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AMYGDALIN IN *PRUNUS* LEAVES

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Key Word Index—*Prunus*; subg. *Padus*; subg. *Laurocerasus*; Rosaceae; cyanogenic glycosides; amygdalin; prunasin; inheritance.

Abstract—The cyanogenic diglucoside amygdalin was found for the first time in the leaves of mature trees of several *Prunus* taxa: *P. serotina* and *P. virginiana* cv. Schubert of subg. *Padus* and *P. ilicifolia* and *P. lyonii* of subg. *Laurocerasus*. Leaves of other taxa in both subgenera contained only the monoglucoside prunasin. Amygdalin production was inherited in hybrids between *P. padus* cv. Grandiflorus and *P. virginiana* cv. Schubert. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Two cyanogenic glycosides have been found in various tissues of *Prunus* species. The monoglucoside prunasin probably occurs in all cyanogenic plant parts and is also found in many unrelated genera and families. Amygdalin, a diglucoside, is restricted to the Rosaceae and, up until now, has been reported only from seeds [1]. Most of the research on cyanogenesis in *Prunus* has involved those taxa noted for their fruits and/or flowers and which are classified botanically in subg. *Prunus* (= *Prunophora* (Neck.) Focke), subg. *Amygdalus* (L.) Benth. & Hook. f., or subg. *Cerasus* (Adans.) Focke. It must be noted here that the subgeneric scheme and authorities used in this paper are from Krüssmann [2] and the species epithets and authorities are those recognized in GRIN (Germplasm Resources Information Network - USDA, ARS).

On 25 July 1995, during preliminary studies of the effects of cyanogenesis in *Prunus* foliage on feeding by adult Japanese beetles (*Popillia japonica* Newman), it was found that leaves of *P. serotina* Ehrh. contained both prunasin and amygdalin in the ratio 16:1. Amygdalin was also determined in the leaves of *P. virginiana* L. cv. Schubert (NA 35892) but not in *P. padus* L. cv. Grandiflorus (NA 37041). However, amygdalin was found in two hybrids resulting from controlled pollination between these two cultivars. On 11 September 1995, the ratio of prunasin to amygdalin in *P. serotina* was 34:1. Thus, we decided to monitor the levels of prunasin and amygdalin in the taxa and hybrids of subg. *Padus* (Moench) Focke noted above through the 1996 growing season. Further, late in 1996, we had the opportunity to examine the leaves of several taxa of subg. *Laurocerasus* (Ser.) Rehd.

RESULTS AND DISCUSSION

The newly-emerging leaves of *P. serotina* produced 7432 μg HCN/g when sampled on 19 March 1996, but prunasin was the only cyanogenic glucoside found in this species, the cultivars of *P. padus* and *P. virginiana*, and the interspecific hybrids through 16 April 1996. It was not until 6 May 1996 that amygdalin was detected in *P. serotina*, *P. virginiana*, and the hybrids between the *P. padus* and *P. virginiana* cultivars. From early June to early September, spanning the period of Japanese beetle feeding, the HCN levels were as follows: *P. serotina* (2 trees), 2644 ± 236 μg/g; P. *virginiana* cv. Schubert, 3776 ± 526 μg/g; *P. padus* cv. Grandiflorus, 1655 ± 125 μg/g; and the two hybrids, 2702 ± 416 μg/g. Thus, the HCN levels of the hybrids appeared to be roughly intermediate between the two parent cultivars.

Prunasin was the only cyanogenic glucoside found in *P. padus* cv. Grandiflorus, *P. padus* (NA 40350), and in two other taxa of subg. *Padus*: *P. buergeriana* Miq. (NA 59190) and *P. grayana* Maxim. (NA 46329). The ratios of prunasin to amygdalin in the other taxa and hybrids were not related to time of harvest and varied, somewhat erratically, from 8:1 to 34:1. *P. virginiana* cv. Schubert always had the lowest levels of amygdalin (av. 29:1) but the hybrids had more amygdalin (av. 16:1), even higher than *P. serotina* (av. 22:1).

By 21 October 1996, the HCN levels in the amygdalin-containing trees noted above had dropped to

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less than 50% of their June to September averages and the ratio of prunasin to amygdalin was 8:1 (*P. virginiana* cv. Schubert not tested). On 13 November 1996, following two nights of below-freezing temperatures, green leaves could be sampled only from the two *P. serotina* trees, and the HCN levels had been reduced to an average of 209 μg/g. Apparently prunasin had disappeared from these trees, since only amygdalin was detected. Strong HCN-picrate reactions with added prunasin indicated, however, that the enzymes capable of cleaving the glucosides were still present.

