The experimental approach described in the present study must not be confused with pathogen inoculation studies. The rapid wound response of clone V68101 relative to HW233, for example, demonstrates that one clone rapidly reestablishes a preformed barrier responsible for disease resistance. Where the time the wound is open (infectible) is decreased, the probability for infection is decreased, and this is reflected in fewer cankers. This argument presumes that both a susceptible clone and a less susceptible clone, in the absence of wounds, would appear resistant. The literature does not contradict this view. Reestablishment of suberin continuity as measured by suberin autofluorescence intensity in impervious tissue and necrophylactic periderm, could be an effective measure of plant susceptibility to peach canker fungi. Studies are in progress to determine the relationship, if any, between xylem and bark responses. Xylem ray cell suberization, assessed with techniques described herein, may provide an accurate measure of compartmentalization, where gumming and discoloration are too variable to be useful.

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Method for Quantifying the Cyanogenic Glucoside, Prunasin in Peach Trees

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Additional index words. β-glucosidase, gas liquid chromatography, *Prunus persica*

Abstract. **A method was developed to determine the concentration of prunasin, a cyanogenic glucoside, in bark and twigs of peach [*Prunus persica* (L.) Batsch] trees. The procedure allowed handling of large numbers of samples with concurrent estimations of reducing sugars and ninhydrin positive materials. Prunasin was extracted with 80% ethanol, concentrated, reacted with β-glucosidase, and the resulting benzaldehyde was detected by gas liquid chromatography (GLC). The technique was rapid and did not require specialty glassware or derivitization of prunasin prior to GLC.**

The genus *Prunus* is characterized by the presence of 2 cyanogenic glucosides, prunasin and amygdalin. Recent studies confirm that prunasin, rather than amygdalin as previously reported (9), is the primary cyanogenic compound in *Prunus* (3). Amygdalin appears only in the seed (3), and is responsible for the human toxicity of such kernels.

In the presence of β-glucosidase, both prunasin and amygdalin break down to form initially mandelonitrile and ultimately benzaldehyde and cyanide. These metabolites are relatively toxic to plants and animals. Cyanide is a potent inhibitor of respiration and certain oxidation/reduction enzymes such as nitrate reductase (2, 8). The parent cyanogenic compounds apparently are compartmentalized away from degradative enzymes, and disruption of the cells initiates cyanogenesis (1). The release of these toxins after injury has led to speculation that they are involved in plant defense mechanisms against pathogens, insects, and herbivores (1). There is some indication that nematode feeding can release cyanide in peach roots (5). Since cyanide is toxic to plants as well as pests, it has been proposed as a factor in replant or short-life problems with *Prunus*, particularly peach (2, 7).

Currently available methods for measuring prunasin are either laborious or indirect (6). Also, other compounds of possible interest such as reducing sugars, amino acids, and phenols are lost in the isolation procedure.

A rapid, yet sensitive method of measuring levels of cyanogenic glucosides (in conjunction with other compounds from the same sample) in peach tissue would allow correlative studies between levels of these compounds and the health and decline of peach

Table 1. Reaction time of β-glucosidase digestion of prunasin.

Treatment[z]	Prunasin detected (mg ± SE)
Prunasin (−) β-glucosidase	0
Prunasin (+) β-glucosidase-boiled[y]	0
β-glucosidase (−) prunasin	0
β-glucosidase (+) prunasin	
0 time	0.52 ± 0.03
5 min	1.05 ± 0.04
10 min	1.08 ± 0.07
20 min	1.03 ± 0.02
30 min	0.99 ± 0.01
60 min	0.91 ± 0.08

[z]The reaction mixture contained 1 mg prunasin in 2.0 ml distilled H_2O and was initiated by the addition of 1 ml of 1 mg/ml β-glucosidase. The reaction was terminated by the addition of 2.0 ml of ethyl acetate and the mixture shaken virgorously.

[y]β-glucosidase (1 mg/ml) was placed in boiling water for 10 min prior to addition to this treatment.

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Table 2. Thin layer chromatography R_f values of benzaldehyde, prunasin, prunasin reaction products, peach stem tissue extract, peach stem tissue extraction products, and mandelonitrile.

Compound	R_f values - solvent system[z] 1	2	3
Benzaldehyde	0.65	0.61	0.31
Prunasin	0.53	0.50	0.00
Prunasin reacted with β-glucosidose	0.65	0.61	0.31
Stem tissue reacted with β-glucosidose	0.65	0.61	0.31
Stem tissue unreacted	0.53	0.50	0.00
Mandelonitrile	0.66	0.69	0.09

[z]Solvent systems used to develop TLC plates 1) 40 n-butanol: 40 acetic acid: 20 water (by volume), 2) water saturated n-butanol, 3) 80 hexane: 20 ether (v/v). Location of spots was first visualized by UV light then plates were sprayed with 1 95% ethanol: 1 conc sulfuric acid (v/v), and heated at 100° C for 10 min to aid in further visualization.

trees. This report describes a rapid and sensitive method of measuring prunasin levels in various tissues of peach trees which also allows for the concurrent extraction of free amino acids, reducing sugars, and phenolic compounds. Up to 120 samples can be taken and analyzed in as little as 3 days.

