the first, angling down to connect with it. The chip of rootstock is removed and discarded.

Identical cuts are made on the scion to remove the desired bud. The bud should be centered on the chip. The bud chip is placed in the rootstock, resting on the lip of the prior cuts. Cambiums match easily if the wood is of similar caliper. The chip bud is wrapped with the chip bud tape, covering the entire bud, tying above the bud. The buds knit in 30 to 35 days. When a small ring of callus is noticeable around the edge of the chip bud, the tape is removed. The budded rootstock is allowed to go dormant in fall.

In February, the upper rootstock is pruned back just above the chip bud. The cut should angle away from the chip bud to prevent the spring sap flow from "drowning" the bud. As the days warm, the bud breaks, producing a single stem whip. When the shoot from the bud is about one foot tall, it is staked. The first season, the main emphasis is on producing height in the maple. The second season the main emphasis is on building caliper and branching.

By utilizing these three methods of Japanese maple propagation, we can spread the work load for the grafting crew, and produce "Distinctively Better" Japanese maples in containers.

## GRAFT INCOMPATIBILITY: EFFECT OF CYANOGENIC GLYCOSIDES ON ALMOND AND PLUM CALLUS GROWTH

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**Abstract.** The effects of the cyanogenic glycosides, amygdalin and prunasin, and their breakdown products, cyanide and benzaldehyde, on callus from 'Marianna 2624' plum (*Prunus cerasifera* Ehrh. × *P. munsoniana* Wight & Hedr.), and on that from two almond cultivars (*P. dulcis* Mill. 'Nonpareil' and 'Texas') were compared. Prunasin inhibited the growth of 'Marianna 2624' plum and 'Nonpareil' almond callus but not 'Texas' almond. Amygdalin inhibited 'Marianna 2624' plum callus growth but promoted growth of both almond cultivars. All 3 cultivars were inhibited to the same extent by sodium cyanide; however, benzaldehyde was strongly inhibitory to 'Marianna 2624' plum callus at 0.05 mM, but a concentration of 5 mM was required to similarily inhibit growth of either almond callus. The greater sensitivity of 'Marianna 2624' plum callus to the cyanogenic glycosides and benzaldehyde suggests that benzaldehyde is an important factor in the almond/plum incompatibility.

Tissue compatibility or incompatibility in plants can be regarded as a physiological tolerance or intolerance, respectively, between the protoplasts of different cells (7). Although substantial research on stock/scion incompatibility has accumulated (4,8), little

attention has been directed at mutual physiological influences underlying vegetative graft incompatibility.

Cyanogenic glycosides have been implicated as causal agents in graft incompatibility. Gur, *et al.* (3) concluded that the anatomical disturbance at the union of incompatible pear/quince graft combinations was caused by seasonal inactivation of the cambium, due to toxic substances liberated by hydrolysis of prunasin near the union. Similarly, Gur and Blum (2) suggested that the accumulation of toxic hydrocyanic acid, which was liberated by hydrolysis of prunasin, causes the death of tissues at the peach/almond graft union in incompatible combinations. Breen (1), however, reported that cyanogenesis was not closely linked with the incompatibility between peach and plum because the prunasin concentration in the peach scion and plum rootstock remained relatively stable even as incompatibility symptoms increased in severity.

In this research, I examine indirectly the possible involvement of cyanogenic glycosides and their catabolites in the almond/'Marianna 2624' plum incompatibility by determining their effects on growth of callus cultures derived from almond and plum.

## MATERIALS AND METHODS

**Callus culture.** Callus cultures were established from nodal explants taken from sections of current season's growth of greenhouse-grown 'Nonpareil' and 'Texas' almonds, and 'Marianna 2624' plum. Cultures were initiated and maintained on Murashige and Skoog salts (9) and the following, in mg/liter: myo-inositol, 100; nicotinic acid, 0.5; pyridoxine HCl, 0.5; thiamine HCl, 0.1; 2,4-D, 1.0; kinetin, 1.0; casein hydrolysate, 200; sucrose, 30,000; and Difco Bacto-agar, 7,000. The pH was adjusted to 5.6 ± 0.1. Erlenmeyer flasks (125 ml) were used as stock culture vessels; each flask contained 50 ml culture medium. Stock cultures were maintained at 26°C under 6 $\mu$mol $sec^{-1}m^{-2}$ (cool white fluorescent lamps, F48T12·CW·HO) for 24 hr daily.

Callus assays were carried out in 120 ml wide mouth, French-square bottles fitted with plastic caps without liners. After sterilization, 10 ml of culture medium was added to each sterile bottle. One piece of callus of approximately 15 mg was transferred to each bottle using sterile technique. The bottles were kept in a lighted incubator as above for 30 days. Ten replicates were used for each treatment. Calli were weighed at the end of the period.

