SA is a hydroxylated aromatic carboxylate and it was one of the putative wound signal tested that had a minimal inductive effect in lettuce leaf tissue (Campos-Vargas and Saltveit, 2002).

Immersion of 5-mm mid-rib segments in aqueous buffered (pH 7.0) solutions of *n*-alcohols ranging from 1 to 7 carbons in length (Choi et al., 2005) or mono-carboxylates ranging from 1 to 10 carbons in length (Saltveit et al., 2005) reduced wound-induced phenolic accumulation (WIPA) in excised lettuce (*Lactuca sativa* L.) leaf tissue. The active *n*-alcohols were maximally effective when applied immediately after excision, and lost effectiveness when treatment was delayed, becoming ineffective after 5 h. In contrast, mono-carboxylates did not loose their effectiveness when treatment was delayed 5 h. If a treatment affected the generation or propagation of a wound signal, then delaying its application would allow the wound signal to induce phenolic metabolism in adjacent non-injured tissue. The 2- and 3-isomers of effective *n*-alcohols (1-alcohols) were ineffective, whereas isomers of mono-carboxylates were equally effective. Unlike the *n*-alcohols, mono-carboxylates do not appear to be interfering with the synthesis or propagation of a wound signal, but appeared to be interfering with subsequent steps in the production and accumulation of wound-induced phenolic compounds.

Increased rates of respiration (e.g., carbon dioxide production) and leakage of ions from excised tissue into an isotonic solution are two indications that lettuce tissue has been subjected to a mechanical, chemical, or thermal stress (Ke and Saltveit, 1989; Saltveit, 2002; Brecht et al., 2004). While many stresses increase phenolic metabolism and subsequent tissue browning, some levels of stress may so perturb cellular metabolism as to suppress a wound response. For example, a heat-shock thermal stress suppresses the ability of wounded lettuce tissue to translate wound-induced PAL mRNA into an active PAL enzyme, and thereby reduces subsequent tissue browning. Measuring rates of respiration and ion leakage from carboxylate treated lettuce tissue will indicate if effective treatments are also causing a level of stress that could, irrespective of the chemical used, alter the wound response.

Research reported in this paper was undertaken to examine the effect of the number and placement of carboxyl group(s) (–COOH) on a molecule's effectiveness in reducing WIPA in excised segments of lettuce leaf tissue. Data presented in this paper suggest that like mono-carboxylates, aromatic and di-carboxylates are not interfering with the synthesis or propagation of the wound signal, but are inhibiting phenolic synthesis and/or accumulation by some subsequent process.

2. Materials and methods

2.1. Plant material

Romaine lettuce (*L. sativa* L. cv. Longifolia) was purchased from local commercial vendors. Outer leaves were discarded, and the undamaged leaves were carefully detached from the stem. The leaf blade was removed and 5 mm segments of the mid-rib tissue were excised starting 10 mm from the base and extending 8 cm up the mid-rib. The freshly excised segments were randomly distributed among treatments, and 9.0 ± 0.2 g FW placed in 15×100 diameter plastic Petri dishes.

2.2. Treatments

Freshly excised mid-rib segments were immersed in gently shaken aqueous solutions of various chemicals. Some compounds were slightly soluble in water and were initially made up in methanol as a bridging solvent. At most, 1 mL of this methanol solution was added to 100 mL of 25 mM potassium phosphate buffer (pH 7.0). The resultant concentration of methanol did not significantly affect WIPA (Choi et al., 2005; data not shown). Solubility of both mono- and di-carboxylates above 14 carbons in length was very limited in buffered aqueous solutions, even when employing the bridging solvent (Saltveit et al., 2005), so experiments were limited to di-carboxylates with 2–14 carbons in length. Tissue was immersed in solutions of various concentration for 60 min with shaking, starting immediately after excision, or for 60 min at 1, 2, 3, or 4 h after excision.

After immersion, the tissue was drained, blotted with paper tissues to remove excess solution and placed in 15 mm × 100 mm diameter plastic Petri dishes. The dishes were placed in 20 cm × 15 cm × 10 cm translucent plastic tubs lined with wet paper towels, the top of the tubs were loosely covered with aluminum foil, and the tubs placed at 10 °C for 48 h.

2.3. Measurement of phenolic content

After 48 h at 10 °C, 3 g of tissue from each Petri dish was put into a 50 mL plastic centrifuge tube along with 20 mL of methanol. The tissue was ground, and the absorbance of a clarified aliquot was measured at 320 nm (Ke and Saltveit, 1989; Loaiza-Velarde et al., 1997; Campos-Vargas and Saltveit, 2002) and expressed as absorbance per gram fresh weight.

2.4. Measurement of ion leakage

The slope of ion leakage was calculated as previously described (Saltveit, 2002, 2005), with slight modifications. The conductivity of a 20 mL isotonic solution (0.2 M mannitol) containing 2 g of tissue was periodically measured. The 50-mL plastic centrifuge tubes were capped and subjected to two cycles of freezing (−20 °C) and thawing before total conductivity was measured at 20 °C. A regression line (R^2 of 0.95 or better) was fitted to the data from 30 to 120 min, and leakage is expressed as a slope with values of percent of total conductivity per min.

