

Institution: University of California at Davis (2)

Participant: Mikal Saltveit, Mann Laboratory, Department of Plant Sciences, University of California, One Shields Ave., Davis, CA 95616-8631; email: mesaltveit@ucdavis.edu

Introduction: This past year, the research emphasis in my lab shifted to a more basic study of the perception, synthesis, and propagation of the signal(s) that produce physiological changes in fresh-cut fruits and vegetables. In the first of a series of four papers we presented data suggesting that phospholipase D (PLD) and its products linolenic acid (LA) and phosphatidic acid (PA) are involved in producing the wound signal responsible for increased wound-induced PAL activity, phenolic accumulation and browning in fresh-cut lettuce leaf tissue (1). In the second paper (2) we used specific inhibitors to further implicate the phospholipid signaling pathway in the generation of a wound signal that induces phenolic metabolism in wounded leaf tissue. We diverged slightly from this line of research in the third paper (3) to study the effect of mono-carboxylates on the wound signal and tissue browning. Subsequent research explored the effectiveness of di-carboxylates and aromatic carboxylates in reducing phenolic metabolism and tissue browning (4).

Activities

Objective 3: Improve understanding of biochemical, physiological and molecular mechanisms that affect fresh-cut product quality.

3.1 Wound-induced phenolic accumulation and browning in lettuce (*Lactuca sativa* L.) leaf tissue is reduced by exposure to n-alcohols. Choi, Y-J, F.A. Tomás-Barberán, and M.E. Saltveit

A wound signal originates at the site of injury in lettuce (*Lactuca sativa* L) leaf tissue and propagates into adjacent tissue where it induces a number of physiological responses which include increased phenolic metabolism with the de novo synthesis of phenylalanine ammonia lyase (PAL, EC 4.3.1.5), the synthesis and accumulation of soluble phenolic compounds (e.g., chlorogenic acid), and subsequent tissue browning. Exposing excised mid-rib leaf tissue to vapors (20 $\mu\text{mol/g}$ FW) or aqueous solutions (100 mM) of n-alcohols inhibited this wound-induced tissue browning by 40% and 60%, respectively. Effectiveness of the alcohol increased linearly from ethanol to the seven-carbon heptanol, and then was lost for the longer n-alcohols 1-octanol and 1-nonanol (Fig. 1). The 2- and 3-isomers of the effective alcohols did not significantly reduce wound-induced phenolic accumulation at optimal 1-alcohol concentrations, but significant reductions did occur at much higher concentrations (100 $\mu\text{mol/g}$ FW) of the 2-, and 3-isomers. The active n-alcohols were maximally effective when applied during the first 2 h after excision, and were ineffective if applied 12 h after excision. Phospholipase D (PLD) and its products linolenic acid (LA) and phosphatidic acid (PA) are thought to initiate the oxylipin pathway that culminates in the production of jasmonic acid, and PLD is specifically inhibited by 1-butanol, but not by 2-, or 3-butanol.

These results suggest that PLD, LA, PA, and the phospholipid signaling pathway may be involved in producing the wound signal responsible for increased wound-induced PAL activity, phenolic accumulation and browning in fresh-cut lettuce leaf tissue.

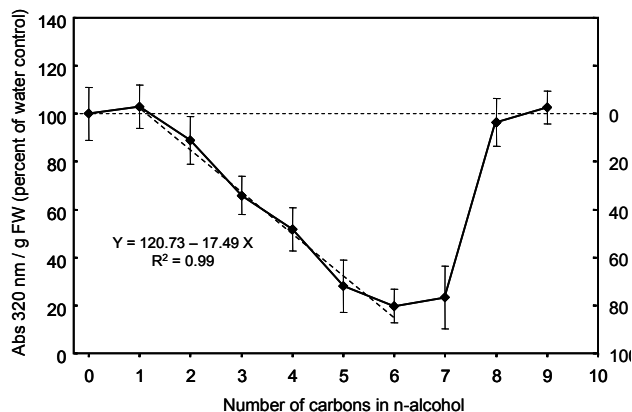


Fig. 1. Phenolic content of excised 5-mm Romaine mid-rib segments exposed to vapors from n-alcohols (from 1-carbon methanol to 9-carbon nonanol). Tissue was exposed to 20 μmol of the alcohol per g FW for 12 h at 10 $^{\circ}\text{C}$. The regression line and linear equation for the normal (1-alcohol) alcohols from 1 to 6 carbons is shown.

