J. Amer. Soc. Hort. Sci. 122(3):399–404. 1997.

Early Wound- and Ethylene-induced Changes in Phenylpropanoid Metabolism in Harvested Lettuce

Francisco A. Tomás-Barberán[1], Julio Loaiza-Velarde, Antonio Bonfanti, and Mikal E. Saltveit[2]
Department of Vegetable Crops, Mann Laboratory, University of California, Davis, CA 95616-8631

Additional index words. ethylene responses, *Lactuca sativa*, phenolic compounds, secondary metabolism, wound responses

Abstract. **The phenolic composition of whole heads and excised midrib sections of iceberg, butter leaf, and romaine lettuce (*Lactuca sativa* L.) was followed at 5 and 10 °C during the first 3 days after wounding or during continuous exposure to 10 μL·L^{-1} ethylene in air. After 3 days of storage at 5 and 10 °C, only 5-caffeoylquinic acid (chlorogenic acid), 3,5-dicaffeoylquinic acid (isochlorogenic acid), caffeoyltartaric acid, and dicaffeoyltartaric acid were detected in wounded lettuce midribs. Of these four compounds, chlorogenic acid accumulated to the highest level in all three lettuce types. The content of caffeic acid derivatives increased 3- and 6-fold after 72 hours of storage at 5 and 10 °C, respectively. The synthesis of caffeoyltartaric acid was not induced by wounding in iceberg lettuce, while chlorogenic acid increased 5-fold at 5 °C and 10-fold at 10 °C. Similar relative phenolic compositions were detected in the three lettuce types studied, although at different concentrations. Changes observed in the content of individual phenolic compounds during the first 3 days of ethylene exposure seemed to follow the same pattern observed during wound induction of the synthesis of phenolic compounds. Chlorogenic acid increased 5-fold and isochlorogenic acid increased 10-fold, while the content of caffeoyltartaric derivatives were not significantly altered by ethylene treatment. Isochlorogenic acid, which was only present in low amounts in the control, was synthesized in the later steps of wound and ethylene induction. Similar kinetics for the induction of phenolic compounds were observed in the three lettuce types studied, suggesting that the mechanisms by which wounding induces phenylpropanoid synthesis are common for the different lettuce types.**

Wounding is one of many biotic and abiotic stresses that alter phenylpropanoid metabolism and increase the production of the plant hormone ethylene. Mechanical wounding of iceberg lettuce during harvest and handling and during preparation of fresh-cut lettuce induces an increase in phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity and in the concentration of several soluble phenolic compounds (Ke and Saltveit, 1989a). These compounds can be oxidized to brown substances by polyphenol oxidase (PPO, EC 1.10.3.2). In addition, wounding also increases peroxidase (EC 1.11.1.7) activity and lignin formation.

Ethylene, whether produced by plant tissue or present as an atmospheric pollutant, affects many plant processes (Abeles et al., 1992). In lettuce tissue, ethylene exposure stimulates phenylpropanoid metabolism and under the right conditions (i.e., 5 ± 2 °C), the development of a postharvest disorder called russet spotting (RS). RS is characterized by the appearance of numerous brown spots on the midrib of iceberg lettuce, as well as an increase in PAL activity and the concentration of soluble phenolic compounds (Hyodo et al., 1978; Ke and Saltveit, 1989a). Increases also occur in the activity of ionically bound peroxidase, indole-3-acetic acid (IAA) oxidase, and the content of lignins (Ke and Saltveit, 1988). Although wounding and ethylene induce phenylpropanoid metabolism in lettuce, and wounding induces ethylene production by lettuce, wound-induced ethylene is not responsible for the increase in phenylpropanoid metabolism following wounding (Ke and Saltveit, 1989a).

Wounding and ethylene cause changes in the phenolic content of lettuce. Lettuce tissue contains 5-caffeoylquinic acid (chlorogenic acid), caffeoyltartaric acid, dicaffeoyltartaric acid, and caffeoylmalic acid (Winter and Herrmann, 1986). Caffeic acid derivatives are the main soluble phenolic compounds that increase after wounding (Ke and Saltveit, 1989a) and RS development in iceberg lettuce (Hyodo et al., 1978; Ke and Saltveit, 1988). Wounding primarily induces the synthesis of chlorogenic and 3,5-dicaffeoylquinic acids (isochlorogenic acid) (Ke and Saltveit, 1989a). The synthesis of these compounds and 4,5-dicaffeoylquinic acid is also induced during RS development (Ke and Saltveit, 1988).

The purpose of the present work was to evaluate induced changes in the individual phenylpropanoid metabolites in three lettuce types (iceberg, butter leaf, and romaine) during the first 3 d after wounding or exposure to ethylene. Characterization of the kinetics of the induction of the various phenolic compounds induced by wounding and ethylene in these three types of lettuce, which differ in their susceptibility to various postharvest browning disorders, will help identify the metabolic pathways and compounds responsible for the various disorders.

Materials and Methods

Plant material. Commercially grown and harvested 'Salinas' iceberg lettuce (*Lactuca sativa*), 'Esmeralda' butter leaf lettuce (*L. sativa* var. *capitata*), and 'Green Tower' romaine lettuce (*L. sativa* var. *longifolia*) were obtained from a local wholesale market, transported to the Mann Laboratory, and stored at 0.5 °C until used. Green coffee beans (*Coffea arabica* L.) were provided by Kerry Sachs (Puroast Coffee, Woodland, Calif.).

