Cyanogenic Glycosides and Graft Incompatibility between Peach and Plum[1]

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Abstract. Peach (*Prunus persica* Batsch. 'Lovell') seedlings and 'Marianna 2624' plum (*P. cerasifera* Ehrh. X *P. munsoniana* Wight & Hedr.?) cuttings were budded with 'Fay Elberta' peach and 'Marianna 2624'. Of the 4 combinations only 'Fay Elberta'/'Marianna 2624' showed foliar symptoms indicative of graft incompatibility; those trees budded in mid-March appeared abnormal by early August. Prunasin, the only cyanogenic glycoside detected in both species, accumulated in young scion bark of 'Marianna 2624' and peach to nearly equal levels. Amounts of prunasin in leaves and bark of 'Fay Elberta' on peach were usually greater than in those on plum. The level in the scion bark of 'Marianna 2624' was similar on both rootstocks. In late summer, the quantity of the glucoside in peach scion bark rose above that in corresponding plum bark; however, the prunasin concentration in both leaves and scion bark of 'Fay Elberta'/ 'Marianna 2624' trees was not correlated with the severity of incompatibility symptoms. The prunasin level in the plum rootstock bark immediately below or 18 cm from the union was unaffected by the scion species or by signs of ill-health in the peach top. Although the rootstock was shown capable of affecting the accumulation of prunasin in scion tissues, the stability of the level of this glucoside in the peach/plum combination suggests that cyanogenesis is not closely linked with their incompatibility.

A wide variety of plants, including many members of the *Rosaceae,* are capable of releasing HCN (5). This poisonous gas is apparently liberated from 1 of several cyanogenic glycosides, which upon breakdown also yield a sugar and a carbonyl compound. Cyanogenic glycosides have been implicated in water-logging (17), bud dormancy (10), and graft incompatibility (7) of fruit trees. Gur et al. (7) concluded that toxic substances liberated by decomposition of the cyanogenic glycoside, prunasin (D-mandelonitrile-β-D-glucoside), present in quince but absent in pear, inactivated the cambium and caused girdling at the union of incompatible pear/quince combinations. Since a) peach as a scion grafted on 'Marianna' plum usually dies within the first growing season, whereas the reciprocal combination is successful (11), and b) peach reportedly contains a higher level of cyanogenic glycoside than plum (9, 13), an argument similar to that offered to explain pear/quince incompatibility has been applied to the failure of peach/plum combinations.

We assessed the possible role of cyanogenic glycosides in peach/plum incompatibility by determining their content in trees of peach/'Marianna 2624' and its reciprocal and in intraspecific graft combinations.

Materials and Methods

Experiment 1. Seeds of 'Lovell' peach and hardwood cuttings of 'Marianna 2624' were lined out in adjacent rows in a nursery during winter, 1969-70. In early June, 1971 alternate trees were "June" budded with 'Fay Elberta' peach and 'Marianna 2624' plum using budwood from trees indexed to be free of virus diseases. A single bud was put in at about 40 cm above the soil, a week later trees were cut back to 13 cm above the bud, and after an additional 10 days they were headed back to the bud. Trees were irrigated and managed in accordance with usual nursery procedure.

Three times during the growing season 4 trees of each scion/rootstock combination were selected at random, cut off at soil surface, and brought to the laboratory where they were prepared for analysis. Bark and the corresponding wood samples were obtained from 3 regions along the main stem: a) from the 15-cm length of scion immediately above the bud-union (scion); b) from the 5-cm segment just below the union (upper rootstock); and c) from a 5-cm segment starting 18 cm below the union (lower rootstock). Bark samples were immediately placed upon dry ice, freeze-dried, and stored in a desiccator at -15°C. Wood segments were split lengthwise and placed in a vacuum oven at 80°C. All samples were ground to pass a 40-mesh screen.