3.2. Involvement of components of the phospholipid signaling pathway in wound-induced phenylpropanoid metabolism in lettuce (*Lactuca sativa* L.) leaf tissue. Saltveit, M.E., Y-J Choi, and F.A. Tomás-Barberán

Exposure to 1-butanol vapors or aqueous solutions inhibited wound-induced increase in PAL activity and phenolic metabolism. Phospholipases D (PLD, EC 3.1.4.4), an enzyme involved in the phospholipid signaling pathway is specifically inhibited by 1-butanol. Re-wounding tissue in which an effective 1-butanol concentration had declined below active levels by evaporation, did not elicit the normal wound response. It appears the 1-butanol treated tissue developed resistance to wound-induced increases in phenylpropanoid metabolism that persisted even when active levels of 1-butanol were no longer present. However, a metabolic product of 1-butanol, rather than 1-butanol itself, may be the active compound eliciting persistence resistance. Inhibiting a subsequent enzyme in the phospholipid signaling pathway, lipoxygenase (LOX; EC 1.13.11.12) with 1-phenyl-3-pyrazolidinone (1P3P) or reducing the product of LOX activity with diethyldithio-carbamic acid (DIECA) also inhibited wound-induced PAL activity and phenolic accumulation.

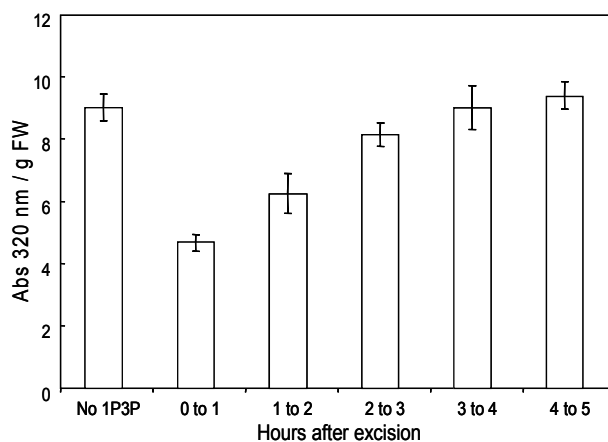


Fig. 2. Phenolic content of mid-rib segments treated with 0.5 mM 1-phenyl-3-pyrazolidinone (1P3P) at different times after excision. Phenolic content was determined by measuring the absorbance of a clarified methanol extract at 320 nm after holding the tissue for a total of 48 h after the initial excision.

The effectiveness of 1-butanol, DIECA, and 1P3P declined as the beginning of the 1 h immersion period was delayed from 0 to 4 h after excision (Fig. 2). This decline is consistent with involvement of the inhibitors in the production or propagation of a wound signal. The wound signal moves into adjacent tissue at ca. 0.5 cm h^{-1} , so delaying application would allow the signal to move into and induce the wound response in adjacent tissue before the delayed application inhibited synthesis of the signal. Salicylic acid (SA) inhibits allene oxide synthase (AOS, EC 4.2.1.92), another enzyme in the phospholipid signaling pathway. Exposure to 1 or 10 mM SA for 60 min reduced WIPA by 26% or 56%, respectively. However, 1 mM SA lost its effectiveness if applied 3 h after excision, while 10 mM SA remained effective even when applied 4 h after excision. At 1 mM, SA may be perturbing the wound signal through inhibition of AOS, while at 10 mM it appears to have some generally inhibitory effect on subsequent phenolic metabolism.

These data further implicate the phospholipid signaling pathway in the generation of a wound signal that induces phenolic metabolism in wounded leaf tissue (Fig. 3).

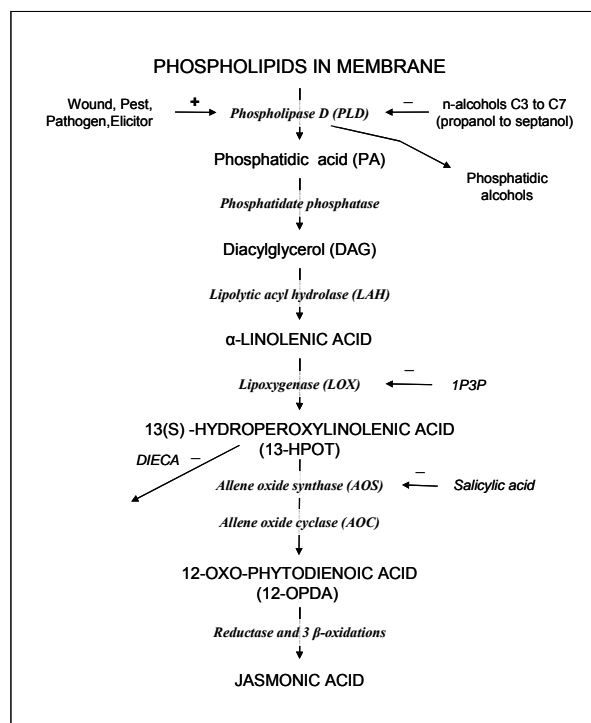


Fig. 3. Hypothetical model for the phospholipid signaling pathway with enzymes, products and inhibitors.

