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Cold Acclimation in Genetically Related (Sibling) Deciduous and Evergreen Peach (*Prunus persica* [L.] Batsch)

I. Seasonal Changes in Cold Hardiness and Polypeptides of Bark and Xylem Tissues

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ABSTRACT

Seasonal patterns of proteins and of cold hardiness were characterized in bark and xylem tissues of genetically related (sibling) deciduous and evergreen peach (*Prunus persica* [L.] Batsch). In contrast with deciduous trees, which entered endodormancy and abscised leaves in the fall, evergreen trees retained their leaves and exhibited shoot elongation under favorable environmental conditions. A successive increase in the cold hardiness of bark and xylem was observed during the fall in both genotypes. This was followed by a subsequent decrease from midwinter to spring. Xylem tissue in both genotypes exhibited deep supercooling and a significant correlation ($r = 0.99$) between the midpoint of the low-temperature exotherm and the subzero temperature at which 50% injury occurred (assessed by electrolyte leakage) was noted. The maximum hardiness level attained in deciduous trees was more than twofold that of evergreens. Seasonal pattern of proteins from bark and xylem of the sibling genotypes was characterized by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Among other qualitative and quantitative changes, accumulation of a 19-kilodalton polypeptide in the bark of both genotypes was observed during fall followed by a decrease in spring. This polypeptide accumulated to higher levels in the deciduous peach compared with the evergreen. Additionally, a 16-kilodalton protein exhibited the same pattern in deciduous trees but not in the evergreen trees. Both the 19- and a 16-kilodalton bark proteins conform to the criteria of a bark storage protein. The relationship of seasonal changes in protein to cold hardiness and dormancy in these genetically related peach genotypes is discussed.

Evergreen genotypes of peach (*Prunus persica*) have been identified in Mexico, where they grow near 18°53′ to 18°11′ N latitude and 98°42′ longitude. They are feral, seed-propagated peaches, and are considered to be descended from introductions by early Spanish settlers. This area ranges from 980 to 2200 m elevation, does not have killing frosts, has about 85% of its rain (1250 mm) from early June to mid-October, and is without supplemental irrigation (6). Terminal growth in these genotypes is continuous under favorable environmental conditions, and leaves are retained until lost due to drought stress or disease (6).

An evergreen genotype (P.I. 442380) was entered into the U.S. Department of Agriculture-Agricultural Research Service stone fruit breeding program at Kearneysville, WV, via the hybridization of P.I. 442380 (pollen source) with OP[1] Empress dwarf. Subsequently, OP seed was collected from the F_1 tree of Empress dwarf OP × evergreen P.I. 442380. Assuming that most OP seed resulted from selfing (8), the seeds were planted and the growth characteristics of individual trees were evaluated over several years. Of relevance to the present study, approximately 25% of the trees from the F_2 generation exhibited a deciduous habit and a period of endodormancy typical of temperate fruit trees, whereas another 25% of the trees exhibited an evergreen habit with continuous terminal growth under favorable environmental conditions and other features indicative of a lack of endodormancy. The remaining 50% of the trees were presumed to be heterozygotes, which were characterized by late leaf abscission in the fall (R. Scorza, personal communication).

Cold acclimation in deciduous, woody perennials, including fruit trees, is a seasonal process that is marked by an increased cold tolerance in fall, reaching maximum in winter (cold acclimation), followed by a decrease in tolerance during spring, and reaching the minimum in summer (deacclimation) (18). Overwintering deciduous trees also enter into endodormancy during the same time as they develop cold hardiness. Early studies on the seasonal variation in protein content of cortical bark cells of black locust (*Robinia pseudoacacia*) demonstrated the accumulation of soluble proteins in the fall with a parallel increase in freezing tolerance. This was followed by subsequent decline in protein content and loss of the cold hardiness in spring when growth resumed (25). Several studies since then have provided evidence for quantitative and qualitative differences in protein content of bark between nonacclimated and cold-acclimated woody plants (5, 12, 20, 23), including deciduous fruit trees (14, 15, 17, 19). Due to the superimposition of endodormancy and cold acclimation, however, it is not clear from these studies whether or not changes in protein turnover are specifically associated with cold acclimation. The existence of genetically related (sibling) deciduous and evergreen peach genotypes, previously de-

[1] Abbreviations: OP, open pollinated; DTA, differential thermal analysis; LTE, low temperature exotherm; LT_{50}, subzero temperature at which 50% injury occurred; HTE, high temperature exotherm; BSP, bark storage protein.

scribed, may provide a good model system to study mechanisms of cold acclimation in woody perennials that are not a direct result of endodormancy.