Wrapper and cap leaves were removed, and the next six to seven uninjured lettuce leaves were carefully excised. Midribs from the lower third of the leaf were excised and cut into 2 × 2-cm pieces (iceberg and romaine) or into 1 × 1-cm pieces (butter leaf). Excised midribs were washed in 0 °C distilled water and centrifuged to remove surface moisture. They were then placed in 1-L jars with flows of humidified air sufficient to maintain CO_2 levels below 0.15%. Samples for each lettuce type were placed at 5 and 10 °C and replicated samples were taken for analysis at 0, 6, 12, 24, 48, and 72 h.

Received for publication 24 June 1996. Accepted for publication 23 Dec. 1996. We are grateful to Marie Jo Amiot for providing a sample of cynarin, to M.N. Clifford for his help in identification of coffee phenolics, and to Kerry Sachs for providing green coffee beans. FATB is indebted to the NATO for a fellowship. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

[1]Permanent address: CEBAS (CSIC), P.O. Box 4195, Murcia E-30080, Spain.
[2]To whom reprint requests should be addressed.

Table 1. Caffeic acid derivatives from iceburg lettuce and coffee.

	Compound (common name)
a	3-Caffeoylquinic acid (neochlorogenic acid)
b	Caffeoyltartaric acid*
c	4-Caffeoylquinic acid (kryptochlorogenic acid)
d	5-Caffeoylquinic acid (chlorogenic acid)
e	p-Coumaroylquinic acid
f	Feruloylquinic acid
g	Dicaffeoyltartaric acid[z]
h	3,4-Dicaffeoylquinic acid
i	3,5-Dicaffeoylquinic acid (isochlorogenic acid)
j	4,5-Dicaffeoylquinic acid

[z]Tentatively identified by comparison with previously reported chromatograms of lettuce extracts and by their UV spectra recorded with diode array detector. The structures were numbered following the IUPAC rules (Clifford, 1986).

Whole lettuce heads were placed in 20-L jars at 5 or 10 °C with flows of humidified air, with ethylene at 10 $\mu L \cdot L^{-1}$ or without ethylene (control) at sufficient rates to maintain CO_2 levels below 0.15%. Two heads were taken from each treatment at 0, 6, 12, 24, 48, and 72 h. Wrapper and cap leaves were discarded and the lower third of the next six uninjured leaves was used to prepare 2 × 2-cm midribs pieces (iceberg and romaine), or 1 × 1-cm pieces (butter leaf). Replicate samples of 10 g from each treatment were frozen at –80 °C until extracted and analyzed. Each experiment was performed at least twice, with similar results.

Extraction of phenolic compounds. Phenolic compounds were extracted from midrib tissue as previously described (Ke and Saltveit, 1988). These extracts were concentrated to dryness under nitrogen at 40 °C, redissolved in 1 mL 1 methanol : 1 H_2O (v:v), and filtered through a 0.45-µm filter before high-pressure liquid chromatographic (HPLC) analysis. Green coffee beans were ground with an electric grinder, and 10 g of the powder was extracted with 20 mL 70% methanol overnight. The extract was then decanted, filtered through Whatman no. 1 filter paper, and concentrated under reduced pressure at 40 °C. The concentrate was redissolved in 70% methanol (HPLC grade), filtered through a 0.45-µm filter, and analyzed by HPLC.

HPLC analysis of phenolic compounds. HPLC analyses were done with a Beckman model 332 gradient liquid chromatograph and a Beckman model 160 UV detector at 326 and 254 nm. The chromatograms were recorded and integrated with a Hewlett-Packard HP 3394 integrator. Separations were achieved on a Bio-Rad Bio-Sil ODS-5S column (25 × 0.4 cm; 5-µm particle size) using 19 water : 1 formic acid (v:v) (A) and 19 methanol : 1 formic acid (v:v) (B) as mobile phases. The solvent flow rate was 1 $mL \cdot min^{-1}$, and a linear gradient was used starting with 10% B in A to reach 70% B in A in 50 min. Compounds were identified by HPLC analyses carried out on a Merck-Hitachi gradient liquid chromatograph with a model L-3000 pump and a Shimadzu 6200 photodiode array detector. The characteristic spectra of caffeic acid derivatives had a maximum at 328 nm and a shoulder at 290 nm. The column and elution conditions were the same as described above.

The monocaffeoylquinic acid derivatives and caffeoyltartaric acid (b) were quantified as chlorogenic acid (5-caffeoylquinic acid) using an authentic marker (Sigma). The dicaffeoylquinic derivatives and dicaffeoyltartaric acid (g) were quantified as cynarin (1,3-dicaffeoylquinic acid), which was provided by Marie Jo Amiot (INRA, Montfavet, France).

