

Effects of Cyanogenic Glycosides and Their Breakdown Products on Callus Growth of Three *Prunus* Species[®]

Charles W. Heuser, Jr.

Department of Horticulture, The Pennsylvania State University, University Park, Pennsylvania 16802 U.S.A.

The effects of the cyanogenic glycosides, amygdalin and prunasin, and their breakdown products, cyanide and benzaldehyde, on callus derived from peach (*Prunus persica* 'Poly'), sand cherry (*P. besseyi*), and Nanking cherry (*P. tomentosa*) were compared. Prunasin (D-mandelonitrile- β -D-glucoside) inhibited the growth of sand and Nanking cherries but not peach. Amygdalin (D-mandelonitrile- β -D-gentiobioside) did not show strong inhibition of the callus cultures. All three species were inhibited by sodium cyanide with sand cherry and Nanking cherry showing the strongest inhibition. Sand cherry and Nanking cherry similarly showed greater sensitivity to benzaldehyde, another catabolite of prunasin. The greater sensitivity of sand and Nanking cherries to the cyanogenic glycoside, prunasin, suggests that it may be an important factor in the peach graft incompatibility with sand and Nanking cherries.

INTRODUCTION

Tissue compatibility or incompatibility in plants can be regarded as a physiological tolerance or intolerance, respectively, between the protoplasts of different cells (Moore, et al., 1981). Although substantial research on stock/scion incompatibility has accumulated (Hartmann et al., 2002; Mosse, 1962), 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. (1968) 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 (1973) 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. In addition, callus cultures derived from the plum understock 'Marianna 2624' have been shown to have increased sensitivity to added cyanogenic glycosides and the catabolic product benzaldehyde (Heuser, 1985; 1986). Breen (1974), 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.

A problem in conducting studies on graft incompatibility is that substances cannot be administered under controlled conditions. Callus cultures provide a unique system for investigating factors regulating plant growth and development and in this study were adapted to study graft incompatibility. Complications resulting from microbial contamination and nutritional and environmental variation are eliminated. In addition, callus cultures allow the incorporation of compounds under controlled conditions.

In this paper, I examine indirectly the possible involvement of cyanogenic glycosides and their catabolites in the peach/sand cherry and peach/Nanking cherry incompatibilities by determining their effects on growth of callus cultures derived from these plants.

MATERIALS AND METHODS

Callus Culture. Callus cultures were established from nodal explants taken from sections of current season's growth of greenhouse grown *Prunus persica* 'Poly' (peach), *P. besseyi* (sand cherry), and *P. tomentosa* (Nanking cherry). Cultures were initiated and maintained on Murashige and Skoog salts (1962) and the following, in mg·L⁻¹: 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 7000. The pH was adjusted to 5.6 ± 0.1 before the addition of sugar and agar. All medium components except the sucrose and agar were filter-sterilized with a Millipore filtration apparatus with a pore size of 0.45 μm. Sucrose and agar were autoclaved for 15 min at 121 °C and combined with the filter-sterilized component. Erlenmeyer flasks (125 ml) were used as stock culture vessels; each flask contained 50 ml culture medium. Stock cultures were maintained daily at 26 °C under 6 μmol s⁻¹·m⁻² (cool white fluorescent lamps, F48T12.CW.HO) for 24 h. Cultures were subcultured every 30 days from callus maintained in culture for approximately 4 years.

General Conditions for Tissue Culture Experiments. 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 about 15 mg was transferred to each bottle using sterile technique. The bottles were kept at 26 °C in a lighted incubator as above for 30 days. Calli were weighed at the end of the period and fresh weight recorded.

Prunasin and Amygdalin Experiments. The objective of this study was to determine effects of the cyanogenic glycosides prunasin (D-mandelonitrile-β-D-glucoside) and amygdalin (D-mandelonitrile-β-D-gentiobioside) on callus growth of peach, sand cherry, and Nanking cherry. 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 compound prior to filter sterilization. Ten replicates were used for each treatment.

Sodium Cyanide Experiment. Sodium cyanide (NaCN) was added at 0.1, 0.5, 1, and 2 mM before pH adjustment and filter sterilization. Ten replications were used for each treatment.

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. Ten replicates were used for each treatment.

RESULTS

Prunasin and Amygdalin Experiments. Prunasin inhibited callus growth of sand and Nanking cherries at 1 and 2 mM but had little effect on the growth of peach (Fig. 1). Amygdalin was tested on Nanking cherry and peach with little growth differences found (results not shown).