The present study was conducted to obtain data on cold hardiness in the deciduous and evergreen peach genotypes to lay a groundwork for subsequent, more detailed studies. Therefore, seasonal patterns in the cold hardiness and protein changes of bark and xylem tissues of both genotypes were characterized. The objective of the protein studies was to elucidate quantitative and qualitative changes that were associated with cold acclimation.

MATERIALS AND METHODS

Plant Material

Current-year shoots from 2-year-old sibling, deciduous and evergreen peach (*Prunus persica* [L.] Batsch) trees were collected approximately every 4 weeks from August 21, 1990 to May 21, 1991 at the Appalachian Fruit Research Station, Kearneysville, WV. The samples were randomly collected from four to five trees of each genotype. Pooled samples of each genotype were packed on ice, brought to the laboratory, and processed for cold hardiness determination, DTA, and protein extraction. The bark from collected samples was scraped with a razor blade, ground with mortar and pestle under liquid nitrogen, and stored at −80°C until used for protein extraction.

Cold Hardiness Determination

Cold hardiness of the bark and xylem tissues was evaluated by the method of Ashworth *et al.* (1) with few modifications. Twig pieces 6 to 8 cm long were wrapped in aluminum foil along with moistened paper and placed in prechilled Dewar flasks. The flasks were transferred to a manually controlled low-temperature freezer (ULT 1090, REVCO, Asheville, NC). Plant tissue temperature was monitored with a copper-constantan thermocouple inserted in the foil pouch. Samples were cooled at approximately 1.5°C/h to −4°C and at 5 to 7°C/h thereafter. Ice nucleation was accomplished at about −1.5 to −3°C by applying a small quantity of a locally obtained isolate of ice nucleation-active bacteria (*Pseudomonas syringae*) to the bottom of the twig of all samples before they were wrapped in the foil (29). Flasks were removed at treatment temperatures and placed at 4°C overnight for slow thawing. Unfrozen control samples were maintained at 4°C. At each temperature, bark and xylem from three separate twigs were sampled for the estimation of freezing injury, which was evaluated by an electrolyte leakage method (1). Three internodal strips of bark (1 × 1 cm) were removed from each twig, placed in a 2.5 × 20-cm test tube containing 20 mL of deionized water, and vacuum infiltrated for 3 min. Two debarked internodal sections (1 cm long, 3–4 mm in diameter) of xylem were also transferred to separate test tubes each containing 20 mL of deionized water and vacuum infiltrated. Test tubes containing bark and xylem tissues were shaken on a gyratory shaker (250 rpm) at room temperature for 1.5 and 18 h, respectively. Subsequently, conductivity of the effusate was recorded with a conductivity meter (YSI model 35, Yellow Spring Instruments, Yellow Springs, OH). Samples were then heat-killed in the same solution and conductivity was once again recorded at room temperature. Ion leakage was calculated as a percentage of total (after heat-killing). Percentage injury was then calculated according to the method of Zhang and Willison (30) using the expression:

$$[\%\ \text{injury} = \%L(t) - \%L(c)/100 - \%\mathrm{L}(c)] \times 100$$

where $\%L(t)$ and $\%L(c)$ are the measurements of percentage of ion leakage for the respective freeze-treatment temperature and unfrozen control, respectively. LT_{50} was defined as the subzero temperature at which 50% injury occurred. All measurements were replicated three times.

DTA

The system utilized to obtain freezing profiles of xylem tissue was as described previously (29). Samples of debarked twig internodes (approximately 0.5 g) were utilized to characterize freezing. A 1-cm section of freeze-dried peach xylem tissue was used as a reference. No marked differences were observed between the freezing profiles obtained at the cooling rates of 5 and 20°C/h (data not shown), so samples were cooled linearly at 20°C/h. Three samples from each treatment were subjected to DTA. The correlation coefficient between LTE and LT_{50} values was determined.

Protein Extraction

Protein from bark tissue was extracted by the method of Wetzel *et al.* (27) with few modifications. Bark tissue (3 g fresh weight) was homogenized at 4°C in borate buffer (50 mM sodium borate, 50 mM ascorbic acid, 1% β-mercaptoethanol, 1 mM PMSF, pH 9.0) for 30 s followed by 60 s at half-maximum speed with a Kinematica Polytron (Kriens-Luzern, Switzerland). Xylem proteins were extracted from lyophilized and powdered tissue by the same procedure. Samples were then centrifuged at 26,000*g* at 4°C for 1.5 h. The resulting supernatant was collected, filtered successively through 0.4- and 0.2-μm filters, and assayed for protein content by a micro-Lowry method using Sigma protein assay kit.