2.5. Measurement of carbon dioxide production

Carbon dioxide production was calculated from changes in the head space concentration as previously described (Saltveit, 2005), with slight modifications. Tissue (2 g) was enclosed in a 50-mL plastic centrifuge tube for 1 h at 20 °C. One-mL gas samples were withdrawn through a rubber serum stopper inserted in the tube's lid and analyzed using an infrared carbon dioxide analyzer as previously described (Saltveit, 2005).

2.6. Chemicals used

All chemicals were reagent grade or better and were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.7. Statistical analysis

Each experiment was done at least three times with two replicates per treatment. Means and standard deviations were calculated from all the replicates, and where appropriate, the data were combined and treatment effects subjected to further analysis.

3. Results

3.1. Di-carboxylates

Excision of 5-mm mid-rib segments from Romaine lettuce leaves induced a three- to five-fold increase in phenolic content (absorbance of methanol extract at 320 nm) after 48 h at 10 °C (Choi et al., 2005). The di-carboxylates had variable effects in reducing this wound-induced increase in phenolic accumulation (WIPA) (Fig. 1). The 2–5 carbon di-carboxylate

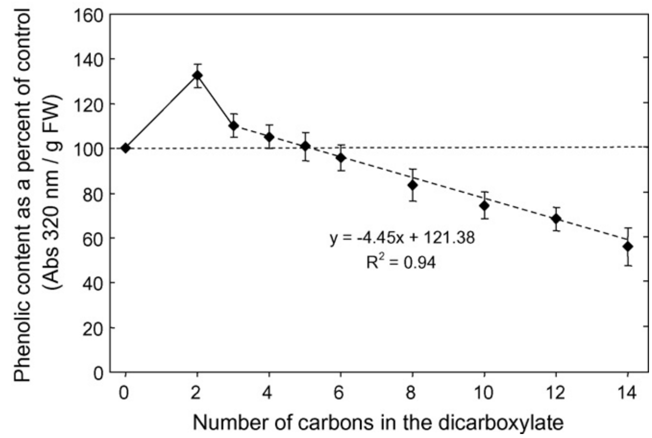

Fig. 1. Phenolic content of excised Romaine lettuce leaf mid-rib segments exposed to di-carboxylates with from 2 to 14 carbons. Excised 5-mm mid-rib segments were exposed to 10 mM for 1 h and then held at 10 °C for 48 h. Absorbance of a clarified methanol extract was read at 320 nm after holding the tissue for 48 h at 10 °C. The vertical line associated with each point represents the standard deviation about that mean.

either stimulated or had no effect on WIPA. There was a linear decrease ($R^2 = 0.94$) in phenolic content as the number of carbons increased from 3 to 14, with the phenolic content decreasing below that of the control at 8 carbons. Treatment with the 10, 12, or 14 carbon di-carboxylate reduced WIPA by 26, 32 and 44%, respectively.

The effectiveness of di-carboxylates in reducing WIPA increased with increasing concentration and with increasing number of carbons (Fig. 2). Except for the C6 di-carboxylate, increased concentrations of each of the C8–C14 di-carboxylate produced greater reductions in WIPA and also produced greater rates of ion leakage. At 10 mM, increasing the carbon number from 6 to 14 produced both a linear reduction ($R^2 = 0.99$) in

