Graft Transmissible Agents Affect Membrane Fatty Acid Saturation during Dormancy Release in Peach

Philip G. Gibson¹
Environmental Horticulture, Gwinnett Technical College, Lawrenceville, GA 30046

Gregory L. Reighard
Department of Horticulture, Clemson University, Clemson, SC 29634

Gary L. Powell
Department of Biological Sciences, Clemson University, Clemson, SC 29634

Thomas C. Jenkins
Department of Animal and Veterinary Sciences, Clemson, SC 29634

Additional Index Words. Prunus persica, viroid, desaturase, linoleic, linolenic, inoculation

Abstract. Peach [Prunus persica (L.) Batsch (Peach Group)] trees infected with peach latent mosaic viroid (PLMVd) have been associated with phenological changes including delay in bloom, reduced shoot vigor, and early autumn defoliation. In order to further characterize the changes occurring in trees inoculated with PLMVd, total fatty acid content was measured for floral buds during release from dormancy in ‘Coronet’ peach trees. Palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids were the major fatty acids in dormant and releasing peach buds of both control and PLMVd-inoculated (VI) trees. The degree of unsaturation increased immediately following release from dormancy in both the control and VI trees. However, desaturation of linoleic acid to linolenic acid was significantly inhibited in VI trees, which was accompanied by a concomitant delay in the resumption of growth. The disparity between the control and VI trees in the progression of increased fatty acid unsaturation continued through petal fall. The presence of PLMVd in ‘Coronet’ peach trees slowed membrane fatty acid desaturation during release from dormancy and suggested that metabolic pathways involving fatty acid desaturation were linked to the delayed phenology of the VI trees.

Plant response to temperature patterns in the environment requires an internal mechanism that is responsive to temperature fluctuations, but includes a variety of control processes which prevent inappropriate responses to minor fluctuations. The resumption of growth following dormancy is accompanied by a change in membrane composition permitting increased permeability of solutes and water (Brockerhoff, 1974; Goad, 1983; Grunwald, 1975; Oldfield and Chapman, 1972). The degree of unsaturation in fatty acids of membrane lipids is a cellular response to changes in temperature which facilitates maintenance of an optimal degree of membrane fluidity (Cossins, 1983; Harris and James, 1968; Lynch and Steponkus, 1987; Thompson, 1979; Yoshida, 1984). Membrane permeability is partially controlled by fatty acid composition, which can limit or promote cellular metabolic activity (Sikorska and Farkus, 1982). Desaturase enzyme activity is correlated with the membrane changes taking place during endodormancy (Faust and Wang, 1993). The association between membrane fatty acid desaturation and a plant’s adaptation to environmental temperature changes is well established (Murakami, et al., 2000; Yoshida, 1984; Wang and Faust, 1988). Specifically, Wang and Faust (1990) found an accumulation of linolenate as apple buds released from dormancy. In peach, the relative proportions of linolenate and linoleate parallel the effects of temperature on dormancy and resumption of growth (Erez et al., 1997). Both of these studies found that linoleate accumulation during chilling followed by an accumulation of linolenate during exposure to temperatures favorable to bud development after rest completion. The rise in linolenate concentration upon resumption of growth is accompanied by a reduction in linoleate concentrations. This can be explained by the activity of specific desaturase enzymes, which insert the third double bond into the linoleate substrate.

Peach latent mosaic viroid (PLMVd) is present as a graft transmissible agent in the peach cultivar ‘Ta Tao 5’ (Diener, 1987). Phenological effects attributed to PLMVd are graft transmissible and include a delay in flowering and fruit maturity, increased fruit firmness, higher yield efficiency, earlier fall defoliation, and reduced tree vigor (Gibson and Reighard, 2002; Reighard, 1998). The objective of this study was to investigate the seasonal change during dormancy of fatty acids in cell membranes of peach floral buds infected with PLMVd.

Materials and Methods

Plant Material. Floral buds were collected from ‘Coronet’ peach trees planted Jan. 1996 as a high-density, Y-trained orchard system. The ‘Coronet’ trees were in four rows that were divided into a completely randomized block design consisting of six blocks of six trees in each row. Half the blocks were randomly selected for PLMVd inoculation with ‘Ta Tao 5’ peach vegetative chip buds in Aug. 1997. The trees were maintained under normal, commercial production cultural practices. The presence of PLMVd was confirmed in the inoculated ‘Coronet’ trees (VI)
used in this study by dot blot cRNA probe hybridization (Gibson et al., 2001b; Shamloul, 1995). Floral buds were collected from both of the two outside trees (tree 1 and 6) in each block during Jan., Feb., and Mar. 1999 and 1999–2000. Buds from each outside tree of each VI or control block in rows one to four were pooled and duplicated to yield a total of eight VI tree samples and eight control tree samples for each collection date. Buds were stored at –80 °C until the samples were processed in each year. Frozen buds were ground in a coffee grinder, weighed and processed immediately for fatty acid analysis.