Sample Preparation and SDS-PAGE

Samples for SDS-PAGE were prepared by the method of Wetzel *et al.* (27). The appropriate volume of extraction buffer containing 200 μg of protein was diluted to 1 mL with deionized water. Protein was precipitated from the diluted sample by adding 0.1 mL of TCA (final concentration of about 10% [v/v]). The precipitate was collected by centrifugation at 16,000*g* for 15 min at 4°C, washed with acetone, and dried. The pellet was resuspended in 65 mM Tris-HCl, 10% (v/v) glycerol, 2% (w/v) SDS, pH 6.8, containing 5% β-mercaptoethanol (16), boiled for 3 min, and cooled to room temperature. Discontinuous SDS-PAGE was performed with a PROTEAN II electrophoresis unit (Bio-Rad) using 4% stacking gel and a 12.5% running gel. Gels were stained with 0.1% Coomassie brilliant blue R in methanol:water:acetic acid (4.2:4.2:1.6, v/v/v) and destained with water:methanol:acetic acid (8:1:1, v/v/v).

RESULTS

Seasonal Patterns of Cold Hardiness in Deciduous and Evergreen Peach

Bark

Seasonal changes in the LT_{50} of bark tissues from deciduous and evergreen peach trees are presented in Figure 1A. Cold hardiness of bark from deciduous peach trees increased from −5°C in August to a maximum of about −50°C in February and then decreased to −4°C by May. In evergreen trees, the LT_{50} changed from −3°C in August to −22°C in January followed by a decrease to −3°C by May. There was an approximate fourfold increase in the cold hardiness of bark between September and October in deciduous peach trees. In contrast, there was no change in bark hardiness of evergreen peach during the same time period.

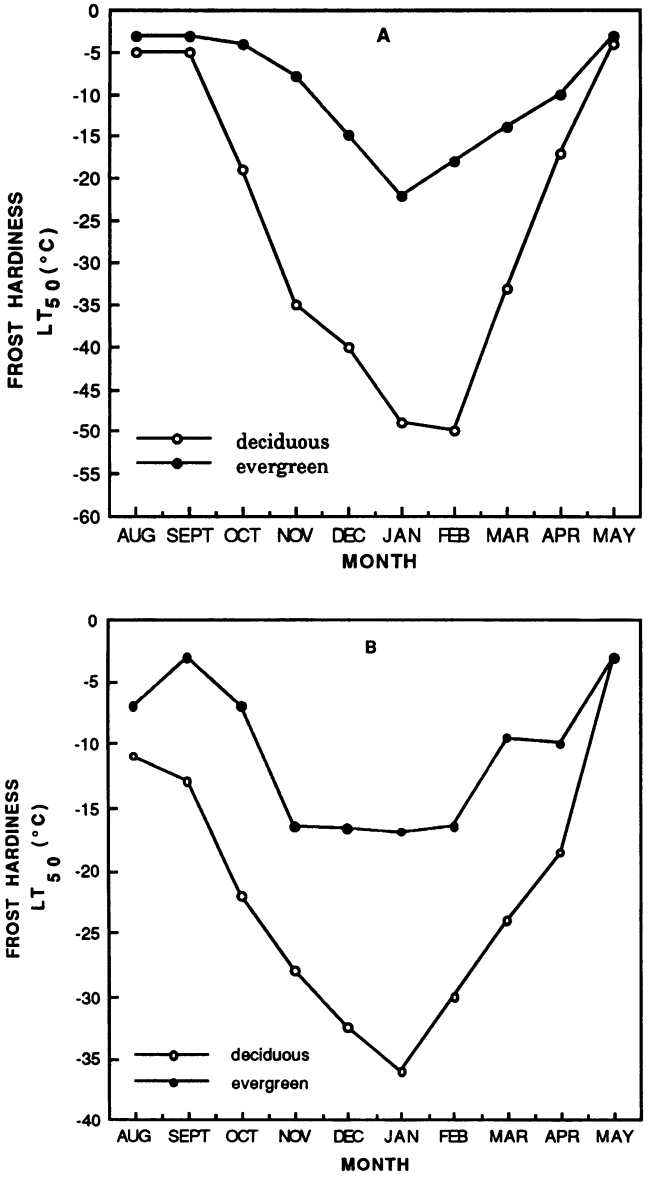

Figure 1. Seasonal changes in the cold hardiness of bark (A) and xylem (B) tissues of sibling deciduous and evergreen peach trees. LT_{50} was assessed by electrolyte leakage.