Cyanogenic glycoside analysis. A 10-20 mg sample of bark was placed in the outer well of a 7-cm Conway microdiffusion dish while 2 ml of 0.1 N KOH were added to the center well. Three ml of water and 2 mg of β-glucosidase in 2 ml of 0.1 M citrate buffer (pH 4.6) were added to the sample and the cover of the dish secured. After gently shaking, the dish remained overnight at room temperature to allow HCN to diffuse into the KOH. To assure complete hydrolysis of the glycoside, 1 ml of 20% H_2SO_4 was then added and the mixture allowed to stand an additional 4 hr. An aliquot of the solution in the center well was removed and brought to 1 ml with KOH and diluted with 2 ml of water. One min after rapidly adding 1 ml of phenolphthalin reagent (16) the absorbance at 550 nm was determined. A standard curve for CN^- was obtained with KCN.

Since the low level of cyanogenic material in wood necessitated samples too large for the Conway dish, the method was modified. One-half to 1 g of wood sample was boiled in 70 ml of 80% ethanol for 30 min and the filtered extract evaporated to dryness under reduced pressure at 45°C. The residue was taken up with water and quantitatively transferred to a Conway dish and analyzed as described above. In a preliminary trial the amount of HCN liberated directly from a wood sample was found to be equivalent to that recovered from an ethanolic extract.

Identification of cyanogenic glycosides. Aliquots of 80% ethanolic extracts from peach and plum bark were streaked on Whatman 3 MM paper and co-chromatographed with authentic amygdalin (D-mandelonitrile-β-D-gentiobioside) and prunasin. Prunasin was isolated from peach leaves according to the method of Trim (19). After the paper was developed ascendingly with n-butanol-pyridine-water (6:4:3, v/v/v) (1) and dried, it was sprayed with β-glucosidase (0.1 mg/ml). A sheet of paper treated with picrate reagent was separated from the chromatogram with plastic window screen and the 2 papers were then sanwiched between glass sheets (3). Within a few hr sufficient HCN was released from the cyanogenic glycosides on the chromatogram to produce red-orange spots on the picrate-treated paper. R_f values for amygdalin and prunasin were 0.54 and 0.78, respectively.

Experiment 2. 'Lovell' and 'Marianna 2624' rootstocks were grown as in the previous experiment and 1-year-old trees were

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spring-budded in mid-March 1972, and headed back a week later. Dormant buds from indexed trees of 'Fay Elberta' and 'Marianna 2624' were T-budded into alternate trees along the nursery row. Four budded trees of each combination were harvested on 8 occasions during the growing season and once the following spring (1973). Bark was removed from an 8-cm segment of the scion immediately above the bud-union (lower scion bark) as well as from upper and lower rootstock segments as described before. When the scion attained adequate girth, bark was also collected from a 10-cm segment starting 50 cm above the union (upper scion bark). Samples were dried in a vacuum oven and ground.

This experiment was carried out in conjunction with a detailed study of carbohydrate levels, and extraction and analytical procedures will be more extensively described elsewhere. Briefly, a composite bark sample from 4 replicate trees on a single sampling date was extracted in a micro-Soxhlet with 80% ethanol. An aliquot of the ethanolic extract was dried, dissolved in pyridine, and the trimethylsilyl derivative of prunasin formed and quantitized by gas chromatography (14).

As a test of this method, and in order to assess the variability within the samples, bark from individual trees was also occasionally assayed for prunasin by the microdiffusion procedure described above. The correspondence of values obtained by the different procedures was excellent.

Results and Discussion

When peach is spring-budded into an incompatible plum rootstock, the take is usually high and the scions grow vigorously for several months; but in mid-summer symptoms of ill-health suddenly appear. The March-budded 'Fay Elberta'/'Marianna 2624' trees started to show foliar symptoms of incompatibility at the beginning of August; at this time the peach scions on both rootstocks had grown to a mean length of 140 cm. Within 10 days many of the leaves on a majority of the peach/plum trees were folded upward along the midrib and much of the foliage was pale green to yellow and frequently slightly bronzed in appearance. Leaves soon withered and some defoliation occurred. The number of afflicted trees and severity of symptoms increased as the season progressed. Peach leaves from scions on 'Lovell' seedlings, however, remained healthy and green into October, while 'Marianna 2624' leaves did not senesce until November. A few peach/plum trees started to leaf out the following spring, but soon died, whereas all of the trees of the other combinations survived.