Sodium Cyanide Experiment. All three species were inhibited by NaCN with the sand and Nanking cherries completely inhibited at 2 mM (Fig. 2). Peach was only

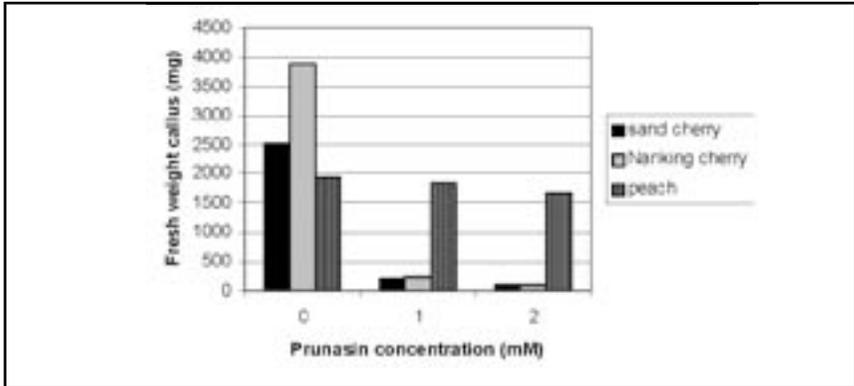


Figure 1. Average fresh weight of callus cultures of sand cherry, Nanking cherry, and peach in the presence of 1 and 2 mM prunasin.

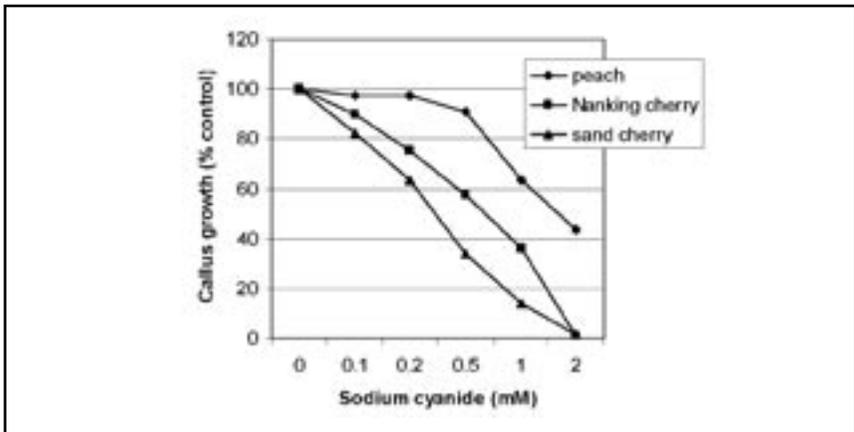


Figure 2. Growth of callus cultures of sand cherry, Nanking cherry, and peach in the presence of sodium cyanide.

inhibited approximately 60% at 2 mM. As shown in Fig. 2, both cherry understocks were inhibited to a greater degree at each NaCN concentration than was peach.

Benzaldehyde Experiment. Benzaldehyde at 0.5 mM completely inhibited sand cherry callus growth and reduced callus growth for Nanking cherry by approximately 80% (Fig. 3). Peach callus showed only slight growth inhibition at 0.5 mM but ceased growth at 1 mM.

DISCUSSION

In the present study, the cyanogenic glycoside prunasin severely inhibited callus growth of the two cherry understocks, sand and Nanking cherries. However, prunasin did not inhibit the peach scion. Amygdalin, the disaccharide cyanogenic glycoside, failed to similarly inhibit callus growth. Therefore, the callus cultures may have lower levels of an enzyme necessary for hydrolyzing amygdalin as was noted

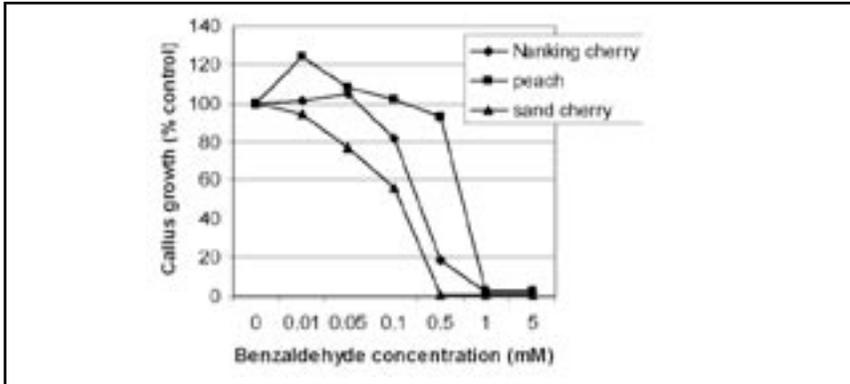


Figure 3. Growth of callus cultures of sand cherry, Nanking cherry, and peach in the presence of benzaldehyde.

in other unpublished results from my lab with two cultivars of almond ('Mission' and 'Nonpareil') (Heuser, 1985, 1986).