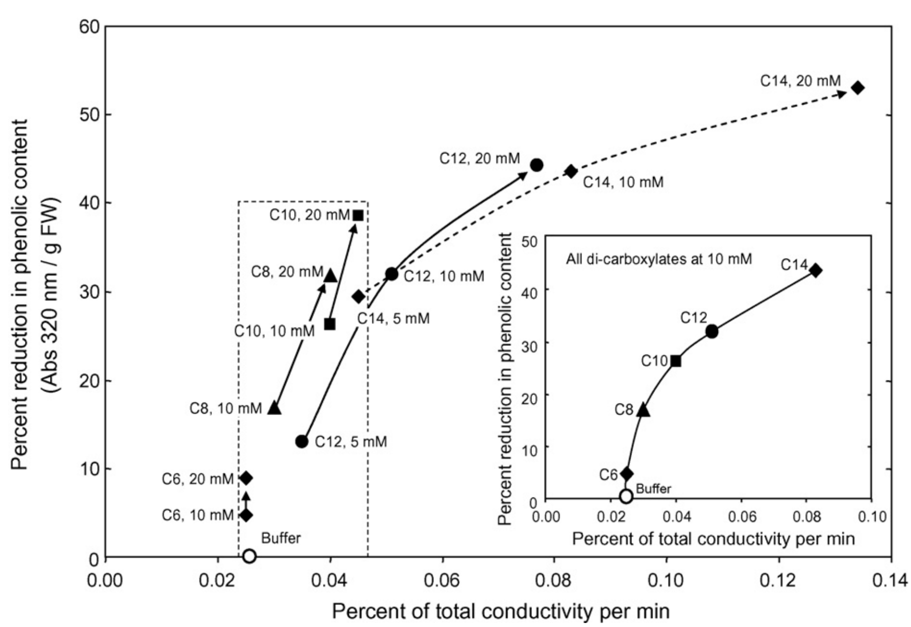

Fig. 2. Phenolic content and rate of ion leakage from excised Romaine mid-rib segments exposed to various di-carboxylates. Excised 5-mm mid-rib segments (10 g) were immersed in 100 mL of 25 mM potassium phosphate buffer containing 5, 10, or 20 mM C6, C8, C10, C12 or C14 di-carboxylates at pH 7 for 60 min (see Section 2 for details of absorbance, and ion leakage measurements).

WIPA, and a quadratic increase ($R^2 = 0.98$) in ion leakage. When compared, increases in ion leakage were related by a quadratic equation to reduction in WIPA ($R^2 = 0.97$) (Fig. 2, inset).

The percent reduction in phenolic content ranged from 0 to 39% within a range of ion leakage from 0.025 to 0.045% of total conductivity per hour (Fig. 2, vertical dashed lines). Within this range, a 26–39% reduction in WIPA was produced by C8 at 20 mM, C10 at 10 or 20 mM, C12 at 10 mM, and C14 at 5 mM, and was accompanied by rates of ion leakage from 0.040 to 0.045% of total conductivity per hour. The greatest reduction in WIPA (39%) was produced by C10 at 20 mM which also increased leakage 80% over the buffer control to 0.045% of total conductivity per hour.

The effectiveness of the 10 carbon di-carboxylate at 20 mM was not related to the time of application after excision (Fig. 3). Delaying the 1 h treatment for up to 4 h did not significantly reduce its effectiveness with an average of a $33 \pm 4\%$ reduction in WIPA over the range of treatment times. Similar results were obtained with 10 mM C8, C10 and C12 di-carboxylates (data not shown).

3.2. Aromatic carboxylates with additional hydroxyl groups

Aromatic carboxylates with from 0 to 3 hydroxyl groups were variable in their ability to reduce WIPA (Fig. 4). The most effective aromatic carboxylates were those with a hydroxyl at the 2 position and another at an even position (e.g., 4, or 6, or 4, 6). They produced an average 46% reduction in WIPA. In comparison, aromatic carboxylates with no hydroxyl groups (benzoate), or groups at the odd position (e.g., 3, or 3, 5), or with groups close together (e.g., 2, 3, or 3, 4, or 2, 3, 4) only produced an average 12% reduction in WIPA.

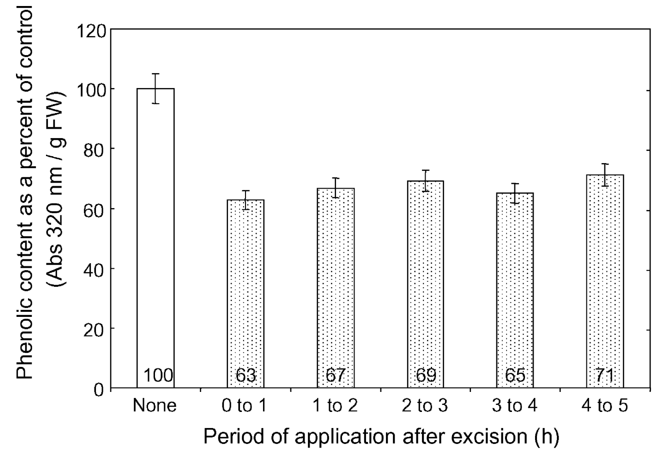

Fig. 3. Phenolic content of excised Romaine lettuce leaf mid-rib segments exposed to 20 mM of the 10 carbon di-carboxylate for 60 min at various times after excision and then held at 10 °C for 48 h. Absorbance of a clarified methanol extract was read at 320 nm after holding the tissue for 48 h at 10 °C. The vertical line atop each bar represents the standard deviation about that mean.

Hydroxylated aromatic carboxylates effectively reduced WIPA at concentrations that did not greatly increase ion leakage (Fig. 5). The percent reduction in phenolic content ranged from 0 to 34% within a range of ion leakage from 0.030 to 0.040% of total conductivity per hour (Fig. 5, dashed vertical lines). There was a range of reduction in WIPA from 24 to 34% within a range of ion leakage from 0.030 to 0.035% of total conductivity per min for 4-hydroxybenzoate (4-HB), 2,4-, and 2,6-dihydroxybenzoate (2,4-DHB, 2,6-DHB), and 2,4,6-trihydroxybenzoate (2,4,6-THB). The greatest reduction in WIPA (34%) was produced by 2,4-DHB at 10 mM, which also had a rate of ion leakage (0.030% of total conductivity