**Amygdalin and prunasin experiment.** Individual cyanogenic glycosides were added at 1 and 2 mM. The pH of the medium was adjusted to 5.6 ± 0.1 after addition of the cyanogenic glycoside and prior to filter sterilization.

**Sodium cyanide experiment.** Sodium cyanide (NaCN) was added at 0.1, 0.5, 1 and 2 mM before pH adjustment and filter sterilization.

**Benzaldehyde experiment.** Benzaldehyde was added at 0.01, 0.05, 0.1, 0.5, 1.0 and 5.0 mM after pH adjustment and filter sterilization.

**Statistical analyses.** Effects of amygdalin and prunasin were evaluated using orthogonal comparisons. Effects of NaCN and benzaldehyde were evaluated using Scheffe's test (5% level).

## RESULTS

**Amygdalin and prunasin experiment.** Amygdalin promoted callus growth of 'Texas' and 'Nonpareil' but inhibited growth of 'Marianna 2624' (Table 1). The difference between the control and the mean of the amygdalin treatments was significantly different from 'Texas' ($P = 0.05$, $F = 6.83$) but not 'Nonpareil' using orthogonal comparisons between control and amygdalin treatments (1 and 2 mM). Both almond cultivars showed increased fresh weight with 2 mM amygdalin. There was a significant effect with the 2 amygdalin concentrations for 'Nonpareil' ($P = 0.05$, $F = 6.7$) but not 'Texas'. In contrast, the growth of 'Marianna 2624' was significantly reduced by the amygdalin treatments. The difference between the control and the mean of the amygdalin treatments was significantly different ($p = 0.01$, $F = 239.5$), and there was a significant effect for the 2 amygdalin concentration ($P = 0.05$, $F = 5.49$).

**Table 1.** Influence of amygdalin and prunasin concentration on fresh weight of callus cultures from 'Marianna 2624' plum, and 'Texas' and 'Nonpareil' almonds.

| Callus culture | Mean fresh weight (g/culture) | | |
|---|---|---|---|
| | Amygdalin conc. (mM) | | |
| | Control | 1 | 2 |
| 'Nonpareil' | 2948.3 | 2706.1 | 3807.2 |
| 'Texas' | 636.4 | 1083.5 | 1035.5 |
| 'Marianna 2624' | 754.7 | 158.2 | 31.7 |
| | Prunasin conc. (mM) | | |
| | Control | 1 | 2 |
| 'Nonpareil' | 2165.3 | 1751.0 | 1786.1 |
| 'Texas' | 669.6 | 680.1 | 673.3 |
| 'Marianna 2624' | 956.5 | 20.5 | 16.9 |

Prunasin inhibited callus growth of 'Nonpareil' and 'Marianna 2624' but did not affect 'Texas' callus growth (Table 1). Prunasin at both 1 and 2 mM severely and equally inhibited the growth of 'Marianna 2624' callus. The difference between the control and the mean of the prunasin treatments with 'Marianna 2624' was significantly different ($P = 0.01$, $F = 88.71$) using orthogonal comparisons. 'Marianna 2624' did not show a significant effect for the 2 prunasin concentrations and prunasin was more inhibitory than amygdalin. Callus from 'Nonpareil' was significantly inhibited ($P = 0.01$, $F = 22.7$) at the higher concentration (2 mM) but not lower

prunasin level, and callus growth of the control was significantly different (P = 0.01, F = 11.18) from the mean of the 2 prunasin concentrations using orthogonal comparisons. 'Texas' callus growth was unaffected by the presence of prunasin. There was no difference between the control and the mean of the 2 prunasin concentrations, nor between the two prunasin concentrations.

**Sodium cyanide experiment.** All 3 cultivars were inhibited to the same extent by 2 mM NaCN, although cultivar differences were observed over the range of concentrations tested (Table 2). Mean separations using Scheffes test (5% level) showed the following in the NaCN study. With 'Nonpareil' treatments up to 1 mM were not different. A break occurred above 1 mM, and 2 mM NaCN was different from the lower concentrations. Callus fresh weight means for 'Texas' not significantly different from each other include: 0 and 0.1 mM; 0.1, 0.5 and 1.0 mM; and 0.5, 1.0 and 2.0 mM. With 'Marianna 2624' 1 and 2 mM NaCN were different from all lower concentrations and from each other.