The greater sensitivity of sand and Nanking cherries callus cultures to applied prunasin is interesting. In the peach/almond incompatibility system, it was reported that almond types with a low cyanogenic glycoside content also hydrolyze little cyanogenic glycoside even when additional glycoside is supplied by the peach scion (Gur and Blum, 1973).

Cyanogenic glycosides do not cause the incompatibility directly but must be decomposed to release a toxic product (Gur and Blum, 1973; Gur, et al., 1968). It is well established that plants containing cyanogenic glycosides almost invariably contain enzymes capable of decomposing them, and unpublished results from our lab indicate that all species contain an enzyme that will hydrolyze prunasin. The enzymatic hydrolysis of prunasin proceeds consecutively in a two-step process: prunasin is hydrolyzed to mandelonitrile and glucose; mandelonitrile is hydrolyzed to hydrogen cyanide (HCN) and benzaldehyde. Of the three breakdown products (glucose, HCN, and benzaldehyde), only HCN and benzaldehyde could be considered as potential toxic products. Hydrocyanic acid has been shown (Gur, et al, 1968) 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 (Gur and Blum, 1973).

In the present study, NaCN inhibited the two understock species at a lower concentration than the peach. At the highest level of NaCN used (2 mM), peach callus cultures were not completely inhibited, indicating that cyanide may have a role in the graft incompatibilities as was found with the pear/quince incompatibility (Gur, et al. 1968) or proposed in the peach/almond (Gur and Blum, 1973) incompatibility. The lower cyanide toxicity in peach may indicate that it is better able to metabolize HCN into amino acids, as reported with many plants.

Benzaldehyde essentially stopped all growth of sand and Nanking cherries callus cultures at 0.5 mM, but a greater concentration was required in order to cause a similar growth reduction in peach callus (1 mM). The increased sensitivity of the

two cherry understock callus cultures to benzaldehyde indicates that this may be a second hydrolytic product from prunasin, inhibiting their growth.

In conclusion, the sensitivity of the two cherry understocks callus cultures to the cyanogenic glycoside, prunasin, and the catabolic products, benzyldehyde and cyanide, suggests that cyanogenesis should be examined as a causal factor in the peach/sand cherry and peach/Nanking cherry incompatibilities. The presence of cyanogenic glycosides in woody plants is restricted to a relatively few genera. Hence, this mechanism can not be considered a universal mechanism, but offers the suggestion that other small molecular compounds, after possibly undergoing metabolism, may be responsible for graft incompatibilities in other woody plants.

LITERATURE CITED

- Breen, P.J.** 1974. Cyanogenic glycosides and graft incompatibility between peach and plum. *J. Amer. Soc. Hort. Sci.* 99(5):412-415.
- Gur, A. and A. Blum.** 1973. The role of cyanogenic glycoside in incompatibility between peach scions and almond rootstocks. *Hort. Res.* 13:1-10.
- Gur, A., R.M. Samish, and E. Lifshitz.** 1968. The role of the cyanogenic glycoside of the quince in the incompatibility between pear cultivars and quince rootstocks. *Hort. Res.* 8:113-134.
- Hartmann, H.T., D.E. Kester, F.T. Davies, Jr., and R.L. Geneve.** 2002. *Hartmann and Kester's plant propagation: principles and practices.* 7th (Ed). Prentice-Hall, Inc., Upper Saddle River, New Jersey.
- Heuser, C.W.** 1972. β -Glucosidase from 'Marianna' plum. *Phytochemistry* 11:2455-2457.
- Heuser, C.W.** 1985. Effect of cyanogenic glycosides on growth of callus from almond and plum. *J. Amer. Soc. Hort. Sci.* 110:467-470.
- Heuser, C.W.** 1986. Graft incompatibility: Effect of cyanogenic glycoside on almond and plum callus growth. *Comb. Proc. Intl. Plant Prop. Soc.* 36:91-97.
- Moore, R. and D.B. Walker.** 1981. Studies of vegetative compatibility-incompatibility in higher plants. 11. A structural study of an incompatible heterograft between *Sedum telephoides* (Crassulaceae) and *Solanum pennellii* (Solanaceae). *Amer. J. Bot.* 68: 831-842.
- Mosse, B.** 1962. Graft-incompatibility in fruit trees. *Tech. Commun. No. 28, Comm. Bureau Hort. Plant Crops, East Malling, England.*
- Murashige, T. and F. Skoog.** 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* 15:473-497.