

Effect of Cultivar on Endogenous Ethylene Evolution and Its Relationship to Increases of Soluble Protein in Peach Mesocarp Tissue

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Abstract

Differences in ethylene evolution rate among 'Biscoe', 'Belle of Georgia', 'Loring', and 'Redskin' peach (*Prunus persica* L.) and 'Fantasia' nectarine (*Prunus persica* L., v *nuciperscia*) fruits were investigated. The effect of cultivar on ethylene evolution rate was highly significant. The mean ethylene evolution rate in 'Belle of Georgia' peach fruits was higher than in any other cultivar. The lowest mean ethylene evolution rate was observed in 'Loring' peach fruits. Ethylene evolution rates were compared with ripening associated changes in accumulation of soluble mesocarp proteins. The soluble protein fraction extracted from fruits at three time points during ripening were resolved by denaturing polyacrylamide gel electrophoresis. Protein band density was determined by scanning laser microdensitometry. Cultivar and ethylene evolution rate were related to protein density and significant gel protein band by cultivar interactions were observed. Analysis of band variance tested the effect of cultivar on protein accumulation during maturation. Sixteen bands showed significant differences and five bands showed no significant differences among cultivars. For 'Belle of Georgia' fruits, the ethylene evolution rate was related to increased accumulation of two proteins with estimated molecular masses of 39.0 and 56.5 kDa. No increased accumulation of 39.0 or 56.5 kDa protein was observed in the three remaining *Prunus* cultivars with suppressed ethylene evolution rates, or in ripening 'Loring' fruits treated with (2-chloroethyl)phosphonic acid (ethephon).

Introduction

Ripening is a controlled developmental sequence of coordinated metabolic events (10, 11). These events are regulated by genetic determinates and

environmental factors (18) and are intimately associated with hormonal interactions (16). Ethylene is considered the single phytohormone capable of coordinating the diverse metabolic events characteristic of fruit ripening (1, 5). Frenkel (9) noted the association between increased ethylene evolution and increased protein biosynthesis during ripening. Recently, ethylene mediated induction of ripening or senescence associated changes in protein accumulation have been demonstrated in fruits of avocado (21), tomato (3, 7, 17), peach (6), and in carnation petals (14). In addition, Speirs et al. (20) demonstrated cultivar based differential accumulation of a specific protein, endopolygalacturonase, during ripening in tomato fruit.

This study demonstrates both ethylene and cultivar related increases of two proteins in peach mesocarp tissue during ripening. The horticultural implications of cultivar related differences in ethylene induced protein accumulation are discussed.

Materials and Methods

Four commercially diverse peach cultivars ('Biscoe', 'Belle of Georgia', 'Loring', and 'Redskin') and 'Fantasia' nectarine were selected to test for cultivar differences in ethylene evolu-

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tion rate. Twelve Stage III (12) fruit from a single tree, spanning the range of natural maturity, were selected from each cultivar. Fruits were equilibrated in the laboratory at 25°C for 18 h. Ethylene evolution rates were measured by flame ionization gas chromatography using a static system with a single fruit per 475 ml sampling jar. The ethylene evolution rate for each fruit was calculated assuming a constant rate of production during the 2 h sampling period. Ethylene data were subjected to standard analysis of variance procedures (ANOVA) after log₁₀ transformation (SAS Version 5). The Student-Newman-Keuls (SNK) multiple unplanned comparisons procedure was used to compare means among cultivars.

Forty-eight early Stage III (12) 'Loring' peach fruits were harvested approximately 12 days prior to commercial harvest, at early shipping maturity. Fruits were randomized, divided into treatment or control groups, and dipped into a treatment solution containing 250 $\mu\text{l} \cdot \text{liter}^{-1}$ ethephon and a non-ionic surfactant (15). Fruits were allowed to dry prior to ethylene determination. Four fruits per treatment were sampled at 4 days, 10 days and 14 days after treatment.