**Table 2.** Influence of sodium cyanide concentration on fresh weight of callus cultures from 'Marianna 2624' plum, and 'Texas' and 'Nonpareil' almonds.

| Sodium cyanide conc (mM) | Mean fresh weight (g/culture) | | |
|---|---|---|---|
| | 'Nonpareil' | 'Texas' | 'Marianna 2624' |
| Control | 3957.5a[1] | 1304.0a | 1100.5a |
| 0.1 | 3879.0a | 1046.6ab | 952.4a |
| 0.5 | 3483.7a | 836.0bc | 906.2a |
| 1.0 | 3566.9a | 712.9bc | 647.3b |
| 2.0 | 1386.2b | 496.6c | 360.7c |

[1]Means followed by the same letter or letters are not significantly different.

**Benzaldehyde experiment.** The response of the almond cultivars to benzaldehyde was distinctly different from that of 'Marianna 2624' (Table 3). Benzaldehyde at 0.05 mM inhibited growth of the 'Marianna 2624' callus, but a hundred fold greater concentration was required to elicit a similar level of inhibition with the almond cultivars. Mean separations using Scheffe's test (5% level) showed the following in the benzaldehyde study: 'Texas' callus fresh weight means at 0.5 and 1.0 mM benzaldehyde were significantly different from all lower concentrations and the highest level, 5 mM, was significantly different from all other concentrations. Callus fresh weight means for 'Nonpareil' not significantly different from each other include: 0, 0.01, 0.05 and 0.1 mM; 0.05, 0.1 and 0.5 mM; and 0.5 and 1.0 mM. Benzaldehyde at 5 mM was different from all lower concentrations with 'Nonpareil'. With 'Marianna 2624' fresh weight means at control and 0.01 mM were different from all higher concentrations which were not different from each other.

**Table 3.** Influence of benzaldehyde concentration on fresh weight of callus cultures from 'Marianna 2624' plum, and 'Texas' and 'Nonpareil' almonds.

| Benzaldehyde conc (mM) | Mean fresh weight (g/culture) | | |
|---|---|---|---|
| | 'Nonpareil' | 'Texas' | 'Marianna 2624' |
| Control | 3236.0a[1] | 1072.4a | 831.2a |
| 0.01 | 3179.8a | 1295.8a | 901.5a |
| 0.05 | 2706.8ab | 1121.6a | 35.4b |
| 0.1 | 2525.3ab | 1113.3a | 26.9b |
| 0.5 | 2225.1bc | 719.1b | 17.1b |
| 1.0 | 2036.3c | 587.6b | 14.8b |
| 5.0 | 32.2d | 26.5c | 14.4b |

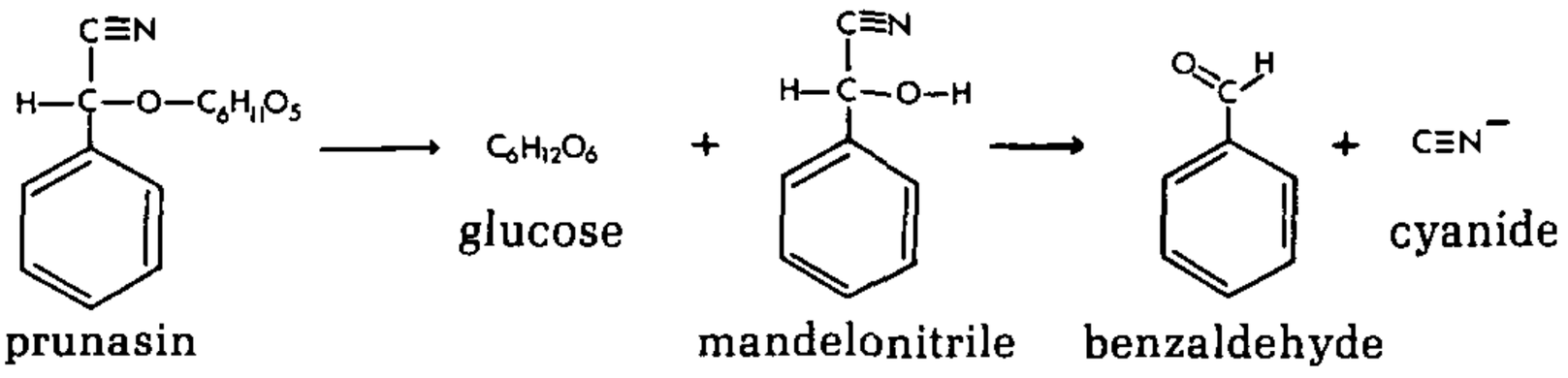
[1]Means followed by the same letter or letters are not significantly different.