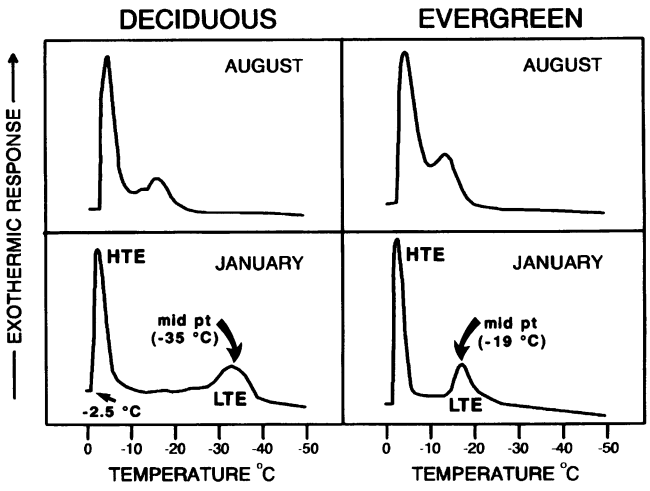

Figure 2. DTA profiles of xylem tissues from nonacclimated (August) and cold acclimated (January) deciduous and evergreen peach shoots. For each DTA profile, the large peak represents the HTE from freezing of the bulk water, and the smaller LTE is indicative of deep supercooling of xylem parenchyma cells. Freezing of nonacclimated xylem tissue typically resulted in the LTE occurring as a shoulder adjacent to the HTE. Acclimation, observed in both genotypes, progressed to markedly different levels as observed by the difference in LTE ranges and midpoints shifting to colder temperatures. Each curve represents the average of two to three separate samples.

Xylem

Seasonal changes in the LT_{50} of xylem tissues from deciduous and evergreen peach trees are presented in Figure 1B. Cold hardiness of xylem from deciduous peach trees increased from −11°C in August to −36°C by January and then decreased to −3°C by May. In evergreen trees, cold hardiness increased from −7°C in August to −16.5°C in November and then remained constant until February. Cold hardiness then decreased to −3°C by May. Similar to the response in bark, xylem cold hardiness increased by twofold between August and October in deciduous peach but remained unchanged in evergreen trees.

DTA profiles of xylem tissue from deciduous and evergreen siblings obtained in August (Fig. 2) displayed no clear separation of the HTE from the LTE. As acclimation progressed, however, the two exotherms became clearly distinct and separate in both genotypes. DTA profiles obtained in January (Fig. 2) displayed an HTE around −2.5°C in both genotypes. At that time period, a small, broad LTE was present from −28°C to −41.6°C in the deciduous genotype and from −14.5°C to −30°C in the evergreen genotype. Separation of the LTE from the HTE occurred 1 month later in the evergreen trees as compared with the deciduous trees (Table I). Conversely, overlap of the HTE and LTE reoccurred in evergreen trees 2 months earlier than in the deciduous trees. The data in Table I indicate that, when the LTE was clearly separated

Table I. *Comparison of Cold Hardiness of Xylem Tissue from Sibling Deciduous and Evergreen Peach Genotypes as Characterized by the LT_{50} (Determined by Electrolyte Leakage) and Midpoint of LTE*

Month	Deciduous		Evergreen	
	LTE	LT_{50}	LTE	LT_{50}
	°C		°C	
August	−16.3[a]	−11.0	−13.5[a]	−7.0
September	−17.0[a]	−13.0	−14.0[a]	−3.0
October	−22.7	−22.0	−14.9[a]	−7.0
November	−28.0	−28.0	−17.0	−16.5
December	−32.7	−32.5	−19.0	−16.6
January	−35.0	−36.0	−19.0	−17.0
February	−29.3	−30.0	−17.5	−16.5
March	−22.2	−24.0	−16.0[a]	−10.0
April	−20.7	−18.5	−16.0[a]	−10.0
May	−7.0[a]	−3.0	−7.0[a]	−3.0

[a] LTE was not separated distinctly from the HTE and appeared as a shoulder of the HTE. Values represent temperatures corresponding to the peak of the shoulder.

from the HTE, there was a significant correlation ($r = 0.99$) between the LT_{50}, as determined by electrolyte leakage, and the midpoint of the LTE. These two parameters, however, did not show close correspondence when the two exotherms overlapped each other.