Total soluble protein was extracted from mesocarp tissue in buffer containing 100 mM Tris-HCl (pH 7.6), 0.3 M mannitol, 10 mM MgCl₂, 20 mM ethylene-diaminetetraacetic acid, 0.5 mM 2-mercaptoethanol, 5 mM dithiothreitol, 1% (v/v) Triton X-100, 5.0 mM diethyldithiocarbamic acid, and 5.0 mM phenyl-methylsulfonyl fluoride. Cellular debris was removed by filtration and centrifugation. The protein concentration of each sample was quantitatively measured using the Coomassie blue binding assay of Bradford (4).

Protein homogenates were separated using a discontinuous denaturing polyacrylamide gel electrophoresis (PAGE) and buffer system (13). Samples were

prepared for electrophoresis following protein quantification by mixing extracted mesocarp tissue homogenate with 3x sodium dodecylsulfate (SDS) sample buffer in a 2:1 (v/v) ratio. Sample buffer consisted of 3.75% (v/v) Tris-HCl (pH 6.8), 3.0% (v/v) glycerol, 1.5% (v/v) 2-mercaptoethanol, and 0.9% (w/v) SDS. Samples in buffer were vortexed and heated to 100°C for 90 sec prior to loading ~ 40 g protein per well onto each PAGE gel. Following electrophoresis, gels were stained for 2 h with a solution containing 0.125% (w/v) Coomassie Blue R-250, destained, and stored at 4°C in 5% (v/v) acetic acid. Proteins in the bands were analyzed using an LKB model 2202 Ultrascan laser microdensitometer. The instrument used a helium-neon laser densitometer controlled by LKB 2190 GelScan interface and software run on an Apple IIe computer. Both ends and two middle positions of individual bands were scanned and average peak values were recorded.

PAGE gel density values were statistically analyzed by band using ANOVA and the SNK means comparison procedure. Each lane of a given electrophoretic gel represented a single fruit replicate. Ethylene evolution rate was analyzed as a continuous variable. The M_r of each protein band was estimated by comparison of band migration distance to that of a commercially supplied mixture of prestained electrophoretic protein standards (Bio-Rad Laboratories, Melville, NY) with a M_r range of 14.4-200 kDa.

Results

Significant cultivar by ethylene evolution rate interactions were observed (Table 1). The mean ethylene evolution rate in 'Belle of Georgia' fruits, a white flesh cultivar noted for preharvest drop, was significantly higher than all other cultivars studied. 'Loring' fruits, a modern shipping peach cultivar virtually incapable of preharvest drop, exhibited the lowest mean ethylene evolution rate. Mean ethylene

evolution rates intermediate between that of 'Belle of Georgia' and 'Loring' were observed in 'Biscoe' and 'Redskin' peach fruits and in 'Fantasia' nectarines (Table 1).

Twenty-one protein bands separated by PAGE and assayed by scanning laser microdensitometry were selected for statistical analysis. Band density, representing the quantity of protein per band, was significantly affected by cultivar or ethylene evolution rate in sixteen of twenty-one bands (Table 2). Band density varied significantly between lanes. Significant band by cultivar interactions were observed in some lanes. This interaction was examined by ANOVA to determine the effect of cultivar on band density. Thirteen bands were found to differ significantly at the 0.01 level and three bands showed significant differences at the 0.05 level (Table 2). Five bands showed no significant differences among cultivars, signifying that these proteins are constitutively expressed in all four cultivars during ripening and confirming that all gel lanes were loaded with equivalent quantities of protein. The density of two bands, representing proteins with a M_r of 56.5 kDa and 39.0 kDa, changed significantly in response to ethylene evolution rate (Table 2). Visual examination of electrophoretic gels showed that accu-

mulation of two proteins with a corresponding M_r of 56.5 and 39.0 kDa occurred only in 'Belle of Georgia' fruits whose ethylene evolution rates exceeded 15 nl/gFW/h (Fig. 1). At maturity, some 'Biscoe' fruits evolved ethylene at the rate of ~10 nl/gFW/h. However, no concomitant accumulation of either 56.5 or 39.0 kDa protein was observed in these fruits (Fig. 1).

Table 2. Twenty-one analyses of variance examining the effect of cultivar and ethylene evolution rates on soluble mesocarp protein of five *Prunus* cultivars separated by denaturing polyacrylamide gel electrophoresis. Statistical analyses for cultivar and ethylene were performed for each protein band.