Five-year-old 'Elberta' peach trees on 'Nemaguard' rootstock were used as source material and all samples taken in November and December. Trunk samples were 1.3 cm diameter cylinders (bark, phloem, and 5mm of xylem), removed using an arch punch from the south side of the trees about 20 cm above groundline. Some samples were cut longitudinally into equal segments about 2 mm thick and prunasin extracted. Twig samples consisted of 1 cm sections of each twig taken 20 cm from the terminal tip with sections from 4 twigs pooled for each sample.

Trunk and twig samples were prepared similarly. Samples were weighed fresh and transferred to 24 × 150 mm culture tubes containing 10 ml of 80% ethanol, prepared using absolute ethanol. Samples were ground with a Polytron (setting #7 for 15 sec) and the probe washed with an additional 10 ml of 80% ethanol. Ethanol fractions were combined, heated at 80°C for 20 min to aid in prunasin extraction, filtered through Whatman #1 filter paper and concentrated under a stream on N_2 at 80° to a final volume of 4.0 ml. Prunasin (Sigma Chemical Co.), was used as an external standard and was treated in a like manner. A 2 ml fraction of the 4 ml concentrated sample was mixed with 1 ml of 1 mg/ml β-glucosidase (Sigma Chemical Co., Type II) and reacted at 23° for 30 min in a sealed vial. The remaining 2 ml were used for determination of ninhydrin positive compounds (4). Two milliliters of ethyl acetate then were added and the vial shaken vigorously to extract benzaldehyde from the aqueous fraction.

Prunasin was quantitated using gas liquid chromatography (GLC). Reacted prunasin standard was used as an external standard. A 6′ × 1/8″ stainless steel column containing 10% Alltech AT-1000 on 80/100 Chromasorb H-AW (Alltech Associates, Deerfield, IL 60015) was used for separation. Chromatography conditions were: oven 150°C; injector 225°; detector 250°; air 300 ml/min; hydrogen 30 ml/min and helium 20 ml/min, on a Varian 2800 series gas chromatograph with a flame ionization detector.

Thin layer chromatography (TLC) of prunasin, mandelonitrile, benzaldehyde (Sigma Chemical Co.), and reaction products was conducted on 20 × 20 cm, 0.2 mm thick silica gel plates on glass backing (Silica gel 60 F −254). TLC plates were developed in 1) water saturated n-butanol, 2) 40 n-butanol: 40 acetic acid: 20 water (by volume), 3) 80 hexane: 20 ether (v/v). Chromatograms were visualized with UV light and by spraying with 1 part conc. sulfuric acid: 1 part 95% ethanol (v/v), then heating at 100° for 10 min. Spectral scans from 350–220 nm of prunasin, mandelonitrile, benzaldehyde, and the prunasin reaction product after the reaction with β-glucosidase in ethyl acetate were conducted with a Beckman DU-8 spectrophotometer.

Prunasin concentration of various peach tree tissue could be calculated by quantitation of benzaldehyde released after β-glucosidase reaction with the tissue extract. The extraction efficiency of prunasin from bark tissue approached 100%. Therefore, attempts with a 2nd and 3rd extraction of the residue of the primary extraction did not yield detectable amounts of prunasin.

The reaction of prunasin with β-glucosidase was completed within 5 min (Table 1). No reaction product was detected when prunasin or β-glucosidase were omitted from the reaction mixture or if the enzyme was heat treated.

The reaction product detected by GLC was benzaldehyde, as confirmed by TLC using 3 solvent systems (Table 2). Prunasin standard and the tissue extract from peach twigs had the identical R_f values in all solvent systems. Upon reaction with β-glucosidase, prunasin and the tissue extract reaction products co-chromatographed with benzaldehyde in the 3 TLC systems. Mandelonitrile and benzaldehyde had equal retention times (4.98 min) under the GLC conditions employed. GLC detection was from 0.05 μg to 10 μg prunasin equivalents, covering the range found in peach tissue samples.

Comparison of spectral scans in ethyl acetate of known compounds (prunasin, benzaldehyde, and mandelonitrile) to reac-

Table 3. Absorption peaks for prunasin, benzaldehyde, mandelonitrile, and prunasin reaction products.

Compound[z]	Absorption (nm)		
Prunasin	268	261	256
Mandelonitrile	263	251	
Benzaldehyde	253	279	
Reaction product	253	279	

[z]Each compound was dissolved in ethyl acetate and scanned from 350 −220 nm.

Table 4. Location and concentration of prunasin in layers of trunk samples from the trunk of 5-year-old peach trees.

Layer[z]	Prunasin[y] (mg/cm² trunk ± SE)	Percent total
Bark	0.323 ± 0.101	19.8
Outer phloem	0.558 ± 0.050	34.2
Inner phloem	0.752 ± 0.088	46.1
Xylem	ND	0.0

[z]Sample cut into 4 equal parts, each layer about 2 mm thick.
[y]Mean of 3 samples from the same tree.