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

Exposure of excised 5-mm mid-rib segments of romaine lettuce leaf tissue to vapors of mono-carboxylic acids or aqueous solutions of mono-carboxylic acids or their salts inhibited wound-induced phenolic accumulation (WIPA) and subsequent tissue browning. The decline in phenolic content followed a quadratic curve with increasing concentration, reaching a maximum inhibition after 60 min of $74 \pm 8\%$ for 50 mM sodium acetate (2 carbons, C2), and $91 \pm 4\%$ for 20 mM sodium decanoate (capric acid, C10). Respiration (i.e., carbon dioxide production) was unaffected by concentrations of formic, acetic, or propionic acids that reduced wound-induced phenolic content or that increase ion leakage from the tissue into an isotonic mannitol solution. However, WIPA was suppressed up to 70% at concentrations (20 mM acetate) that did not increase ion leakage over that of water controls. Various acetate salts (i.e., ammonium, calcium, magnesium, sodium) all produced the same level of inhibition. The effectiveness of the compounds increased with increasing number of carbons in the molecule from 1 to 10, but was unaffected by whether the carbons were a straight chain or branched, or whether the treatment was delayed by up to 6 h.

An unexpected and difficult to explain result is that the effectiveness of butyrate (C4) in reducing WIPA (27% reduction at 20 mM) was significantly less than that predicted from the response of the two adjacent mono-carboxylates similarly applied (Fig. 4); propionate (C3) (62%) and valerate (C5) (73%). A similar deviation in effectiveness did not occur

with n-butanol in the n-alcohol series from 1 to 7 carbons (Choi et al., 2005). The physiological cause for the anomalous behavior of butyrate is under investigation.

It appears that, unlike the n-alcohols, mono-carboxylates are not interfering with the synthesis or propagation of a wound signal, but are interfering with subsequent steps in the production and accumulation of wound-induced phenolic compounds.

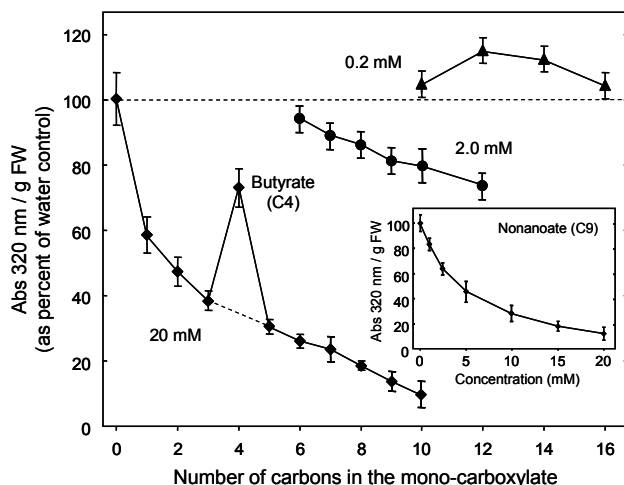


Fig. 4. Phenolic content of excised 5-mm Romaine mid-rib segments exposed to 20, 2.0, or 0.2 mM aqueous solutions of various mono-carboxylates. Phenolic content was determined by measuring the absorbance of a clarified methanol extract at 320 nm after holding the tissue for 48 h at 10 °C. Vertical lines associated with each bar represent the standard deviation about that mean.

Publications

1. Choi, Y.J., F. A. Tomás-Barberán, and M.E. Saltveit 2005. Wound-induced browning in lettuce (*Lactuca sativa* L.) leaf tissue is reduced by exposure to n-alcohols. *Postharvest Biology and Technology*. 37: 47-55.
2. Saltveit, M.E., Y.J. Choi, and F. A. Tomás-Barberán. 2005. Involvement of components of the phospholipid signaling pathway in wound-induced phenylpropanoid metabolism in lettuce (*Lactuca sativa* L.) leaf tissue. *Physiologia Plantarum* 125: 345-355.
3. Saltveit, M.E., Y.J. Choi, and F. A. Tomás-Barberán. 2005. Mono-carboxylic acids and their salts inhibit wound-induced phenolic accumulation in excised lettuce (*Lactuca sativa* L.) leaf tissue. *Physiologia Plantarum* 125: 454-463.
4. Saltveit, M.E. and Y-J. Choi, Y-J. 2006. Aromatic- and di-carboxylates inhibit wound-induced phenolic accumulation in excised lettuce (*Lactuca sativa* L.) leaf tissue. *Postharvest Biology and Technology* (In Review).