Seasonal Patterns in Proteins of Sibling Deciduous and Evergreen Peach

SDS-PAGE analysis of the monthly samples of bark and xylem proteins was repeated at least three times with similar results. Data from a single, representative analysis are presented.

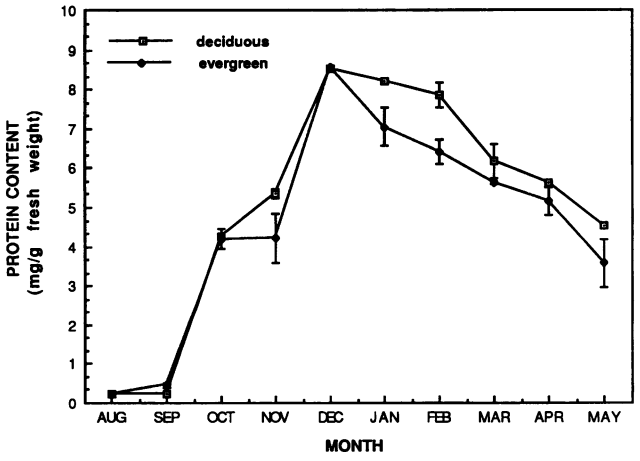

Figure 3. Seasonal changes in the amount of total protein extracted from the bark of sibling deciduous and evergreen peach trees. The bars represent ± SD based on means of duplicates, except where SD is smaller than the symbol.

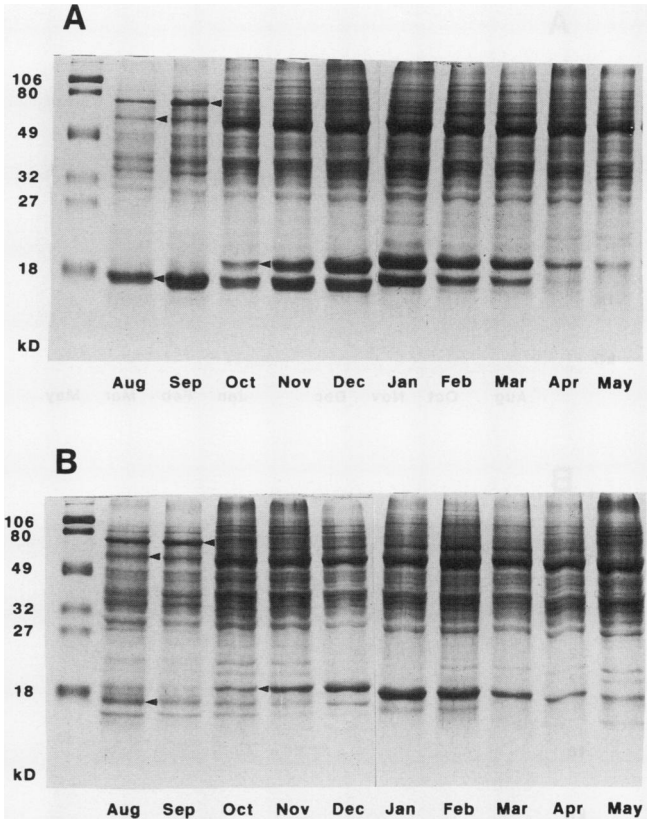

Figure 4. Monthly SDS-PAGE protein profiles of the bark of sibling deciduous (A) and evergreen (B) peach trees. A total of 20 μg of protein was loaded in each lane. Molecular mass markers and their molecular masses are shown on the left side. Arrows indicate the position of 78-, 60-, 19-, and 16-kD polypeptides.

Bark

Total protein content in the bark tissues of both genotypes increased linearly from September through December followed by a successive decline through May (Fig. 3). Seasonal changes were also observed in the protein patterns obtained from deciduous and evergreen genotypes by SDS-PAGE (Fig. 4, A and B). In general, there was a marked similarity between the SDS-PAGE profiles of proteins from the two genotypes, although some qualitative and quantitative differences were noted. Data indicate that, in both genotypes, a polypeptide with an estimated molecular mass of 19 kD became prominent in early fall, accumulated during winter, and decreased during spring. During the months of November, December, and January, this polypeptide accumulated to higher levels (about twofold) in deciduous tress compared with evergreens (Fig. 4, A and B). In addition, a 16-kD protein, which appeared to constitute a major portion of total protein, accumulated to high levels in deciduous trees during fall and winter followed by a complete disappearance in spring (Fig. 4A). In evergreen trees, however, this protein was only faintly detected and showed no distinct seasonality (Fig. 4B). An accumulation of a 60-kD polypeptide and a reduction of a 78-kD polypeptide were also noted in both genotypes as fall progressed (Fig. 4, A and B).