'Fay Elberta'/'Marianna 2624' combinations budded in June (Expt. 1) developed only slight incompatibility symptoms by the end of the season. Similarly, Booth (2) reported that 'Marianna' trees budded with peach during the summer did not die until the second season. Possibly symptom development is in part dependent upon the relative sizes of the scion and rootstock. If, as McClintock (11) concluded, a lack of phloem continuity across the union effects an exhaustion of food reserves in the plum rootstock which in turn restricts root growth and water absorption, severe water stress and wilting likely would develop more rapidly in larger peach scions.

Hydrocyanic acid was recovered from bark and wood samples of 'Marianna 2624', 'Fay Elberta', and 'Lovell' seedlings upon treatment with β-glucosidase (Table 1). Paper chromatograms of ethanolic extracts of 'Fay Elberta' and 'Marianna 2624' bark showed only a single area which generated HCN and this had the same R_f as authentic prunasin. Gas chromatography of the extracts of bark from different stock/scion partners revealed a component with a retention time identical to that of prunasin, whereas no peak corresponding to amygdalin was observed. Since prunasin was the only cyanogenic glycoside detected in shoots of young 'Lovell' seedlings (1), it appears to be the predominant cyanogenic substance in stems of both peach and 'Marianna 2624' plum; amygdalin may be restricted solely to generative parts (14).

Table 1. Prunasin, recovered as cyanide, in bark and wood of peach and 'Marianna 2624' plum adjacent to the bud union on 3 dates.

Scion	Rootstock	µmole prunasin/g dry wt Sampling date July 21	Aug. 17	Sep. 16
		Scion bark		
Marianna 2624	Marianna 2624	143.6a[z]	96.3a	81.2ab
	Lovell	144.5a	112.2ab	62.2a
Fay Elberta	Marianna 2624	145.1a	131.4b	79.3ab
	Lovell	155.2a	144.2b	106.7b
		Scion wood		
Marianna 2624	Marianna 2624	6.5a	9.6a	0.3a
	Lovell	14.4ab	17.5a	0.6a
Fay Elberta	Marianna 2624	8.8a	12.6a	1.0a
	Lovell	21.2b	21.9a	2.6b
		Rootstock bark		
Marianna 2624	Marianna 2624	23.9a	21.8a	18.5a
Fay Elberta		23.7a	17.1a	17.4a
Marianna 2624	Lovell	79.6b	47.2b	59.1b
Fay Elberta		77.1b	47.2b	52.8b

[z]Means within a bark or wood sample and column not sharing the same letter are significantly different at the 0.05 level according to Duncan's multiple range test.