Estimated Molecular Mass (kDa)	Analysis of Variance (F-Value)	
	Cultivar	Ethylene
79.0	4.47°	0.11ns
70.0	4.99**	0.97ns
68.0	10/80**	0.18ns
66.0	2.02ns	0.30ns
63.0	5.59**	0.95ns
62.0	1.01ns	0.01ns
60.5	15.04**	0.16ns
58.0	3.22°	3.37ns
56.5	6.86**	9.79**
55.5	1.55ns	0.06ns
44.5	14.50**	0.16ns
43.5	0.63ns	0.00ns
43.0	8.65**	0.02ns
41.5	5.52**	0.44ns
41.0	4.94**	1.17ns
39.0	12.74**	5.05°
38.5	14.31**	0.99ns
37.5	7.60**	1.18ns
37.0	4.44°	0.07ns
31.5	1.00ns	0.16ns
31.0	5.46**	0.53ns

ns, °, ** Non significant (ns) or significant at $p = 0.05$ (°) or 0.01 (**), respectively.

Table 1. Effect of cultivar on mean ethylene evolution rate in five commercially diverse cultivars of *Prunus persica*.

Cultivar	Maturity ^z	Ethylene Evolution (nl/gFW/h)	
		Range	Mean
Belle of Georgia	AM	8.0-25	16.14a ^y
Biscoe	AM	0.7-10	2.57b
Fantasia	AM	0.8-5.7	2.30b
Redskin	AM	0.05-2.1	1.73b
Loring	M	0.1-0.97	0.51c

^zMaturity range (as defined by Sims and Comin, 1963): AM = Advanced Mature (5-6 kg); M = Mature (7 kg).

^yWithin columns, any two means having a common letter are not significantly different at the 5% level by the Student-Newman-Keuls multiple unplanned comparisons procedure.