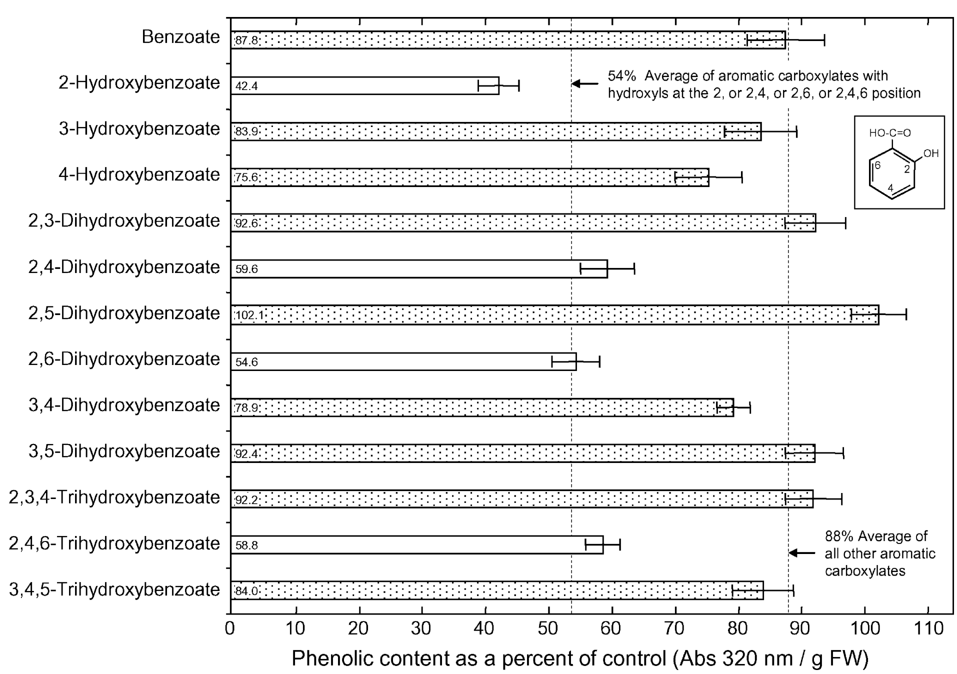

Fig. 4. Phenolic content of excised Romaine lettuce leaf mid-rib segments exposed to aromatic carboxylates with from 0 to 3 hydroxyl groups. Excised 5-mm mid-rib segments were exposed to 10 mM for 1 h and then held at 10 °C for 48 h. Absorbance of a clarified methanol extract was read at 320 nm after holding the tissue for 48 h at 10 °C. The horizontal line atop each bar represents the standard deviation about that mean.

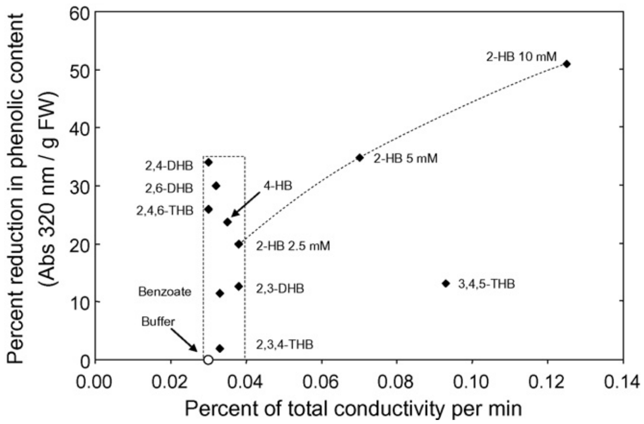

Fig. 5. Phenolic content and rate of ion leakage from excised Romaine mid-rib segments exposed to aqueous solutions of aromatic carboxylates. Excised 5-mm mid-rib segments (10 g) were immersed in 100 mL of 25 mM potassium phosphate buffer containing 10 mM benzoate, 2-hydroxybenzoate (2-HB), 4-HB, 2,3-dihydroxybenzoate (2,3-DHB), 2,4-DHB, 2,6-DHB, 2,3,4-trihydroxybenzoate (2,3,4-THB), or 2,4,6-THB, or 3,4,5-THB at pH 7 for 60 min. Solutions of 2.5 and 5 mM 2-HB were also used (see Section 2 for details of absorbance, and ion leakage measurements).