Results

Phenolic compound identification. Soluble phenolic compounds were identified by their UV spectra and by co-chromatography with authentic markers (chlorogenic acid = 5-caffeoylquinic acid) and with green coffee bean extracts (Clifford, 1986). The different phenylpropanoids identified in lettuce and/or green coffee beans are shown in Table 1. Green coffee beans are characterized by the presence of 3-, 4-, and 5-caffeoylquinic acids (a, c, and d); 5-p-coumaroylquinic acid (e); 5-feruloylquinic acid (f); 3,4-dicaffeoylquinic acid (h); 3,5-dicaffeoylquinic acid (i); and 4,5-dicaffeoylquinic acid (j) as main constituents (Clifford, 1986) (Fig. 1A).

Synthesis of caffeic acid and the flavonoids catechin, epicatechin, and phloridzin, which had been previously reported in ethylene-exposed lettuce tissues (Ke and Saltveit, 1988, 1989b), was not detected during the early stages studied in the present work. However, when extracts from iceberg lettuce showing severe RS (i.e., after 10 d in 10 $\mu L \cdot L^{-1}$ ethylene) were analyzed, a more complex phenolic compound profile was observed (Fig. 1B). In this case 3-, 4-, and 5-caffeoylquinic acids (a, c, and d) were detected as well as 3,4-; 3,5-; and 4,5-dicaffeoylquinic acid derivatives (h, i, and j) and caffeoyltartaric (b) and dicaffeoyltartaric (g) acids.

The analysis of extracts from wounded and ethylene-exposed iceberg lettuce tissue held for 72 h at 10 °C revealed the presence of 5-caffeoylquinic acid (d) and 3,5-dicaffeoylquinic acid (i) as well as caffeoyltartaric acid (b) and dicaffeoyltartaric acid (g) (Fig. 2). Quinic acid derivatives were identified by co-chromatographic comparison with chlorogenic acid and coffee extracts. Tartaric

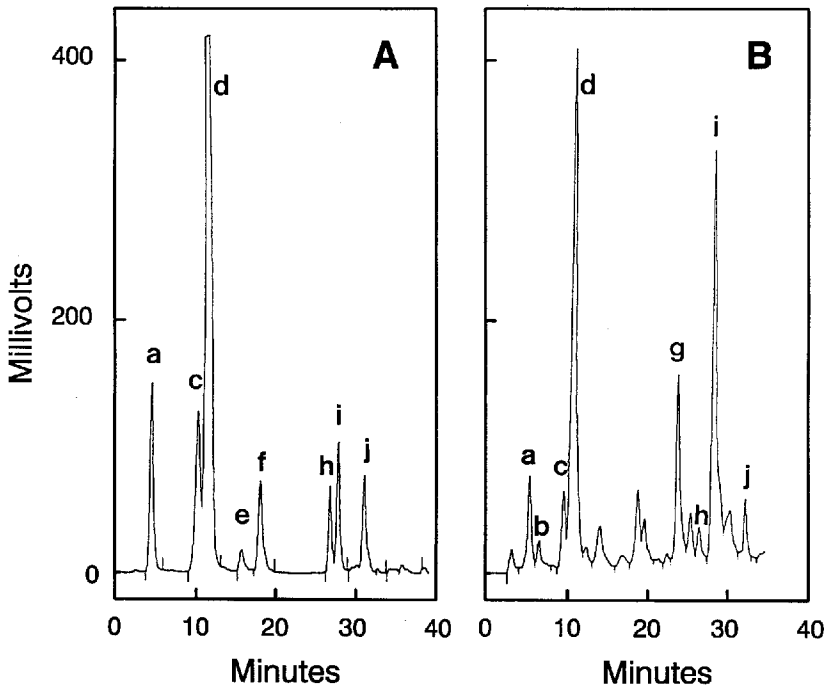

Fig. 1. High-performance liquid chromatography chromatograms of phenylpropanoids from green coffee beans (**A**) and iceberg lettuce (**B**) with severe ethylene-induced browning (russet spotting). Chromatograms were recorded at 320 nm. For compound identification see Table 1.

acid derivatives were tentatively identified by comparisons with previously reported data on the occurrence of these compounds in lettuce, endive, and chicory (HPLC relative retention times and UV spectra) (Winter and Herrmann, 1986). None of the other green coffee caffeic acid derivatives were detected in these extracts.

Changes obviously occur in the metabolism of phenolic compounds in iceberg lettuce during the early stages of ethylene exposure when RS lesions are not yet apparent (Fig. 2). A more complex phenylpropanoid profile was observed when lesions had developed (Fig. 1B). For example, the first caffeic acid derivatives synthesized after ethylene exposure were chlorogenic and isochlorogenic acid, while derivatives in which caffeic acid is linked to the hydroxyl located at the 4-position of quinic acid (4-caffeoylquinic; 3,4-dicaffeoylquinic acid; and 4,5-dicaffeoylquinic acid) were synthesized during the later stages of RS development.

Wound-induced changes in phenylpropanoids. After 3 d of storage at 5 and 10 °C, only caffeoyltartaric acid (b), chlorogenic acid (d), dicaffeoyltartaric acid (g), and isochlorogenic acid (i) were detected in wounded lettuce midribs (Fig. 2). The same phenolic compounds were detected in the three lettuce types studied, although at different concentrations (Fig. 3). The zero time levels for each of the four compounds shown in Fig. 3 were maintained in the controls throughout the duration of the experiment.