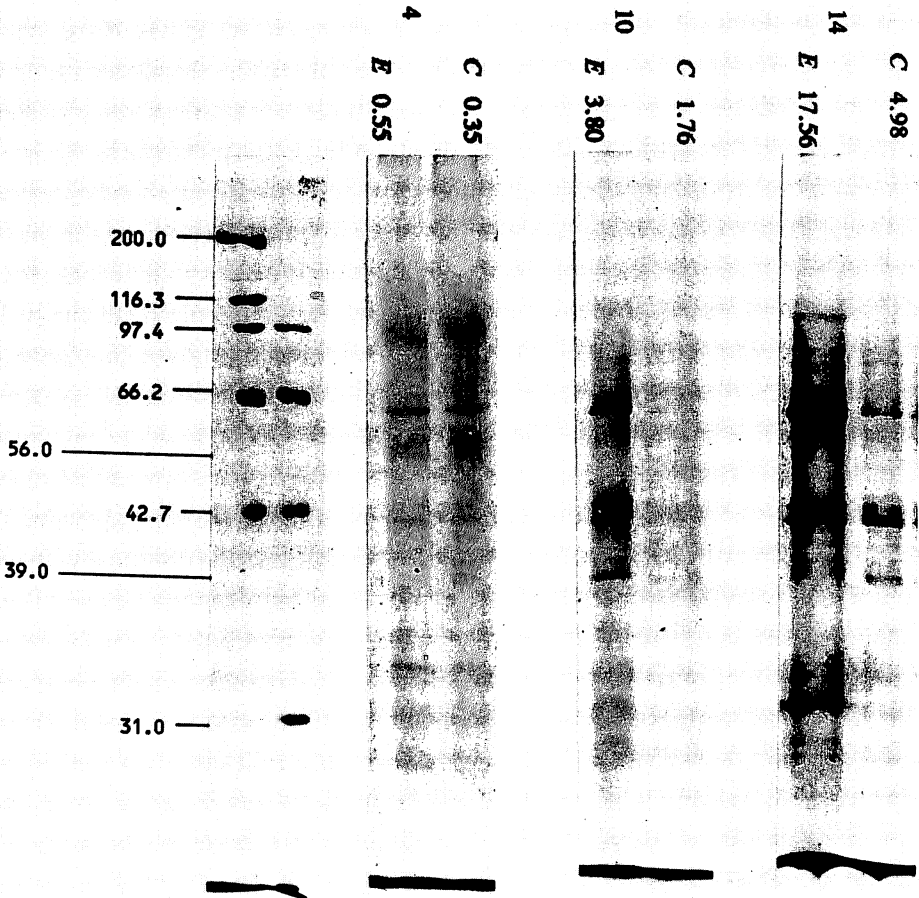


Figure 1. Denaturing polyacrylamide gel electrophoresis gel resolving soluble proteins extracted from mesocarp tissue of replicate fruits of 'Redskin' (R), 'Belle of Georgia' (BG), and 'Biscoe' (B) peach and from 'Fantasia' (F) nectarine at three representative levels of ethylene evolution during ripening. Ethylene evolution rates (nl/gFW/h) of individual fruit are indicated.

To determine whether accumulation of these ripening associated proteins were inducible by ethylene in early-shipping mature fruits (19), soluble protein homogenates obtained from mature, ethephon treated 'Loring'

mesocarp tissue were electrophoretically resolved as described above. Such fruit were previously reported to evolve ethylene at rates significantly higher than untreated control fruit (15). After 14 d at 25°C, treated 'Loring'

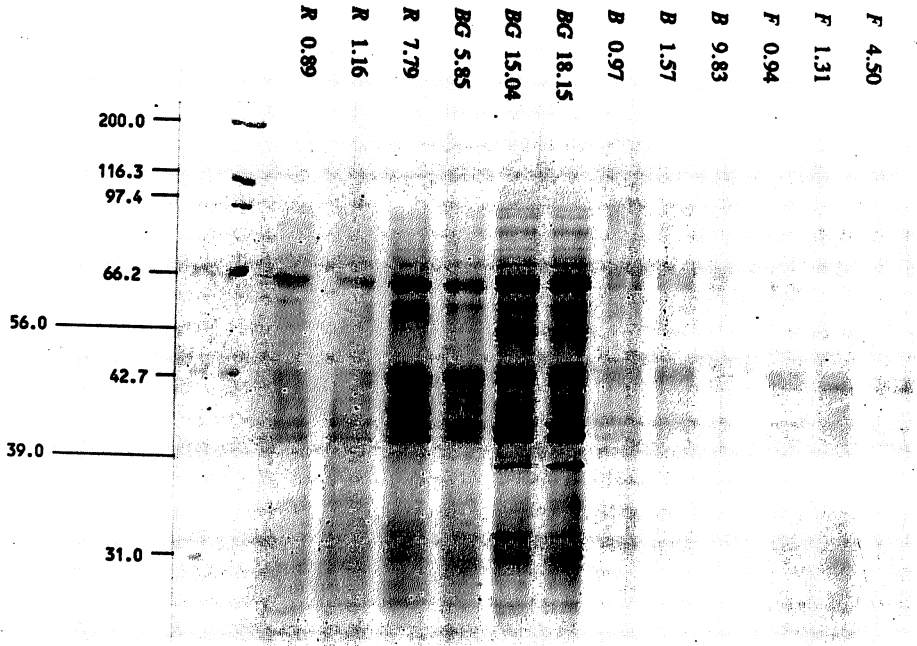


Figure 2. Denaturing polyacrylamide gel electrophoresis gel resolving soluble proteins extracted from mesocarp tissue of 'Loring' peach fruits at 4, 10, and 14 days after treatment with $250 \mu\text{l} \cdot \text{liter}^{-1}$ ethephon (E) and from untreated controls (C) sampled at the same time. The ethylene evolution rate (nl/gFW/h) of fruit at each sampling date is indicated.

fruits evolved ethylene at a rate comparable to that of 'Belle of Georgia' fruit. While the quantity of protein per gram fresh weight of tissue increased in treated 'Loring' fruits in response to the presence of exogenous ethylene, no qualitative changes in soluble mesocarp protein occurred during the period of observation (Fig. 2). Further, no accumulation of either 56.5 or 39.0 kDa protein was observed in ethephon treated 'Loring' fruits, indicating the cultivar specificity of the ripening associated induction of these proteins.