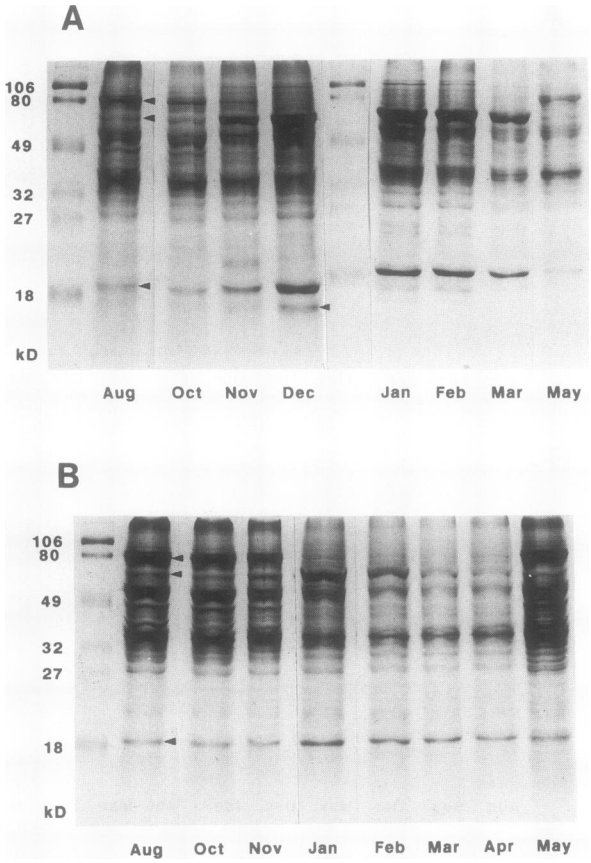

Figure 5. Monthly SDS-PAGE protein profiles of the xylem tissues from sibling deciduous (A) and evergreen (B) peach trees. A total of 20 μg of protein was loaded in each lane. Molecular mass markers and their molecular masses are shown on the left side. Arrows indicate the position of 80-, 70-, 19-, and 16-kD polypeptides.

Xylem

Seasonal changes in xylem proteins were generally similar in both genotypes (Fig. 5, A and B). A distinct seasonality was noted, however, for a few polypeptides. This may have been partly due to the presence of lanes with unusually faint protein bands in the February, March, and April samples of evergreen trees and in the March and May samples of deciduous trees. The reason for this anomaly was not clear; however, similar results were obtained following repetitive protein estimation and SDS-PAGE. Although the SDS-PAGE analysis should be interpreted with caution because of the problems of accurate protein estimation, a conservative evaluation indicated that polypeptides with estimated molecular masses of 70 and 19 kD accumulated in the fall and winter followed by a decrease in the spring in both genotypes. These proteins accumulated to a higher level in deciduous trees compared with evergreens. During the same period, the band intensity of an 80-kD protein decreased in both genotypes. A 16-kD protein, which was present in the deciduous trees during December, was not apparent in evergreen trees during this period (Fig. 5, A and B).

DISCUSSION

Seasonal Patterns of Cold Hardiness of Bark and Xylem in Deciduous and Evergreen Peach

The sibling deciduous and evergreen peach genotypes exhibited an increase in cold hardiness during the fall, which reached a maximum in midwinter and then gradually decreased (Fig. 1, A and B). Although this pattern of cold hardiness has been reported in many woody plants (15, 19, 22, 26), this is the first report to our knowledge of a comparative study on deciduous and evergreen siblings of *Prunus* species.

A fourfold increase (−5 to −19°C) in the freezing tolerance of bark of deciduous peach trees occurred from September 18 through October 27 (Fig. 1A), during which time the mean average air temperature was 15°C (only 4 d in the last week with mean temperatures of 5–8°C and with the first light frost on October 21). Therefore, it is tempting to speculate that this increase in hardiness was primarily due to inductive short photoperiods. A subsequent, nearly twofold increase (−19 to −35°C) occurred in hardiness from October 27 through November 27, during which time the mean average air temperature was 8.5°C (with about 10 occurrences of frost). This latter increase in cold hardiness may have been due to both short photoperiods and colder temperatures. Previous studies (9, 13) have also indicated that, in nature, cold acclimation in deciduous woody perennials is a two-stage process. The first step is induced in the fall by short days and temperatures of 10 to 20°C, while the second stage is induced by temperatures below 5°C (24). Although the data are not definitive because acclimation in the absence of either low temperatures (frost events) or short photoperiods could not be determined, our results are consistent with this view.