The concentration of total soluble phenolic compounds in the initial tissue samples was the smallest in iceberg lettuce midribs (10 to 20 $\mu g \cdot g^{-1}$ fresh mass), higher in romaine midribs (20 to 30 $\mu g \cdot g^{-1}$), and highest in butter leaf midribs (40 to 60 $\mu g \cdot g^{-1}$). Wounding induced changes in the content of soluble caffeic acid derivatives after 3 d of storage at 5 °C; iceberg lettuce midribs had accumulated 40 $\mu g \cdot g^{-1}$ of soluble phenolic compounds, while romaine midribs had accumulated 65 $\mu g \cdot g^{-1}$ and butter leaf to 115 $\mu g \cdot g^{-1}$. As would be expected because of higher metabolic activity at higher temperatures, accumulation of phenolic compounds was higher in wounded lettuce midribs stored at 10 than at 5 °C. Thus, at 10 °C, the concentration of soluble phenolic compounds reached 80 $\mu g \cdot g^{-1}$ in iceberg lettuce, 135 $\mu g \cdot g^{-1}$ in romaine lettuce, and 185 $\mu g \cdot g^{-1}$ in butter leaf lettuce.

In butter leaf lettuce, wounding induced an 87% increase in the four quantified phenolic compounds from 47 to 88 $\mu g \cdot g^{-1}$ at 5 °C, and a 2.8-fold increase from 47 to 130 $\mu g \cdot g^{-1}$ at 10 °C (Fig. 3). The increase at 5 °C was almost exclusively made up of a 5.3-fold increase in chlorogenic acid and a 33% increase in dicaffeoyltartaric acid. The increase at 10 °C was composed of a 6.5-fold increase in chlorogenic acid, a 17-fold increase in isochlorogenic acid, and a 75% increase in caffeoyl and dicaffeoyltartaric acid.

At 5 °C, the first caffeoyl acid derivatives (chlorogenic acid and caffeoyltartaric acid) began to increase in butter leaf lettuce 12 h after wounding (Fig. 3). The subsequent compounds (isochlorogenic acid and dicaffeoyltartaric acid) showed only a slight increase after 24 h. At 10 °C, the first caffeoyl acid derivatives began to increase within 6 h of wounding, while the subsequent compounds did not start to increase until after 24 h.

In iceberg lettuce, wounding induced a 2.8-fold increase from 11 to 31 $\mu g \cdot g^{-1}$ in the four quantified phenolic compounds at 5 °C, and a 6-fold increase from 11 to 67 $\mu g \cdot g^{-1}$ at 10 °C (Fig. 3). The increases at 5 °C were composed of a 4.7-fold increase in chlorogenic acid, a 3.8-fold increase in isochlorogenic acid, a 16% increase in caffeoyltartaric acid, and a 3-fold increase in dicaffeoyltartaric acid. The increases at 10 °C were composed of a 10.3-fold increase in chlorogenic acid, a 20-fold increase in isochlorogenic acid, a 33% increase in caffeoyltartaric acid, and a 5.2-fold increase in dicaffeoyltartaric acid.

At 5 °C, the first caffeoyl acid derivatives began a slight increase in iceberg lettuce 24 h after wounding, while the subsequent compounds showed no significant increase (Fig. 3). At 10 °C, chlorogenic acid began to increase within 12 h of wounding, while the other three phenolic compounds did not start to increase until after 48 h.

In romaine lettuce, wounding induced a 2.6-fold increase from 24 to 62 $\mu g \cdot g^{-1}$ in the four quantified phenolic compounds at 5 °C and a 4.8-fold increase from 24 to 114 $\mu g \cdot g^{-1}$ at 10 °C (Fig. 3). The increases at 5 °C were composed of a 5.8-fold increase in chlorogenic acid, an increase from undetectable limits to 2.9 $\mu g \cdot g^{-1}$ for isochlorogenic acid, a 16% increase in caffeoyltartaric acid, and a 67% increase in dicaffeoyltartaric acid. The increases at 10 °C were composed of a 10-fold increase in chlorogenic acid, an increase from undetectable limits to 17 $\mu g \cdot g^{-1}$ for isochlorogenic acid, a 47% increase in caffeoyltartaric acid, and a 2.8-fold increase in dicaffeoyltartaric acid.

At 5 °C, only chlorogenic showed a pronounced increase in romaine lettuce, which started 24 h after wounding (Fig. 3). The other three caffeoyl acid derivatives showed only slight increases, which occurred within 48 h. At 10 °C, chlorogenic acid began a pronounced increase after 6 h, while isochlorogenic acid and dicaffeoyltartaric acid started to increase after 12 h. Caffeoyltartaric acid also increased, but the increase was small and did not start until after 24 h.