It was, perhaps, coincidental that the appearance of amygdalin in the leaves of mature plants grown outdoors occurred during the onset of flowering. Amygdalin was also found (52:1) in the leaves of 1-year-old seedlings of *P. serotina* that had remained in a greenhouse and that were sampled on 3 September 1996. It is of some interest that Swain and Poulton [3] found that the amygdalin levels in the cotyledons of *P.serotina* seeds declined by more than 80% during the first 3 weeks following imbibition. The first true leaves that appeared above the cotyledons were apparently not analyzed.

On 31 October 1996, we tested fresh leaves of single individuals of several evergreen taxa of subg. *Laurocerasus*: *P. ilicifolia* (Nutt. ex Hook. & Arn.) Walp. (A69. 0046) and *P. lyonii* (Eastw.) Sarg. (A69. 0044) from the University Arboretum in Davis, CA and *P. lusitanica* L., *P. laurocerasus* L. cv. Otto Luyken, and *P. caroliniana* Ait. cv. Bright 'N Tight from a local Maryland nursery. Even at this late date, *P. lyonii* had an HCN content of 4364 μg/g and contained amygdalin in the prunasin: amygdalin ratio of 24:1. A prunasin: amygdalin ratio of 4:1 was found in *P. ilicifolia* with an HCN content of 3320 μg/g. Neither of the cultivars of *P. caroliniana* nor P. *laurocerasus*, with HCN levels of ca 3100 μg/g, contained amygdalin. Only a trace of HCN (109 μg/g) from prunasin was detected in *P. lusitanica*.

At this juncture, it is not possible to determine whether the presence of both amygdalin and prunasin in the leaves of some *Prunus* taxa have any effect on feeding by adult Japanese beetles.

EXPERIMENTAL

Plant material

Unless otherwise noted, all plants were either growing wild or were contained in experimental plantings at the U.S. National Arboretum.

HCN concentration

The amounts of HCN evolved from 0.05g to 0.1 g fresh leaf tissue were quantified by a modified sodium picrate method described earlier [4], and are expressed as μg/g dry wt.

Identification of cyanogenic glucosides

Aliquots of 70% EtOH extracts of *Prunus* leaves were banded on Whatman 3MM paper and subjected to ascending PC in *n*-BuOH–EtOH–H_2O (7:2:2). The R_f values of 0.60 for prunasin and 0.18 for amygdalin reported earlier [5] were not achieved in our system but the movements of the extracted glycosides were identical to those of pure prunasin and amygdalin (Sigma). After drying, the papers were sprayed with (β-glucosidase (0.1 mg/ml) or (β-glucuronidase 6% v/v). A sheet of paper treated with the picrate reagent was separated from the chromatogram by fine nylon mesh fabric and the two papers were sandwiched between glass plates and placed in an oven at 30 for 24 h. Areas containing the reddish HCN-picrate complex were cut from the papers and eluted in dist H_2O for 10 min. The absorbtion of the eluates at 515 nm was determined with a spectrophotometer (Bausch and Lomb "Spectronic 20") and the levels of HCN generated from prunasin and amygdalin calculated by comparisons with a standard curve based on known conc. of KCN. There were no differences in the amount of HCN evolved between β-glucosidase and β-glucuronidase.

The suspected amygdalin was isolated and purified from young leaves of *P. serotinia* and a *P. padus* × *P. virginiana* hybrid by PC in May 1997. Solution state NMR spectra in D_2O using water suppression (presaturation) were recorded with a Bruker QE Plus spectrometer (300 MHz) with 16 acquisitions at a proton spectral width of 3100 Hz. The chemical shifts, coupling patterns, and relative intensities of the sample extract were the same as for authentic amygdalin.

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REFERENCES

1. Seigler, D. S., in *Cyanide in Biology*, ed. B., Vennesland, E. E., Conn, C. J., Knowles, J. Westley, and F., Wissing. Academic Press, New York, 1981, pp. 133–143.
2. Krüssmann, G., *Handbuch der Laubgeholze*, Band II, Paul Parey, Berlin, Hamburg, 1962.
3. Swain, E. and Poulton, J. E., *Plant Physiol.*, 1994, **106**, 437–445.
4. Jacobs, K. A., Santamour, F. S., Jr., Johnson, G. R. and Dirr, M. A., *J. Environ. Hort.*, 1996, **14**, 154.
5. Fikenscher, L. H. and Hegnauer, R., *Planta Med.*, 1977, **31**, 266.