The evergreen trees exhibited no increase in bark cold hardiness during September 18 through October 27 (Fig. 1A). The increase in cold hardiness, when it occurred (October 27 through January 16) in these trees, may have been primarily in response to low temperatures rather than to a short photoperiod. A similar trend was observed for the xylem tissues (Fig. 1B). The apparent lack of responsiveness in evergreens to short photoperiods could be attributed to the fact that the pollen parent (PI 442380) of evergreen trees is native to Chiapas province of Mexico (latitude 18°N), where daylength shows little seasonal shift.

Our data indicate that deciduous trees acclimate sooner and to a greater extent than evergreen trees. This may be associated with both the response to photoperiod and the cessation of growth, which are apparently lacking in evergreen trees. Fuchigami and coworkers (9) have suggested that growth cessation is a prerequisite to cold acclimation in woody perennials and that maximum hardiness is attained during endodormancy or "rest." Our results support the idea that the maximum hardiness in woody perennials occurs after cessation of growth (endodormancy). The cold hardiness of the bark and xylem tissues of fully dormant, deciduous peach trees was more than double that of evergreen trees (Fig. 1, A and B). A considerable degree of cold hardiness, however, was attained by the evergreen peach trees. This

indicates that cessation of growth is not an absolute requirement for cold acclimation to occur.

Xylem of both genotypes exhibited deep supercooling, although the extent of supercooling was much less in the evergreen genotype compared with deciduous trees (Fig. 2). The midpoint of the LTE was closely associated with the LT_{50} of xylem in both genotypes (Table I) only during the time period when the LTE was clearly separated from the HTE. Hong *et al.* (11) suggested that the relationship between LTE and injury to xylem was quantitative, *i.e.* at any percentage of total LTE peak area, that percentage of ray cells was killed. Our results and those of a previous study (1) support this idea in that a strong correlation ($r = 0.99$) between the LT_{50} of xylem tissue and the midpoint of the LTE was noted for both genotypes.

Seasonal Pattern of Proteins in the Bark and Xylem from Deciduous and Evergreen Peach

Bark

The most apparent seasonal pattern in proteins observed in both genotypes was the accumulation of a polypeptide with apparent molecular mass of 19 kD during acclimation followed by a subsequent decrease during deacclimation (Fig. 4, A and B). A 16-kD protein exhibited a similar seasonal pattern in deciduous trees but was either present in low amounts or undetectable in evergreens. In addition, a 60-kD polypeptide accumulated during cold acclimation and declined during deacclimation in both genotypes.

Although both qualitative and quantitative changes in protein content have been reported during acclimation and deacclimation in various woody perennials (2, 5, 7, 20, 26), research on deciduous temperate fruit trees is limited. Moreover, due to the concurrence of development of cold hardiness and other physiological changes such as dormancy, leaf senescence, abscission, etc. in deciduous fruit trees, it is difficult to ensure that changes detected in proteins are specifically related to increased freezing tolerance and not to dormancy.

Our results indicate that evergreen preach, whose shoot tips do not enter into vegetative endodormancy, exhibited a seasonality of cold hardiness similar to deciduous trees that enter into endodormancy in the fall. Moreover, the seasonal fluctuation in the 60- and 19-kD proteins were also commonly observed in deciduous and evergreen genotypes (Fig. 4, A and B). Therefore, these proteins may, in some way, be related to cold acclimation.

It is interesting to note that despite differences in their growth habits, there was a marked similarity between the protein profiles of deciduous and evergreen genotypes. However, this does not rule out subtle differences that might have been revealed by two-dimensional gel electrophoresis. Recently, Hummel *et al.* (12) also noted a close similarity between the SDS-PAGE profiles of bark proteins from deciduous and evergreen species of *Hibiscus*.