Ethylene-induced changes in phenylpropanoid metabolism. The HPLC analyses of extracts from midribs excised from whole lettuce heads that had been exposed to ethylene at 10 $\mu L \cdot L^{-1}$ for 3 d at 5 or 10 °C showed significant differences between their phenolic compound profiles (Fig. 3). Ethylene exposure induced far less synthesis of phenolic compounds than wounding. In butter leaf lettuce, wounding induced a 87% increase in the four quantified phenolic compounds at 5 °C, while ethylene only induced a

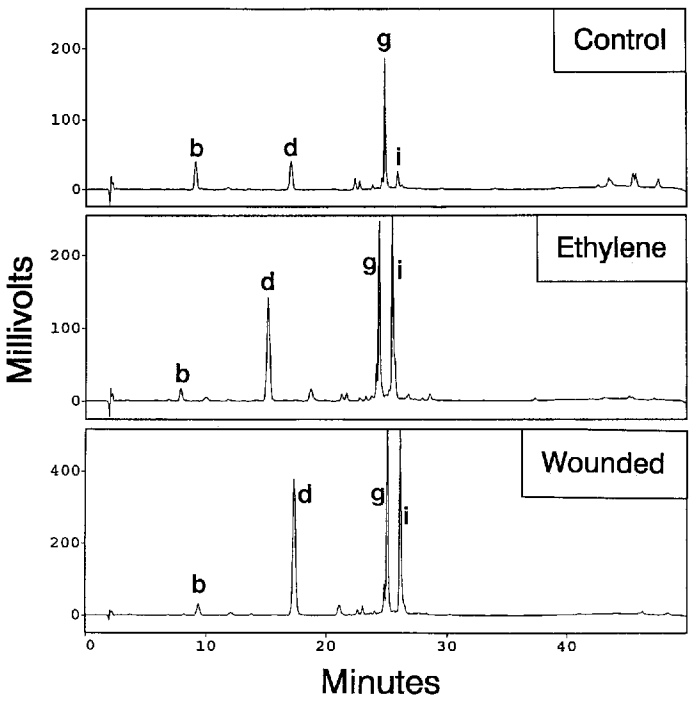

Fig. 2. Effect of ethylene exposure and wounding on the high-performance liquid chromatography phenylpropanoid profiles of iceberg lettuce tissue. Phenolic compounds were extracted from control (uninjured), ethylene exposed (10 $\mu L \cdot L^{-1}$ ethylene in air), and wounded midribs after storage at 10 °C for 72 h. Chromatograms were recorded at 320 nm. For compound identification see Table 1.