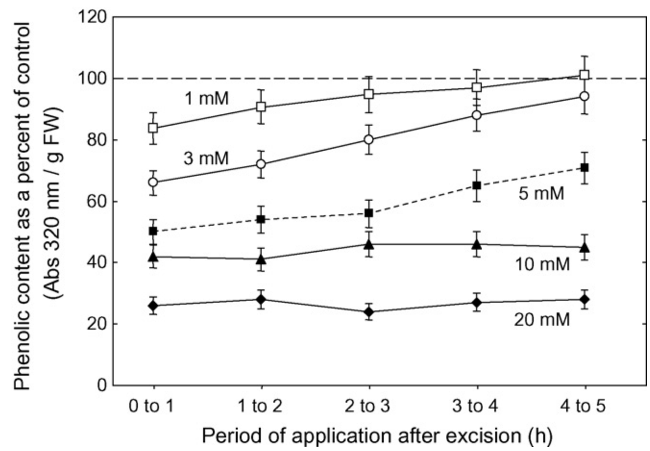

Fig. 7. Phenolic content of excised Romaine lettuce leaf mid-rib segments exposed to various concentration of salicylic acid (2-hydroxylbenzoate; 2-HB) for 60 min at various times after excision and then held at 10 °C for 48 h. Absorbance of a clarified methanol extract was read at 320 nm after holding the tissue for 48 h at 10 °C. The vertical line associated with each point represents the standard deviation about that mean.

per hour) similar to that of the buffer control. In comparison, 2-hydroxybenzoate (2-HB, salicylate) reduced WIPA 51% at 10 mM and increased ion leakage four-fold to 0.125% total per hour, while 3,4,5-trihydroxybenzoate (3,4,5-THB) reduced WIPA only 13% and increased ion leakage three-fold to 0.093% total per hour.

Salicylate (2-HB) was the most effective of the hydroxylated aromatic carboxylates with 2.5, 5.0, 10, or 20 mM treatments producing 21, 36, 59, or 71%, reductions in WIPA, respectively (Fig. 5). The decline in WIPA followed a quadratic curve ($R^2 = 0.99$) saturating at 15 mM and higher concentrations (Fig. 6).

Like the response to the di-carboxylates, delaying the application of 10 and 20 mM solutions of effective hydroxylated aromatic carboxylates (e.g., 2-HB) to freshly excised 5-mm mid-rib segments of Romaine lettuces leaves did not significantly alter their level of effectiveness in reducing WIPA (Fig. 7). However, applying low concentrations of 2-HB (1–3 mM) immediately after excision reduced WIPA by 15 ± 5 and 34 ± 9%, respectively, while delaying application for 4 h effectively reduced the level of inhibition to zero (Fig. 7). Higher concentrations of 2-HB (10 and 20 mM) produced inhibitions of 56 ± 2 and 73 ± 2%, respectively, regardless of whether applied immediately after excision or 4 h later. Exposure to 5 mM 2-HB produced an intermediate response, with immediate application producing a 50 ± 5% inhibition of WIPA, whereas delaying application for 4 h produced only a 34 ± 5% inhibition.

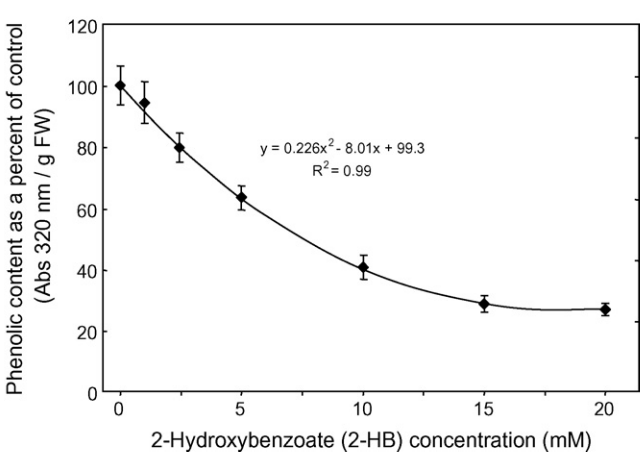

Fig. 6. Phenolic content of excised Romaine lettuce leaf mid-rib segments exposed to various concentrations of salicylic acid (2-hydroxybenzoate, 2-HB). Excised 5-mm mid-rib segments were exposed to 10 mM for 1 h and then held at 10 °C for 48 h. Absorbance of a clarified methanol extract was read at 320 nm after holding the tissue for 48 h at 10 °C. The vertical line associated with each point represents the standard deviation about that mean.

3.3. Aromatic carboxylates with additional carboxyl groups

Aromatic carboxylates that contained additional carboxyl groups were either marginally effective or ineffective in reducing WIPA, or actually stimulated WIPA (Fig. 8). Benzoate (one carboxyl group on the aromatic ring) reduced WIPA 12%, while the addition of another carboxyl group at the 2, 3, or 4 position reduced WIPA 10%, 0.0% or increased WIPA 12%, respectively. Increasing the benzoate concentration to 30 mM produced a 64 ± 3% reduction in WIPA that was unaffected by whether applied immediately after excision or delayed for 5 h (data not shown). WIPA was stimulated 55% by compounds with three carboxyl groups (i.e., trimesate and trimellitate). The addition of carboxyl groups to the aromatic ring did not increase the ability of the compound to reduce WIPA.