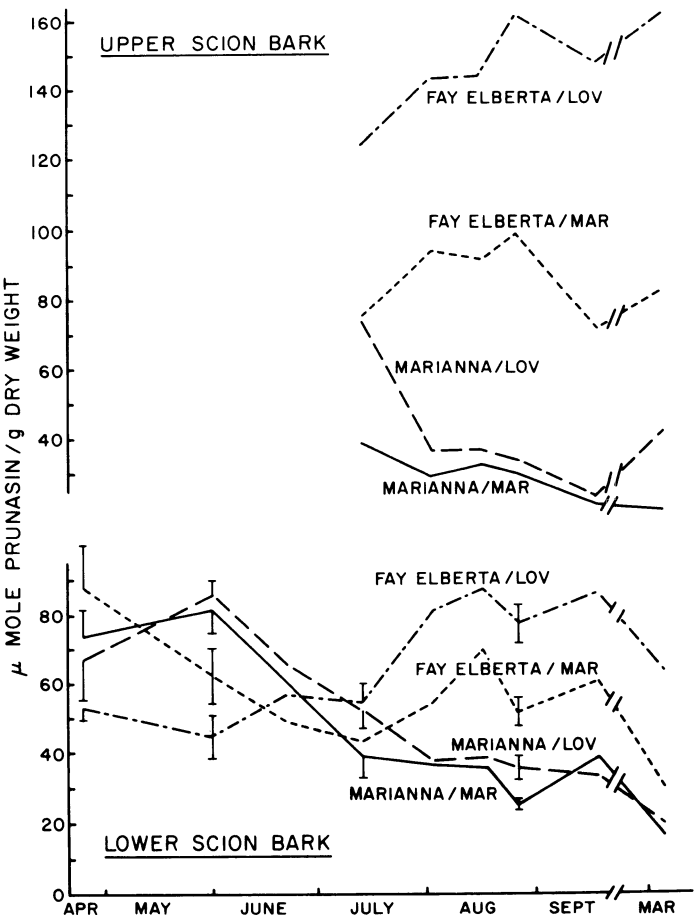

Fig. 1. Prunasin content of upper and lower scion bark segments from 'Fay Elberta' peach and 'Marianna 2624' plum on rootstocks of 'Lovell' peach seedlings and 'Marianna 2624'. Vertical bars indicate the standard error of the mean.

The amount of cyanogenic glycoside in young scion bark of 'Marianna 2624' plum may equal or exceed that found in young peach bark (Table 1, Fig. 1). On the other hand, more mature bark of 'Lovell' peach seedlings may contain much greater amounts of prunasin than plum bark of similar age (Fig. 2). The high concn of prunasin in young plum bark contradicts the results of Heuser (9) who, by an unreported method, was unable to detect cyanogenic substances in shoots of 'Marianna 2624'.

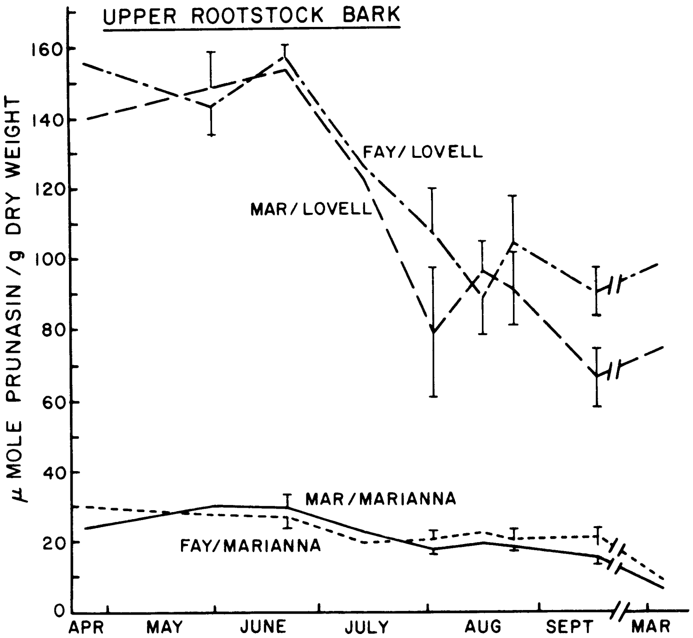

Fig. 2. Prunasin content of the upper rootstock bark from 'Marianna 2624' plum and 'Lovell' peach seedlings with scions of 'Fay Elberta' peach and 'Marianna 2624'. Vertical bars indicate the standard error of the mean.

The amount of HCN liberated from peach or 'Marianna 2624' bark was many times greater than that from corresponding wood samples. Smallest amounts were found in wood below the bud union; the seasonal maximum levels were 0.3 and 1.6 µmole HCN/g dry wt for 'Marianna 2624' and 'Lovell', respectively. Values similar to these were obtained from wood of the 2 scion cultivars collected in September, which earlier contained much higher amounts of cyanogenic substances (Table 1).