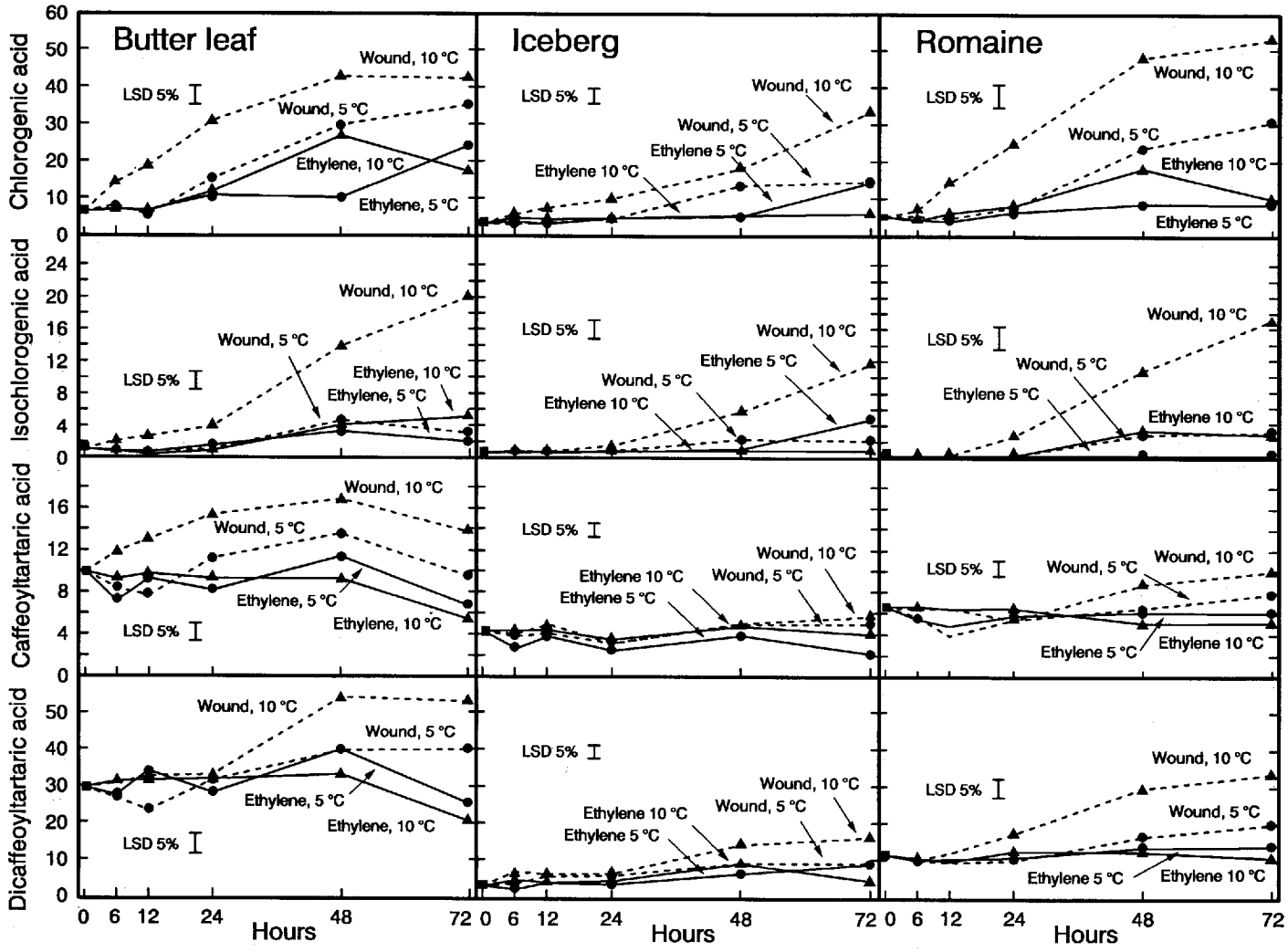

Fig. 3. Changes in four phenylpropanoids compounds (chlorogenic acid, isochlorogenic acid, caffeoyltartaric acid, and dicaffeoyltartaric acid) extracted from butter leaf, iceberg, and romaine lettuce midribs wounded or exposed to 10 $\mu L \cdot L^{-1}$ ethylene in air for up to 72 h at 5 or 10 °C. The amount of each of the four chemicals is given in $\mu g \cdot g^{-1}$ on a fresh mass basis.

38% increase. The increases were closer at 10 °C ,with wounding and ethylene inducing increases of 174% and 155%, respectively. In contrast, in iceberg lettuce wounding and ethylene induced about the same increase in the synthesis of phenolic compounds at 5 °C (182% and 173%, respectively) but vastly different amounts at 10 °C (509% and 82%, respectively). In romaine, wounding induced more synthesis of phenolic compounds than ethylene at both temperatures. At 5 °C, wounding induced a 158% increase while ethylene induced only a 21% increase. A similar pattern occurred at 10 °C, with wounding and ethylene inducing a 375% and a 63% increase, respectively.

An increase in chlorogenic (d) and isochlorogenic (i) acids was observed at 5 °C in iceberg lettuce after 48 h, while no differences from the control were observed in the extracts of midribs stored at 10 °C (Fig. 3). Ethylene induced a 3-fold increase in caffeic acid derivatives after 72 h at 5 °C, while no significant changes were observed even after 72 h at 10 °C. The increase observed at 5 °C started after 48 h of storage. These results agree with previously reported data showing that RS symptoms only occur at 5 °C, while they were not observed when lettuce heads were stored at 0 °C or at 10 to 12.5 °C (Hyodo et al., 1978). Similar temperature effects were observed on the induction of PAL activity (Ritenour et al., 1995).

The kinetics of ethylene-induced synthesis of phenolic compounds in iceberg lettuce stored at 5 °C are consistent with previously published data on ethylene-induced PAL activity. In those reports, PAL activity started to increase after 24 h of ethylene exposure and reached maximum activity after 4 d. Our present results show that the concentration of phenolic compounds started to increase only after 48 h of ethylene exposure, and confirm that the kinetics for wound- and ethylene-induced metabolism of phenolic compounds are different. In addition, different phenolic compounds were detected during the first 3 d of ethylene induction than those synthesized during the later stages of RS development (see above).

Changes observed in the content of individual phenolic compounds in iceberg lettuce during the first 3 d of ethylene exposure were different from the pattern observed during wound induction of the synthesis of phenolic compounds (Fig. 3). For example, the largest increase in chlorogenic and isochlorogenic acid occurred at 5 °C when exposed to ethylene, whereas it occurred at 10 °C in wounded tissue. The content of caffeoyltartaric derivatives was only slightly altered by ethylene treatment. 3,5-Dicaffeoylquinic acid (i), which was only present in very small amount in the control, was synthesized in the later steps of induction (i.e., after 72 h at 5 °C). However, these compounds remained at levels similar to those observed in the control when lettuce heads were exposed to ethylene for 3 d at 10 °C.

When butter leaf heads were exposed to ethylene at 10 $\mu L \cdot L^{-1}$ at 5 and 10 °C, a slight, but significant, increase in caffeic acid