**FATTY ACID ANALYSIS.** Fatty acids were methylated to fatty acyl methyl esters (FAME) using a direct transesterification procedure (Sukhija and Palmquist, 1988). Samples were weighed to 500 mg and added to a 15 × 150-mm culture tube with screw cap and Teflon liner. Heptadecanoic acid (17:0) was added as an internal standard (500 mg·mL–1 benzene) to each tube. Next, 3 mL of 5% v/v methanolic HCl was added, tubes were capped tightly and vortexed. Samples were incubated in a 70 °C water bath for 2 h. Tubes were cooled to room temperature, 7.5 mL of 6% w/v K2CO3 was added, 1 mL hexane, capped and vortexed. After centrifugation at 10,000 g, for 5 min, the organic layer was transferred to a gas chromatograph sampling vial with a Teflon lined cap. FAMEs were analyzed by flame ionization detection gas chromatography (FID-GC) using a Supelco P-2380 (Supelco, Bellefonte, Pa.) fused silica capillary column 30 m x 0.25 mm x 0.2-µm film thickness. The HP5890A gas chromatograph oven temperature was ramped from at 140 °C for 3 min. to 220 °C at 3.7 °C/m and held for 20 min. Helium carrier rate was 20 cm·s–1 at 150 °C with a flame ionization detector temperature of 260 °C. The injector was split 100:1 at 250 °C with column head pressure of 87 kPa. The flow rate was 35.0 He at tank pressure 276 kPa. Individual FAMEs were identified by comparing their retention times with authentic standards (Supelco). The FID FAME identification results were confirmed by mass spectrometry.

**Results and Discussion**

Palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids were the major fatty acids in dormant and releasing peach buds of both the control and PLMVd-inoculated ‘Coronet’ (VI) trees (Fig. 1). Concentrations of palmitic, stearic, and oleic acids remained relatively constant throughout the experiment (Fig. 2). The increase in linolenic acid in the lower buds from the VI trees lagged significantly behind the control trees during release from dormancy in 1999 and 2000 (Fig. 3). A differential change to linolenic and linoleic acids was found between the VI and control trees (Fig. 4). The larger ratio of saturated [palmitic (16:0) and stearic (18:0)] to unsaturated fatty acids [oleic (18:1), linoleic (18:2), and linolenic (18:3)] in the VI trees indicated higher degree of unsaturation in the control trees (Fig. 5).

The presence of PLMVd did not change the chilling requirement of ‘Coronet’ peach (Gibson and Reighard, 2002). The response of peach blossoms to forcing conditions is dependent upon chilling accumulation and subsequent heat accumulation (Richardson et al., 1975). Breaking dormancy requires heat unit accumulation
Arnold, C.Y. 1959. The determination and significance of the base temperature in a linear heat unit system. Proc. Amer. Soc. Hort. Sci. 74:430–445.

Ashcroft, G.L., E.A. Richardson, and D.S. Schuyler. 1977. A statistical method of determining chill unit and growing degree hour requirements for deciduous fruit trees. HortScience 12:347–348.

Brockerhoff, H. 1974. Model of interaction of polar lipids, cholesterol, and proteins in biological membranes. Lipids 9:645–650.

Cossins, A.R. 1983. The adaptation of membrane structure and function to changes in temperature, p. 5–32. In: A.R. Cossins and P. Sheferline (eds.). Cellular acclimation to environmental changes. Cambridge Univ. Press, Cambridge.
Fig. 5. The ratio of saturated [palmitic (16:0) and stearic (18:0)] to unsaturated fatty acids [oleic (18:1), linoleic (18:2), and linolenic (18:3)] in floral buds of control (VF) and peach latent mosaic viroid (PLMVd)-inoculated (VI) trees. Each data point represents the average of eight samples. Differences in the ratio of saturated to unsaturated fatty acids between the control and VI trees for sample dates after 5 Feb. 1999 and 17 Feb. 2000 were significant ($t$ test $P = 0.05$).

Diener, T.O. 1987. In: The Viriods. Plenum Press. New York.