Although tissue degeneration occurs at the junction of incompatible peach/plum combinations, xylem continuity is maintained across this mechanically strong union (11). Because of this and the low amount of HCN released from wood, the prunasin level in wood was considered to be unimportant in peach/plum incompatibility and, therefore, it was not investigated further.

Leaves of both 'Marianna 2624' and 'Fay Elberta' from scions on peach rootstock contained greater amounts of prunasin than when on plum (Fig. 3). On a given rootstock the scion leaves of plum were a richer source of prunasin than peach. In all graft combinations the content of cyanogenic substances was highest in the spring, decreasing to a nearly constant value in June or July. A decrease in cyanide from leaves of peach during the growing season has been reported (10). Throughout our study, leaves from the peach/plum combination contained the least prunasin and its level was unaffected by the development of foliar symptoms of incompatibility.

Prunasin in the leaves of the various combinations did not correspond closely with the levels in the scion bark (Figs. 1 and 3). The leaves of 'Marianna 2624'/'Lovell' always had the highest amount of prunasin of any combination, although during August and September the level in the plum scion bark was much less than that in peach scions. The quantity of cyanogenic glycoside in leaves, therefore, was not a reliable

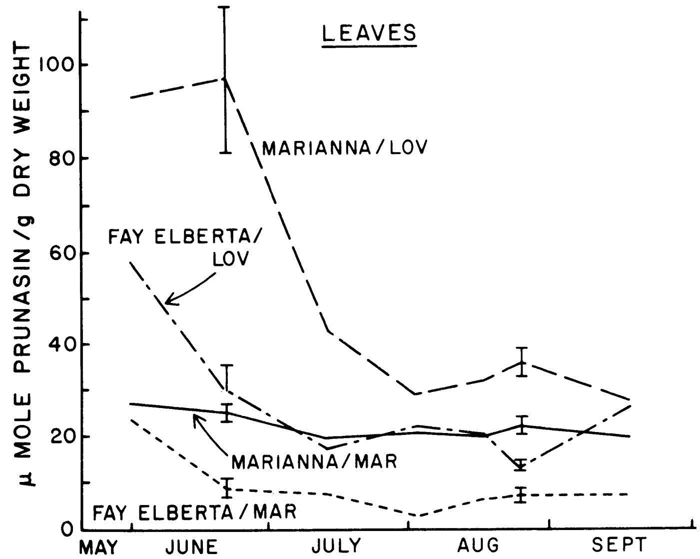

Fig. 3. Prunasin content of leaves from 'Fay Elberta' peach and 'Marianna 2624' plum scions on rootstocks of 'Lovell' peach seedlings and 'Marianna 2624'. Vertical bars indicate the standard error of the mean.

indication of the amount in other parts of the tree.

In June-budded trees the level of cyanogenic substances in scion bark of either 'Marianna 2624' or 'Fay Elberta' was highest in July and subsequently declined (Table 1). When rootstocks were budded in mid-March (Expt. 2) the prunasin content in the lower scion bark of plum attained a maximum in late May and fell to a relatively stable level by early August. Some weeks earlier the amount of prunasin in the corresponding bark segments from 'Fay Elberta' scions rose above that found in plum. The concentration in 'Marianna 2624' scion bark was independent of the rootstock, however, the amount of prunasin in 'Fay Elberta' bark was consistently higher, after the end of May, in scions on peach rather than those on the incompatible plum rootstock. The difference was especially exaggerated in bark segments 50 cm above the union (upper scions bark). Apparently some condition during June allowed the bark of peach scions on 'Lovell' seedlings to accumulate a higher level of prunasin. However, the difference between the amounts of this glucoside in the 2 groups of peach scions did not increase markedly as the season progressed even though the peach/plum trees began to reveal symptoms of incompatability. Parallel fluctuations in the level of prunasin suggest that its metabolism in the bark of 'Fay Elberta' was influenced by similar factors on both rootstocks.