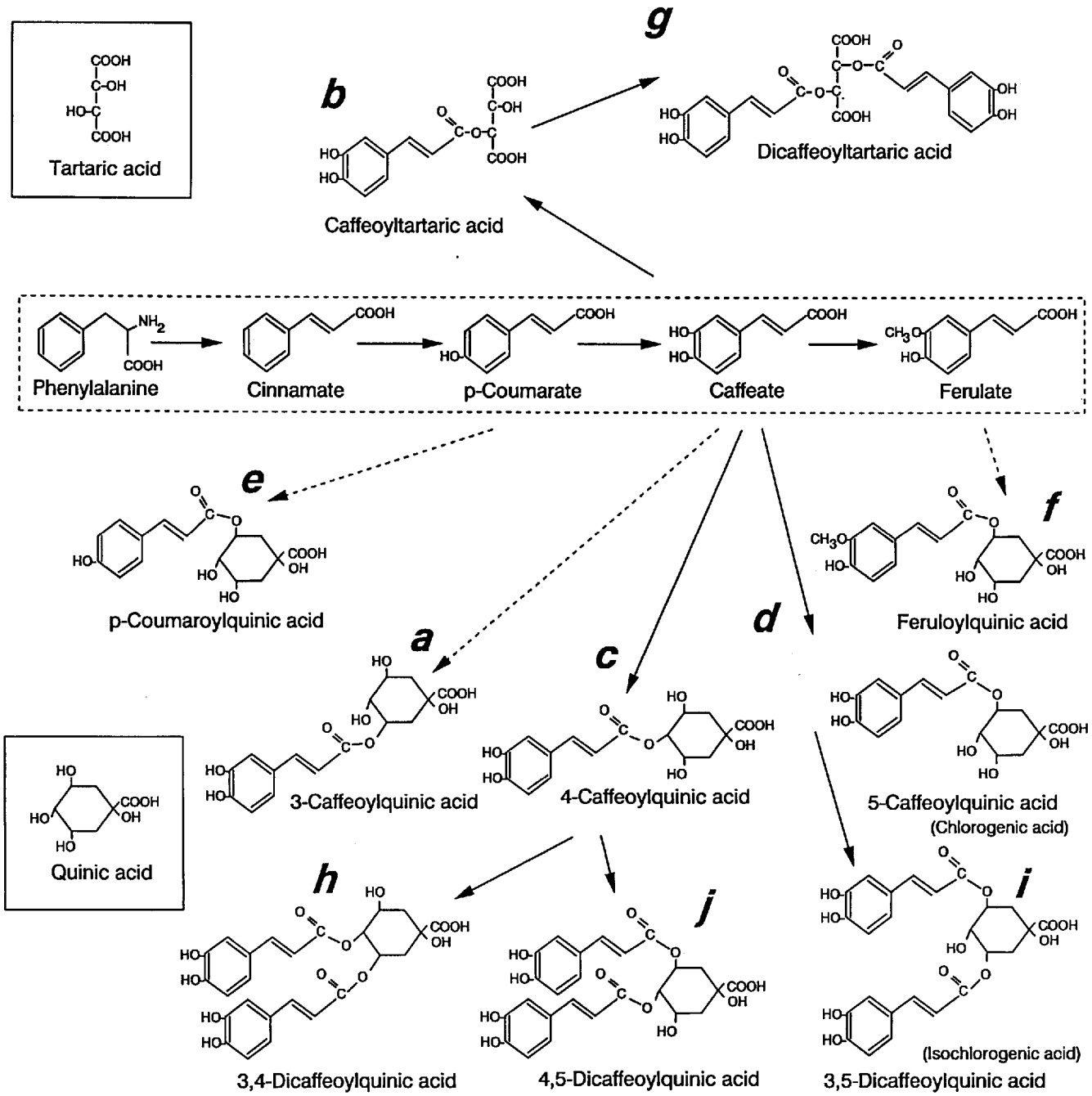

Fig. 4. Phenylpropanoid metabolism in wounded and ethylene-treated lettuce midrib tissue. The basic phenylpropanoid pathway from phenylalanine to ferulate is shown in the center box. Derivative compounds containing tartaric acid are shown above the box, while derivatives containing quinic acid are shown below the box. The letter preceeding each chemical structure refers to the list of compounds in Table 1. Symbols and abbreviations: solid lines show reactions to compounds identified in lettuce after 3 days, while dotted lines show pathways to compounds not found in lettuce after 3 days (i.e., a, e, and f).

derivatives was observed after 48 h, followed by a slight decrease at 72 h. At both temperatures, the caffeoyltartaric derivatives (b and g) did not increase in concentration, and even decreased in content after 72 h. In comparison, the content of chlorogenic and isochlorogenic acids increased 4- and 5-fold, respectively, at 10 °C.

Similar results were observed for romaine lettuce stored in the ethylene atmosphere. A slight increase in total soluble phenolic compounds was observed after 48 h, with a slight decrease at 72 h. The increase was higher in those heads stored at 10 °C than at 5 °C. Again, the caffeoyltartaric derivatives were only slightly affected by ethylene, while the caffeoylquinic derivatives were the main compounds responsible for the observed changes.

Discussion

Plants respond to wounding with increased production of compounds involved in the repair of wound damage and in defense against microbial invasion (Dixon and Paiva, 1995). Wound repair requires the synthesis of lignin, and defense against pathogens is associated with the synthesis of low-molecular-mass antimicrobial compounds. Lignin accumulation is accompanied by the activation of enzymes of lignin synthesis, which include PAL, cinnamate 4-hydroxylase, p-hydroxycinnamate-CoA ligase, chorismate mutase (Dyer et al., 1989), and peroxidase (Ke and

Saltveit, 1989a). In lettuce, wound-induced increases in PAL activity are a response to injury, not to wound-induced ethylene production (Ke and Saltveit, 1989a). Genes encoding key enzymes of lignin synthesis are transcriptionally activated by wounding (Dyer et al., 1989).

PAL is involved in the synthesis of cinnamic acid from phenylalanine. As the metabolism of phenolic compounds continues, cinnamic acid is hydroxylated to p-coumaric acid, which is altered to form caffeic acid; this compound is then coupled either with quinic acid or tartaric acid to produce a number of soluble phenylpropanoids (Dixon and Paiva, 1995), some of which are found in lettuce (Fig. 4). In addition, wounding induces other enzymes earlier in the shikimate pathway. In fact, the first enzyme in the shikimate pathway, 3-deoxy-D-arabino-helptulosonate 7-phosphate (DAHP) synthase, the enzyme controlling carbon flow from carbohydrates to secondary metabolism, is activated by wounding in potato and tomato (Dyer et al., 1989).