3.4. Measurement of carbon dioxide production, ion leakage and phenolic content

The di-carboxylates (C6–C14) and the hydroxylated benzoates (2-HB; 2,3-, 2,4-, and 2,6-DHB; and 2,4,6-, and

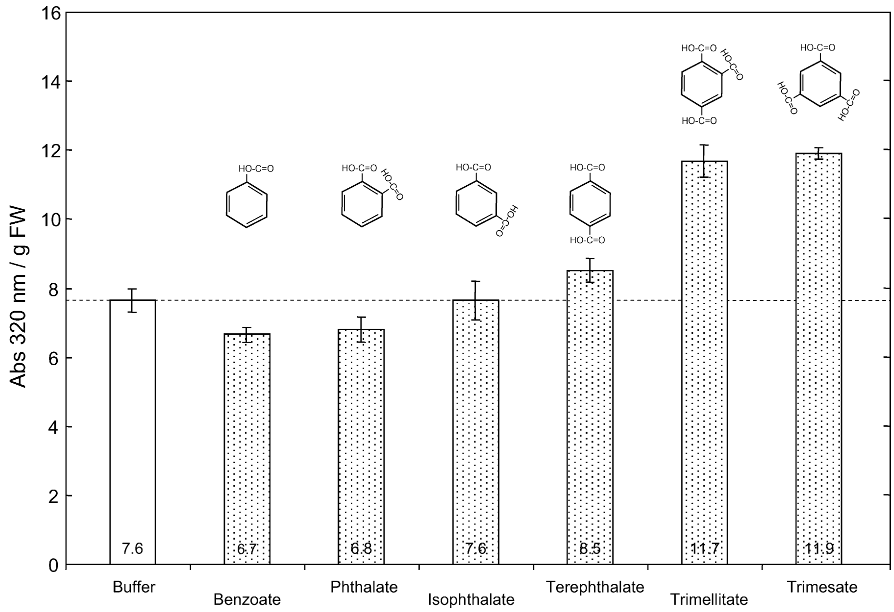

Fig. 8. Phenolic content of excised Romaine lettuce leaf mid-rib segments exposed to aromatic carboxylates with from 1 to 3 carboxyl groups. Excised 5-mm mid-rib segments were exposed to 10 mM for 1 h and then held at 10 °C for 48 h. Absorbance of a clarified methanol extract was read at 320 nm after holding the tissue for 48 h at 10 °C. The vertical line atop each bar represents the standard deviation about that mean.

3,4,6-THB) produced different effects. When applied at 10 mM, the di-carboxylates above C6 increased both ion leakage and carbon dioxide production with increasing chain length (Fig. 9). The rate of carbon dioxide production from tissue treated with the C6–C14 di-carboxylates was highly correlated ($R^2 = 0.98$) with the rate of induced ion leakage. In contrast, although the hydroxyl aromatic carboxylates also increased carbon dioxide production, apart from 2-HB, they had no significant effect on the rate of ion leakage. However, the decrease in phenolic content of mid-rib tissue treated with 10 mM solutions of these carboxylates was linearly correlated ($R^2 = 0.91$) with the rate of carbon dioxide production (Fig. 9, inset).

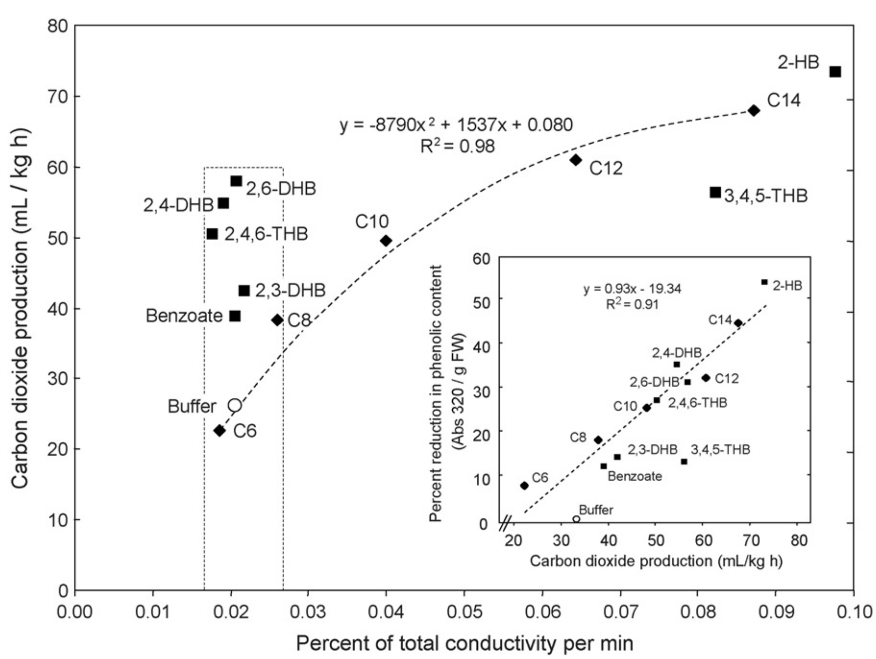

Fig. 9. Production of carbon dioxide as related to ion leakage from 5-mm excised mid-rib segments from Romaine lettuce exposed to 10 mM solutions of di-carboxylates (C6–C14), benzoate, and hydroxylated benzoates 2-hydroxybenzoate (2-HB), 4-HB, 2,3-dihydroxybenzoate (2,3-DHB), 2,4-DHB, 2,6-DHB, 2,4,6-trihydroxybenzoate (2,4,6-THB), or 3,4,5-THB at pH 7 for 60 min. The inset shows the relation between the rate of carbon dioxide production and the percent reduction in absorbance in tissue exposed to the same treatments (see Section 2 for details of absorbance, carbon dioxide, and ion leakage measurements).