Discussion

These results indicate that the mean rate of ethylene evolution in peach fruit is cultivar dependent. 'Belle of Georgia,' a white fleshed peach that

arises from the original Chinese Cling seedlings, was found to evolve ethylene at a rate 6-fold greater than 'Biscoe,' the yellow fleshed cultivar with the highest mean ethylene evolution rate. 'Biscoe' has a white fleshed parent, 'Ruritan Rose.' In previous studies in our laboratory, 'Ruritan Rose' fruits were found to have a mean ethylene evolution rate similar to that of 'Belle of Georgia' (22). In addition, 'Belle of Georgia' fruits evolved ethylene at a rate 7-fold greater than 'Fantasia', 9-fold greater than 'Redskin,' and 26-fold greater than early shipping mature 'Loring' fruits (Table 1).

Significant differences in protein band occurrence and density among the four cultivars examined by PAGE may demonstrate an ethylene-related increase in protein concentration of

ripening peach fruits. The appearance of two novel proteins in 'Belle of Georgia' fruit in response to greatly increased ethylene evolution during maturation and tree ripening may indicate the cultivar dependent nature of this induction.

The measured differences in ethylene evolution rates and in accumulation of soluble proteins are probably not linked solely to flesh color. Rather, they may reflect selection efforts by peach breeders, who may unknowingly have selected reduced ethylene evolving *Prunus persica* genotypes to reduce preharvest drop and improve shipping performance (R. E. Scorza, personal communication). 'Belle of Georgia', the only cultivar utilized in this study which is subject to severe preharvest drop, is capable of attaining horticultural ripeness while attached to the tree. Induction of two proteins only in 'Belle of Georgia' fruit may indicate that their accumulation is associated with fruit color, quality, or with other ripening related physiological processes, such as ethylene biosynthesis. The induction of 39.0 and 56.5 kDa protein may occur in response to evolution of a high concentration of endogenous ethylene in 'Belle of Georgia' fruits and may be necessary for ethylene biosynthesis or transduction of ethylene action. Recently, a ~35 kDa protein encoding ACC oxidase was characterized in muskmelon (2) and apple fruits (8). The ethylene-related 39.0 and 56.5 kDa proteins observed in 'Belle of Georgia' peach fruit may not accumulate to detectable levels in modern, reduced ethylene shipping cultivars.

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Book Review:

Temperate Zone Pomology: Physiology and Culture

By M. N. Westwood

Timber Press has just released a revised edition of the classic pomology textbook. *Temperate Zone Pomology* was first released in 1978 and was the first textbook in a number of years that presented fruit culture from a plant physiology perspective as well as the practical culture. Much of the information; has been expanded and updated in this most recent edition. World wide production figures have been updated as well as information on rootstocks for tree fruits. Additional information on minor fruit crops has also been added, although the information is not meant to be all encompassing it does improve the usefulness of the book.

The addition of chapters on; the *History of Fruit Culture* and *Temperate Fruits in Low Latitudes* give a good cursory examination of these subjects. These two chapters greatly increase the overall comprehensiveness of the text. A final chapter on *Optimizing the Fruit Farm* is especially appropriate because it is useful in allowing individuals to analyze

the overall productivity of an orchard.

There are only a few minor fault that I could find with the layout of the text. I did not understand why the *Biotechnology Methods* was placed at the end of the chapter on *Species and Varieties* and not in the beginning in the discussion of breeding. I enjoyed the addition of the color plates but I was uncertain as to why the particular pictures were chosen and why they were positioned in the text.

In general I would recommend this text as a good second level text for a pomology course. I think it would also be of some value to commercial growers that want to have a reference on some of the latest research work and information and who might want to know more about the physiology of growing fruit.

(Available from Timber Press, Inc., 9999 S. W. Wilshire, Suite 124, Portland, OR 97225. Telephone 1-800-327-5680. Price \$59.95, plus shipping and handling.)

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