A carbohydrate deficiency may encourage increased decomposition of cyanogenic glycosides (7). The lower level of prunasin, however, in the scion bark of 'Fay Elberta' on plum rather than on peach cannot be ascribed to paucity of carbohydrates since their level greatly increases in the scion of an incompatible peach/plum conbination (18, unpublished results).

The quantity of cyanogenic substances in plants is greater under conditions of high N (12), presumably because of increased availability of amino acids which are precursors of the aglycones (5). If severe enough, tree girdling, whether by knife or incompatibility, interferes with normal uptake and transport of nitrogenous substances and limits the quantity reaching the shoot (6, 15). The lower content of prunasin in the scion bark of 'Fay Elberta' when on the plum rootstock may have resulted from a reduction in amino acids in this tissue because of impeded transport across the graft union. Cerletti et al. (4) reported that the content of ninhydrin-positive substances in peach scions was low when grafted to myrobalan plum *(P. cerasifera)* which is also incompatible with peach. If the lower prunasin concn in peach scions on 'Marianna 2624' was caused by a reduction in precursors, this diminished supply apparently preceded the paling of the foliage by at least a month.

In both the upper and lower bark segments from peach rootstock grafted in March the maximum prunasin level occurred in June and then sharply declined. The concn of the glucoside in both these segments from the plum rootstock was similar and constant over the entire season. Since the prunasin contents in both bark segments of a particular rootstock were nearly equal, only the data from the upper segment are presented (Fig. 2). The level of prunasin in 'Marianna 2624' rootstock bark was generally between 15 and 25 μmole/g dry wt in both experiments. However, its concn in bark from June-budded 'Lovell' seedlings was less than that in corresponding tissue from those budded in March. This difference may have been a seasonal one since the studies were not carried out in the same year. On the other hand, budding and heading back 'Lovell' seedlings in March may have temporarily halted both bark development and the corresponding decline in prunasin.

Clearly, 'Fay Elberta' or 'Marianna 2624' scions failed to markedly affect the level of prunasin in the bark of either the peach or plum rootstock (Table 1, Fig. 2). Thus, even in the incompatible peach/plum combination the content of prunasin in the rootstock bark at the union or some distance below was similar to that if the scion was plum. This constancy of prunasin in the 'Marianna 2624' rootstock even when foliar symptoms of incompatibility developed and the level of carbohydrates in both graft components was altered (18), indicates that this glucoside is not sensitive to the metabolic changes which accompany this disorder. Prunasin may have a more or less quiescent role in the metabolism of mature plum bark and may also be resistant to hydrolysis even under adverse conditions. In an anaerobic atmosphere, the rate of cyanogenesis increase with temperature by detached seedling roots was much less with myrobalan plum than with peach (17).

Phloem degeneration in incompatible peach/plum trees is reportedly more extensive immediately below than above the union (8); this, and the compatibility of the reciprocal graft combination, suggests the involvement of a hypothetical toxic component from the peach scion. The failure of the scions in our study to influence the level of prunasin in the rootstock conflicts with the hypothesis that a cyanogenic glucoside is the causal agent which induces incompatibility between peach and plum. That the prunasin concn in the peach scion and plum rootstock remained relatively stable even as incompatibility symptoms increased in severity and the amounts of other metabolites changed (18), suggests that prunasin metabolism may be insulated from the primary metabolic systems. Maintenance of prunasin in incompatible trees showing poor health demonstrated that general cyanogenesis does not occur during symptom development. Furthermore, the results show that prunasin in tissues of 'Marianna 2624' may reach high levels, even exceeding that in peach, and shoots of this plum as well as other *Prunus* species, and even acyanogenic plants, are capable of metabolizing HCN into amino acids (5, 20, and unpublished results). Thus 'Marianna 2624' may not be abnormally sensitive to this glucoside or its decomposition products.

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