4. Discussion

4.1. Period of application affects WIPA reduction

Delaying application of an inhibitor of WIPA can be used to discern whether the compound was acting on generation and propagation of the wound signal, or on some other subsequent aspect of phenolic metabolism. If the compound acted by interfering with the wound signal, then applying it after the signal had had time to be synthesized and stimulate phenolic metabolism in adjacent tissue (i.e., after 4 h) would produce far less inhibition of WIPA than would its application immediately after excision. Delaying the application of di-carboxylates and hydroxylated aromatic carboxylates to excised lettuce leaf tissue did not reduce their ability to suppress WIPA. This suggests that these compounds were not directly interfering with the synthesis or propagation of the wound signal, but were affecting some other subsequent reaction in the synthesis and accumulation of wound-induced phenolic compounds.

4.2. Reduced WIPA and increase ion leakage

While some compounds were able to reduce WIPA without increasing ion leakage (Figs. 2, 5 and 9), the effectiveness of these same compounds was highly correlated with increased ion leakage. Increased rates of ion leakage are an indication that lettuce tissue has been subjected to a level of stress sufficient to alter cellular activity (Ke and Saltveit, 1989; Saltveit, 2002; Brecht et al., 2004). There may be two modes of actions for these chemicals; one interferes with phenolic synthesis without causing increased ion leakage, while the other disrupts cellular functions and increases ion leakage, and thereby reduces phenolic synthesis.

4.3. Relation among carbon dioxide production, ion leakage and phenolic content

Aromatic- and di-carboxylates that ranged in effectiveness in reducing WIPA from less than 10% (C6) to over 50% (2-HB) produced ion leakage rates within a narrow range, yet they were strongly correlated with increasing rates of carbon dioxide production (Fig. 9). It is surprising that an increase in carbon dioxide production is so highly correlated with a reduction in WIPA. Increased rates of carbon dioxide production are often synonymous with increased metabolic activity (e.g., at elevated temperatures), or indicative of increased tissue injury (e.g., respiratory burst associated with cutting) and are followed by increased, not decreased levels of phenolic production.

4.4. Mode of action

Polyphenol oxidase (EC 1.14.18.1; PPO) is involved in the browning of cut lettuce and is present in effective concentrations in tissue before wounding (Tomás-Barberán et al., 1997). Lettuce PPO has a broad optimal pH range (5–8) (Heimdal et al., 1994). Aromatic carboxylic acids are competitive inhibitors of PPO because of their structural similarity with phenolic substrates (Janovitz-Klapp et al., 1990). However, the effective aromatic-, di-, and mono-carboxylates all inhibited the production of phenolic compounds (Abs 320 nm), not just their oxidation to colored compounds. So although these compounds may interfere with subsequent phenolic oxidation, their mode of action seems to be at a more nascent stage in the production of wound-induced phenolic compounds.

The mode of action of salicylate (2-HB) may depend on its concentration. Lower concentrations of salicylate (2-HB) (<5 mM) were more effective in reducing WIPA when applied immediately after excision than when the 1 h application was delayed for up to 4 h. Both 1 and 3 mM became ineffective in reducing WIPA when the start of the 1 h treatment was delayed for 4 h after excision. At low concentration SA may be affecting the wound signal by acting as an inhibitor of AOS (Creelman and Mullet, 1997; Harms et al., 1998), whereas at higher concentration it may be acting as an inhibitor of other cellular processes. SA can also modulate catalase activity (Chen et al., 1993; Durner and Klessig, 1996) and act as a nonspecific inhibitor of cellular kinases (Frantz and O'Neill, 1995).

The wound signal is rapidly synthesized and propagated into adjacent tissue, and delaying application of inhibitors of its synthesis for a few hours greatly diminishes their effectiveness (Choi et al., 2005). Yet delaying the application of the aromatic, mono-, and di-carboxylates for 4 h did not diminish their effectiveness (Figs. 3 and 7 and Saltveit et al., 2005). The carboxylates may be interfering with one of the myriad reactions intervening between the propagation of the wound signal and the accumulation of the newly synthesized phenolic compounds.

References

Brecht, J.K., Saltveit, M.E., Talcott, S.T., Schneider, K.R., Felkey, K., 2004. Fresh-cut vegetables and fruits. Hortic. Rev. 30, 185–251.