Table 5. Prunasin content of twig and trunk samples from 'Elberta' peach trees on 'Nemaguard' rootstocks.

Sample type	No. samples/tree	Mg prunasin/g fresh weight ± SD
	Twigs[z]	
Elberta/Nemaguard (single trees)	3	9.04 ± 0.57
	3	7.41 ± 1.58
	3	8.16 ± 1.13
Elberta/Nemaguard (16 trees)	1	8.98 ± 2.03
	Trunk[y]	
Elberta/Nemaguard (single trees)	3	2.98 ± 0.16
	3	2.06 ± 0.05
	3	2.02 ± 0.17
Elberta/Nemaguard (15 trees)	1	3.22 ± 0.74

[z]Pooled samples using single segments from each of 4 twigs per tree.
[y]Consisted of 1.3 cm diameter cylinders from the bark down to the xylem, which was discarded.

tion products (Table 3) indicated that benzaldehyde was the prunasin reaction product in the procedure.

Prunasin from trunk samples was confined to the area between the xylem and the bark surface (Table 4). The 2 phloem sections contained greater than 80% of the total prunasin detected. The remainder was in the bark section with none detected in the outer xylem section (Table 4).

Twig samples of the 5-year-old 'Elberta' peach trees had a higher concentration of prunasin than did trunk samples (Table 5). Variation between twig samples from the same tree was smaller than the variation between samples from different trees. Variation in trunk samples from the same tree also was small and in the same range as for twig samples. The variation of prunasin content of trunk samples from different trees was no different than that detected for twig samples (Table 5).

This procedure is a rapid and accurate means of monitoring prunasin levels in peach trees. Concentration of this potentially phytotoxic compound can be monitored during critical periods of tree growth and development in order to evaluate the role of prunasin in peach tree short life and other *Prunus* abnormalities.

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Demonstration of the Penetration of Boron in Apple Fruit Using an Enriched Stable Isotope

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Additional index words. *Malus pumila* 'Golden Delicious', spark-source mass spectrometry, boric acid

Abstract. **The uptake and distribution of boron ([^{10}B] boric acid) applied directly to apple fruit (*Malus pumila* L. 'Golden Delicious') was studied by stable isotope ratio measurements, using spark-source mass-spectrometry. Uptake of exogenous boron appeared limited, but the absorbed boron was relatively well distributed in tissues of apple fruit.**

Boron (B) is involved in the quality of apple fruit (5, 7, 8), its effects often interacting with those of Ca (7). To prevent deficiencies, periodic applications of borax to the soil are the standard commercial practice. An alternative is to apply boron to the leaves in soluble form or directly to the fruit because the mobility of boron from leaves to fruit is questionable (1). Physiological studies are still necessary to determine the behavior of B applied to leaves or fruit; however, the lack of a suitable radioactive B isotope has hindered such research. The possibilities of a highly sensitive technique, the spark-source mass spectrometry, to discriminate between the 2 stable isotopes ^{10}B and ^{11}B, were recently demonstrated (2).

Using the same technique, we studied the penetration of B into 'Golden Delicious' apple fruit using boric acid enriched with ^{10}B. Experiments were carried out with carefully selected uniform mature fruit (65–70 mm diameter) harvested at the end of September at the Plant Pathology Station (INRA) in Angers. Polyvinyl wells were fixed to half apples with RTV III adhesive (Rhône-Poulenc). One ml of solution of 20 mM boric acid, enriched with ^{10}B (90.5 ± 0.2 atom %) at

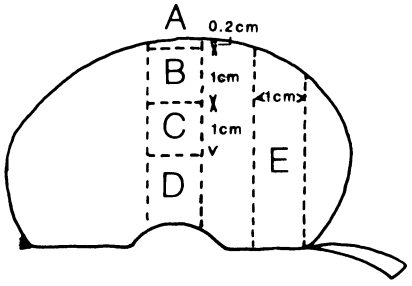

Fig. 1. Analyzed parts in each half fruit. Solution was applied on the surface at A.

Table 1. Boron distribution after 7 days at 12°C following application of [^{10}B] boric acid to 'Golden Delicious' apples. Each value of the isotopic ratio is the mean of 8 analytical measurements.

Analyzed Section[z]	^{11}B/^{10}B Abundance ratios in 4 separate fruit (ppm total B, dry wt basis, in brackets)				Mean value
	1	2	3	4	
A	1.7 (66.3)	1.7 (66.2)	1.6 (68.2)	1.6 (68.1)	1.65**[y]
B	1.8 (64.7)	1.8 (64.7)	1.8 (64.7)	1.9 (63.3)	1.83**
C	4.0 (50.3)	3.3 (52.7)	4.0 (50.3)	4.0 (50.3)	3.83
D	4.1[x]	3.7 (51.2)	3.8 (50.9)	4.1[x]	3.93
E	3.4 (52.3)	3.3 (52.7)	3.6 (51.6)	---	3.43*

[z]See Figure 1.

[y] *,**Value significantly different from naturally occurring ^{11}B/^{10}B ratio (4.1) by Student *t* test at 5%, 1% level, respectively.

[x]ppm values not reliable.

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