Appearance of new proteins and accumulation of other proteins during cold acclimation is thought to be a general response (10). However, no clear mechanistic relationship to cold hardiness has been proposed. Our results indicate that a 60- and a 19-kD protein accumulated to relatively higher levels in deciduous than in evergreen trees (*cf.* Fig. 4, A and B). This is coincident with the relatively higher level of cold hardiness of deciduous bark tissue. Recently, a higher degree of winter hardiness was correlated with the increased amount of a specific group of soluble proteins in the bark of *Malus domestica* (14). These proteins were designated as peak III proteins based on the procedure of elution from a crude extract.

O'Kennedy and Titus (21) first hypothesized that specific proteins in the bark of *M. domestica* may act as overwintering storage proteins for the support of spring growth. They proposed two criteria for the classification of BSP: (a) they accumulate to high levels in dormant shoots; and (b) they completely disappear during growth. However, Wetzel *et al.* (27) suggested that storage proteins, although greatly reduced in the bark of temperate hardwoods, are not completely absent during spring. Our results indicate that a 16-kD protein in the bark of deciduous peach fulfills these criteria (Fig. 4A).

In addition, a 19-kD protein, which appears to constitute a major portion of total protein, also exhibited the characteristics of a BSP. It accumulated to much higher levels (about twofold) in deciduous as compared with evergreen trees during winter months. Recently, a 16-kD protein was shown to accumulate in the bark tissues of sugar maple (*Acer saccharum*) during fall and winter and to decline during spring (27). This was paralleled by the presence of protein-storing organelles within the bark during winter and their absence during spring. It is interesting that the 16-kD bark protein of peach was either undetectable or appeared as a faint band in the protein profile of evergreen trees. Moreover, in contrast with the findings in deciduous trees, this protein did not exhibit a distinct seasonality in evergreen trees. Recent studies by Hummel *et al.* (12) demonstrated the presence of a 16-kD protein in the bark of nonacclimated and cold-acclimated deciduous species of *Hibiscus*. This protein, however, was not apparent in *H. rosa-sinensis*, an evergreen species.

The regulatory mechanism of the differential accumulation of 16- and 19-kD proteins in sibling deciduous and evergreen peach trees is not clear. Recently, Coleman *et al.* (4) documented a substantial accumulation of a 32-kD BSP in poplar (*Populus deltoides*) after SD exposure. LD exposure, however, resulted in either low or no accumulation of BSP. Low accumulation of 16- and 19-kD protein in the bark of evergreen trees in the present study, therefore, may be associated with the apparent insensitivity of evergreen trees to short photoperiods. This can be explained either in terms of a direct effect of short photoperiods on the regulation of protein synthesis or due to the lack of substrate in the bark of evergreen trees resulting from the failure to remobilize leaf nitrogen. In deciduous trees, however, nitrogen is remobilized from senescing leaves to bark, where proteins may be synthesized. Hence, the differences in the banding pattern of these proteins in deciduous and evergreen peach trees may be associated with the differential source/sink relationships in two genotypes during the yearly cycle. The accumulation of BSP is thought to be regulated through SD-induced growth cessation and the resulting altered source/sink relationship (4).

Xylem

Although physiological studies on bark tissue that exhibit equilibrium freezing are numerous, little information exists for xylem tissue that exhibits deep supercooling. This may be because the cellular characteristics that allow deep supercooling are thought to be imparted by physical properties of the cell wall. We showed a close parallel between seasonal fluctuations of three proteins (19, 70, and 80 kD) and cold hardiness of xylem of deciduous and evergreen peach (Fig. 5, A and B). This indicates that, similar to bark, cold acclimation of xylem tissue is a physiologically dynamic process and conforms to the observations of Wisniewski and Ashworth (28) that seasonal changes in the ultrastructure of xylem parenchyma cells are coincident with acclimation and deacclimation. An apparent 16-kD BSP also accumulated in the xylem of deciduous trees during winter (Fig. 5A). Recently, a 32-kD polar storage protein has also been detected and shown to accumulate in the xylem sap during winter (3).

In summary, despite the contrasting deciduous and evergreen growth habit, the latter also exhibiting an apparent lack of endodormancy, seasonal patterns of protein expression were quite similar in the two sibling genotypes. This was surprising given the marked differences in the degree of cold hardiness between the two genotypes. One major difference noted between the deciduous and evergreen trees was the presence and accumulation of a 16- and 19-kD bark protein, both of which conformed to the criteria of a BSP. The 16-kD protein was virtually absent in the evergreen genotype, and the 19-kD protein accumulated to a much lesser degree. Further research is in progress on these two novel genotypes to characterize proteins with greater resolution, document seasonal patterns in mRNA expression, and demonstrate immunolocalization of the 16- and 19-kD BSPs.

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