Erez, A., S.Y. Wang, and M. Faust. 1997. Lipids in peach buds during dormancy, a possible involvement in dormancy control. Adv. Hort. Sci. 11:128–132.

Faust, M. and S.Y. Wang. 1993. Biochemical events associated with resumption of growth in temperate-zone fruit trees. Acta Hort. 329:257–264.

Gibson, P.G. and G.L. Reighard. 2002. Chilling requirement and postrest heat accumulation in peach trees inoculated with peach latent mosaic viroid. J. Amer. Soc. Hort. Sci. 127:333–336.

Gibson, P.G., G.L. Reighard, S.W. Scott, and D.R. Ouellette. 2001a. Using graft transmissible agents in Y-trained peach systems. Acta Hort. 557:139–144.

Gibson, P.G., G.L. Reighard, S.W. Scott, and M.T. Zimmerman. 2001b. Identification of graft-transmissible agents from 'Ta Tao 5' peach and their effects on 'Coronet' peach. Acta Hort. 550:309–314.

Goad, L.J. 1983. How is sterol synthesis regulated in higher plants? Biochem. Soc. Trans. 11:548–552.

Grossman, Y.L. and T.M. Dejong. 1994. Carbohydrate requirements for dark respiration by peach vegetative organs. Tree Physiology 14:37–48.

Grunwald, C. 1975. Plant sterols. Annu. Rev. Plant Physiol. 26:209–236.

Harris, P. and A.T. James. 1968. The effect of low temperature on fatty acid biosynthesis in plants. Biochem. J. 112:325–330.

Lynch, D.V. and P.L. Steponkus. 1987. Plasma membrane lipid alteration associated with cold acclimation of winter rye seedlings (Secale cereale L. cv. Puma). Plant Physiol. 83:761–767.

Murakami, Y., M. Tsuyama, Y. Kobayashi, H. Kodama, and K. Iba. 2000. Trienoic fatty acids and plant tolerance of high temperature. Science 287:476–479.

Norman, H.A., P. Pillai, and J.B. St. John. 1991. In vitro desaturation of monogalactosyldiacylglycerol and phosphatidylcholine molecular species by chloroplast homogenates. Phytochemistry 30:2317–2322.

Oldfield, E. and D. Chapman. 1972. Dynamics of lipid membranes: Heterogeneity and the role of cholesterol. FEBS Lett. 23:285–297.

Reighard, G.L. 1998. Manipulation of peach phenology, growth, and fruit maturity using interstems. Acta Hort. 465:567–572.

Richardson, E.A., S.D. Seely, D.R. Walker, J.L. Anderson, and G.L. Ashcroft. 1975. Phenoclimatography of spring peach bud development. HortScience 10:236–237.

Shamloul, A.M. 1995. Peach latent mosaic viroid: nucleotide sequence of an Italian isolate, sensitive detection using RT-PCR and geographic distribution. Acta Hort. 386:522–530.

Sikorska, E. and T. Farkas. 1982. Sterols and frost hardening of winter rape. Physiol. Plant 56:349–352.

Siller-Cepeda, J.H., L.H. Fuchigami, and T.H.H. Chen. 1992a. Glutathione content in peach buds in relation to development and release of rest. Plant Cell Physiol. 33:439–452.

Siller-Cepeda, J.H., L.H. Fuchigami, and T.H.H. Chen. 1992b. Hydrogen cyanamid-induced bud break and phytotoxicity in 'Redhaven' peaches. HortScience 27:874–876.

Spiegel-Roy, P. and F.M. Alston. 1979. Chilling and post-dormant heat requirement as selection criteria for late flowering pears. J. Hort. Sci. 54:115–120.

Sukhija, P.S. and D.L. Palmaquist. 1988. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agr. Food Chem. 36:1202–1206.

Thompson, G.A. 1979. Molecular control of membrane fluidity. p. 347–363. In: J.M. Lyons, D. Grahas, and J.K. Raison (eds.). Low temperature stress in crop plants. Academic, New York, London.

Wang, S.Y. and M. Faust. 1988. Changes of fatty acids and sterols in apple buds during bud break induced by a plant bioregulator, thidiazuron. Physiol. Plant. 72:115–120.

Wang, S.Y. and M. Faust. 1990. Changes in membrane lipids in apple buds during dormancy and bud break. J. Amer. Soc. Hort. Sci. 115:803–808.

Yoshida, S. 1984. Chemical and biophysical changes in the plasma membrane during cold acclimation of mulberry bark cells (Morus bombycis Koidz. Goroji). Plant Physiol. 76:257–265.