Campos-Vargas, R., Saltveit, M.E., 2002. Involvement of putative chemical wound signals in the induction of phenolic metabolism in wounded lettuce. Physiol. Plant. 114, 73–84.

Campos-Vargas, R., Nonogaki, H., Suslow, T.V., Saltveit, M.E., 2004. Isolation and characterization of a wound inducible phenylalanine ammonia-lyase gene (LsPAL1) from Romaine lettuce leaves. Physiol. Plant. 121, 429–438.

Chen, Z., Silva, H., Klessig, D.F., 1993. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science 262, 1883–1886.

Choi, Y.-J., Tomás-Barberán, F.A., Saltveit, M.E., 2005. Wound-induced browning in lettuce (*Lactuca sativa* L.) leaf tissue in reduced by exposure to *n*-alcohols. Postharvest Biol. Technol. 37, 47–55.

Creelman, R.A., Mullet, J.E., 1997. Biosynthesis and action of jasmonates in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48, 355–381.

Doares, S.H., Narvaez-Vasquez, L., Conconi, A., Ryan, C.A., 1995. Salicylic acid inhibits synthesis of protenase inhibitors in tomato leaves induced by systemin and jasmonic acid. Plant Physiol. 108, 1741–1746.

Durner, J., Klessig, D.F., 1996. Salicylic acid is a modulator of tobacco and mammalian catalases. J. Biol. Chem. 271, 28492–28501.

Frantz, B., O'Neill, E.A., 1995. The effect of sodium salicylate and aspirin on NF-kB. Science 207, 2017–2018.

Gardiner, J., Collings, D.A., Harper, J.D.I., Mare, J., 2003. The effect of the phospholipase D-antagonist 1-butanol on seedling development and microtubule organisation in Arabidopsis. Plant Cell Physiol. 44, 687–696.

Harms, K., Ramirez, I., Peña-Cortés, H., 1998. Inhibition of wound-induced accumulation of allene oxide synthase transcripts in flax leaves by aspirin and salicylic acid. Plant Physiol. 118, 1057–1065.

Heimdal, H., Larsen, L.M., Poll, L., 1994. Characterization of polyphenol oxidase from photosynthetic and vascular lettuce tissues (*Lactuca sativa*). J. Agric. Food Chem. 42, 1428–1433.

Janovitz-Klapp, A., Richard, F., Goupy, P., Nicolas, J., 1990. Inhibition studies on apple polyphenol oxidase. J. Agric. Food Chem. 38, 926–931.

Kang, H.-M., Saltveit, M.E., 2003. Wound-induced increases in phenolic content of fresh-cut lettuce is reduced by a short immersion in aqueous hypertonic solutions. Postharvest Biol. Technol. 29, 271–277.

Ke, D., Saltveit, M.E., 1989. Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. Physiol. Plant. 76, 412–418.

Klessig, D.F., Malamy, J., 1994. The salicylic acid signal in plants. Plant Mol. Biol. 26, 1439–1458.

Lee, S., Hirt, H., Lee, Y., 2001. Phosphatidic acid activates a wound-activated MAPK in Glycine max. Plant J. 26, 479–486.

Loaiza-Velarde, J.G., Tomás-Barberán, F.A., Saltveit, M.E., 1997. Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in Iceberg lettuce. J. Am. Soc. Hortic. Sci. 122, 873–877.

Munnik, T., Arisz, S.A., de Vrije, T., Musgrave, A., 1995. G Protein activation stimulates phospholipase D signalling in plants. Plant Cell 7, 2197–2210.

Peña-Cortés, H., Albrecht, T., Prat, S., Weiler, E.W., Willmitzer, L., 1993. Aspirin prevents wound-induced gene expression in tomato leaves by blocking jasmonic acid biosynthesis. Planta 191, 123–128.

Porta, H., Rocha-Sosa, M., 2002. Plant lipoxygenases. Physiological and molecular features. Plant Physiol. 130, 15–21.

Saltveit, M.E., 2002. The rate of ion leakage from chilling-sensitive tissue does not immediately increase upon exposure to chilling temperatures. Postharvest Biol. Technol. 26, 295–304.

Saltveit, M.E., 2005. Influence of heat shocks on the kinetics of chilling-induced ion leakage from tomato pericarp discs. Postharvest Biol. Technol. 36, 87–92.

Saltveit, M.E., Choi, Y.-J., Tomás-Barberán, F.A., 2005. Mono-carboxylic acids and their salts inhibit wound-induced phenolic accumulation in excised lettuce (*Lactuca sativa* L.) leaf tissue. Physiol. Plant. 125, 454–463.

Senaratna, T., Touchell, D., Bunn, E., Dixon, K., 2000. Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerances in bean and tomato plants. Plant Growth Regul. 30, 157–161.

Tomás-Barberán, F.A., Loaiza-Velarde, J., Bonfanti, A., Saltveit, M.E., 1997. Early wound- and ethylene-induced changes in phenylpropanoid metabolism in harvested lettuce. J. Am. Soc. Hortic. Sci. 122, 399–404.