Quinate can be synthesized in one step from dehydroquinate by quinate dehydrogenase or from shikimate by quinate hydrolyase, both intermediates of the shikimic acid pathway (Herrmann, 1995). An increase in carbon flow through this pathway by activation of DAHP synthase would most likely induce an increase in quinate levels as well. Tartaric acid synthesis is produced by a side chain of carbohydrate metabolism (from glucose), and it is not known to be induced by wounding. Therefore, differences in the observed rate of wound- and ethylene-induced synthesis of caffeoylquinic derivatives and caffeoyltartaric derivatives could be explained by the different availability of the acids to which caffeic acid is linked.

The induction of phenylpropanoid synthesis by wounding after 24 h at 5 °C is consistent with previously published data on wound-induced PAL activity, since the activity induced by wounding starts to increase after 4 h at 5 °C and reaches a maximum after 24 h (Ke and Saltveit, 1989a). There appears to be a lag of 16 to 20 h between the induction of PAL and the synthesis and accumulation of phenolic derivatives in lettuce midribs stored at 5 °C. Similar kinetics were observed for the induction of phenolic compounds in the three lettuce types studied in the present work. This similarity suggests that the mechanism by which wounding induces phenylpropanoid synthesis is common for the different lettuce types. It is interesting to note that the increases observed in phenylpropanoid content when lettuce was exposed to ethylene occur before the brown RS lesions are visible on the midribs. It appears that PAL induction and the synthesis and accumulation of phenolics occur before visual lesions are formed.

As would be expected because of higher metabolism at higher temperatures, wound-induced synthesis of caffeic acid derivatives was always higher at 10 than at 5 °C. Similar effects were observed in ethylene-induced synthesis, with the exceptional behavior of iceberg lettuce, in which phenylpropanoids accumulated at higher levels at 5 than at 10 °C. These results are consistent with previously reported data on RS development and ethylene-induced PAL activity at 5 °C, which is reported to be higher at 5 than at 10 °C (Ritenour et al., 1995).

It has been shown that ethylene exposure increased the levels of PAL mRNA and the mRNAs of other enzymes of the metabolism of phenolic compounds in carrots (Ecker and Davis, 1987). Therefore, it is not surprising that an increase in phenolic constituents was observed in the ethylene-exposed lettuce midribs. However, the content of soluble phenolic compounds at 72 h was often less than at 48 h. This decrease could be explained by a similar effect to that observed in potato tuber discs (Zucker, 1968) and iceberg midrib tissue (Ritenour and Saltveit, 1996), in which ethylene-induced PAL activity decreased supposedly due to an induced PAL inactivating or degrading system.

Literature Cited

Abeles, F.B., P.W. Morgan, and M.E. Saltveit, Jr. 1992. Ethylene in plant biology. 2nd ed. Academic Press, San Diego.

Clifford, M.N. 1986. Coffee bean dicaffeoylquinic acids. Phytochemistry 25:1767–1769.

Dixon, R.A. and N.L. Paiva. 1995. Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085–1097.

Dyer, W.E., J.M. Henstrand, A.K. Handa, and K.M. Herrmann. 1989. Wound induces the first enzyme of the shikimate pathway in Solanaceae. Proc. Natl. Acad. Sci. USA 86:7370–7373.

Ecker, J.R. and R.W. Davis. 1987. Plant defense genes are regulated by ethylene. Proc. Natl. Acad. Sci. USA 84:5202–5206.

Herrmann, K.M. 1995. The shikimate pathway: Early steps in the biosynthesis of aromatic compounds. Plant Cell 7:907–919.

Hyodo, H., H. Kuroda, and S.F. Yang. 1978. Induction of phenylalanine ammonia-lyase and increase in phenolics in lettuce leaves in relation to the development of russet spotting caused by ethylene. Plant Physiol. 62:31–35.

Ke, D. and M.E. Saltveit. 1988. Plant hormone interaction and phenolic metabolism in the regulation of russet spotting in iceberg lettuce. Plant Physiol. 88:1136–1140.

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

Ke, D. and M.E. Saltveit. 1989b. Regulation of russet spotting, phenolic metabolism, and IAA oxidase by low oxygen in iceberg lettuce. J. Amer. Soc. Hort. Sci. 114: 638–642.

Ritenour, M.A. and M.E. Saltveit. 1996. Identification of a phenylalanine ammonia lyase inactivating factor in harvested iceberg lettuce (*Lactuca sativa* L). Physiol. Plant. 97:327–331.

Ritenour, M.A., M.J. Ahrens, and M.E. Saltveit. 1995. Effects of temperature on ethylene-induced phenylalanine ammonia lyase activity and russet spotting in harvested iceberg lettuce. J. Amer. Soc. Hort. Sci. 120:84–87.

Winter, M. and K. Herrmann. 1986. Esters and glucosides of hydroxycinnamic acids in vegetables. J. Agr. Food Chem. 34:617–620.

Zucker, M. 1968. Sequential induction of phenylalanine ammonia-lyase and a lyase-inactivating system in potato tuber disks. Plant Physiol. 43:365–374.