## DISCUSSION

In the present study both cyanogenic glycosides, amygdalin and prunasin, severely inhibited callus growth of 'Marianna 2624' plum. However, neither cyanogenic glycoside inhibited 'Texas', a cultivar that forms a compatible combination with 'Marianna 2624' plum. 'Nonpareil', which is not compatible with plum, showed a 77% inhibition at 2 mM prunasin but not with amygdalin. The increased growth observed with amygdalin in the almond cultures suggests that the callus was able to metabolize this compound. Unpublished results from our lab have shown that both cultivars contain low levels of an enzyme capable of hydrolyzing amygdalin.

The greater sensitivity of 'Marianna 2624' plum callus to applied cyanogenic glycosides is interesting since both the almond scions (1) and the plum understock (2) contain the cyanogenic compound prunasin. In the peach/almond incompatibility system it was reported that almond types with a low cyanogenic glycoside content also have a low ability to hydrolyze cyanogenic glycosides, even when additional glycoside is supplied by the peach scion (2).

Cyanogenic glycosides do not directly cause the incompatibility but must be decomposed to release a toxic product (2,3). It is well established that plants containing cyanogenic glycosides contain enzymes capable of decomposing them and it has previously been reported that shoot tissue of 'Marianna 2624' contains an enzyme capable of hydrolyzing cyanogenic glycosides (5). The enzymatic hydrolysis of prunasin proceeds consecutively in a two-step process: prunasin is hydrolyzed to mandelonitrile and glucose; mandelonitrile is hydrolyzed to HCN and benzaldehyde:




prunasin mandelonitrile benzaldehyde cyanide

Of the 3 breakdown products (glucose, HCN and benzaldehyde) only HCN and benzaldehyde could be considered as potential toxic products. Hydrocyanic acid has been shown (3) to cause the anatomical disturbance at the union of the incompatible pear/quince combination. Hydrocyanic acid, liberated from prunasin, also has been implicated in the incompatibility between peach scions and almond roots (2).

Cyanide, however, inhibited the almond or plum cultivars equally (Table 2). At the highest level of cyanide (2 mM) all 3 cultivars were inhibited to approximately the same extent: 32%, 35% and 38% for 'Marianna 2624', 'Nonpareil', and 'Texas', respectively. This indicates that cyanide is not the sole toxic breakdown product of prunasin or amygdalin inhibiting plum callus growth, as was found with the pear/quince incompatibility (3) or proposed in the peach/almond (2) incompatiblity. The lack of severe cyanide toxicity may indicate that all 3 plants are capable of metabolizing HCN into amino acids as has been reported with many plants.

Benzaldehyde stopped all growth of plum callus at 0.05 mM, but a 100-fold greater concentration was required to cause a similar growth reduction of the almond cultivars (Table 3). The greater sensitivity of 'Marianna 2624' plum callus to benzaldehyde indicates that it is a major hydrolytic product from prunasin inhibiting plum callus growth.

Almond interclonal differences in incompatibility with 'Marianna 2624' plum are apparently inherited. Kester, *et al.* (6) reported that almond cultivars incompatible with 'Marianna 2624' were seedlings of 'Nonpareil' or had a genetic relationship to it. Most of the compatible combinations had a known or suspected relationship to 'Texas.'

The incompatibility reaction between almond and plum also has been reported to be of the translocated type, characterized by phloem degeneration and failure of a mutually compatible interstock to overcome the incompatibility (1,6). Kester, *et al.* (6) suggested from their studies on the compatibility reaction between almond and 'Marianna 2624' that the incompatible scion produced a factor which is translocated in the phloem to the graft union where it produces a toxic reaction with the rootstock.

The mechanism allowing 'Texas' callus to grow in the presence of the two naturally occurring cyanogenic glycosides is unknown, however, it may be the same factor responsible for the successful graft union of this cultivar with 'Marianna 2624' understock. Callus from the understock 'Marianna 2624' appears to have a greater sensitivity to added cyanogenic glycosides and benzaldehyde. This sensitivity suggests that cyanogenesis should be examined as a causal factor in the almond/plum incompatibility.

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# AIR LAYERING: AN ALTERNATIVE METHOD FOR THE PROPAGATION OF *MAHONIA AQUIFOLIUM* 'COMPACTA'


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At the 1985 IPPS, Western Region meeting, Dennis Connor of Monrovia Nursery Co. reported (1) on the production of *Mahonia aquifolium* 'Compacta' via cutting and tissue culture. Since then we have conducted an experiment to determine whether this plant could also be propagated utilizing air layering techniques.

Air layering is an ancient and, under favorable conditions, a very sure method of plant propagation for many plants. This method has been practiced in China and other Asian countries for thousands of years. The method has been used mostly with plants native to the tropics and subtropics; however, some hardy perennial plants such as dogwoods, hemlocks, hollies, rhododendrons, viburnums, and wisterias have also been propagated in this manner.

Basically the method involves the stimulation of root develop-