ALKALOID PRODUCTION IN HEALTHY AND AMPELAMUS-VIRUS INFECTED PERIWINKLE, VINCA ROSEA L.

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If the biochemical syndrome of virus-infected plants differs from the biochemistry of noninfected plants, detection of this difference should be ascertainable with modern techniques. Since many of the hydrolytic products of plant viruses contain nitrogen, it seems likely that viral infections may induce aberrations in the plant's nitrogen metabolism and thus influence the synthesis, assimilation, or accumulation of organic bases such as alkaloids. Since Ampelamus virus invades periwinkle, *Vinca rosea* L., systemically (unpublished data) and since some new alkaloids with antitumor activity have been isolated from periwinkle (Johnson et al., 1960; Neuss et al., 1959; Noble et al., 1958; Svoboda et al., 1959) it seemed worthwhile to compare the production of alkaloids in healthy and Ampelamus-virus infected periwinkle plants. The findings are reported and discussed.

Materials and Methods

Ampelamus virus used in the experiments was isolated from *Ampelamus albidus* (Nutt.) Britt. (honeyvine, bluevine, or sandvine) in 1958 and maintained in *Nicotiana glutinosa* L. The virus, probably a strain of cucumber mosaic virus (*Marmor cucumeris* H.) is tentatively designated a distinct virus awaiting a more accurate identification.

Periwinkle plants, healthy and infected, were a single clone which had been propagated vegetatively since 1956. Infected plants were increased from a single plant of the clone inoculated mechanically. The healthy and infected plants were started in potted soil in a glasshouse and transferred May 21, with the potted soil, to field plots reasonably uniform in fertility, texture, and topography. The plants were harvested October 20.

Results and Discussion

Drying of plant tissues. Samples of periwinkle leaves and roots from healthy and virus infected plants were dehydrated in a vacuum desiccator over potassium hydroxide pellets at room temperature. Three samples of healthy leaves (each 200 grams wet weight) dried to 52, 67, and 32 grams. Four samples of diseased leaves (each 200 grams wet weight) dried to 53, 57, 50, and 59 grams. Two samples of healthy roots (250 and 290 grams wet weight) dried to 55 and 65 grams, respectively. Two samples of diseased roots (206 and 190 grams wet weight) dried to 45 and 43 grams, respectively.

Extraction of alkaloids. The dry-weighed sample of healthy or diseased leaves or roots was blended in a commercial blendor for five minutes with a mixture of 50 ml benzene, 25 ml ethanol, and 1 ml of NH_4OH. After blending, the slurry was transferred to a beaker and stirred for six hours with 200 ml of the above mixture. The liquid was filtered through paper, and the residue was extracted overnight with 200 ml of fresh solvent mixture. The liquid of the second extraction was filtered and combined with the filtrate from the first extraction. After adding 100 ml of 10% acetic acid to the combined filtrates, the liquid was concentrated by evaporation until no organic solvent remained. The

acetic acid solution was alkalized with NH_4OH and extracted with methylene chloride. Alkaloids were obtained from the dried and evaporated solution.

Analysis of alkaloids. The yield of alkaloids was in each case 0.8%± 0.2%. Thin layer chromatography of these fractions on a silica plate containing 100 μg per application showed no qualitative or quantitative difference after developing in a solvent mixture of 3 parts ethyl acetate and 1 part ethanol and spraying with ceric ammonium sulfate in phosphoric acid (Cone, Miller, and Neuss, 1963).

These findings indicate that the production of alkaloids by periwinkle is not grossly, if at all, influenced by Ampelamus-virus infection under the conditions of this experiment. It is conceivable, however, that under other conditions alkaloid production or accumulation in periwinkle might be influenced by Ampelamus-virus infection or by infection by other viruses. Although there was no detectable difference in alkaloid content of healthy and infected periwinkle, the infection may have actually influenced the metabolic pathways involved in alkaloid synthesis or utilization but allowed the same amount of alkaloid to accumulate. From these findings, it seems unlikely that alkaloid production in periwinkle is associated with metabolism influenced by infection with Ampelamus-virus. The findings orient research in other directions.

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Literature Cited

Cone, Nancy J., Ruthanne Miller, and Norbert Neuss. 1963. Alkaloids of *Vinca rosea* Linn. (*Catharanthus roseus* G. Don) XV. Analysis of vinca alkaloids by thin-layer chromatography. J. Pharm. Sci. 52 (7):688-692.

Johnson, I. S., H. F. Wright, G. H. Svoboda, and Janet Vlantis. 1960. Antitumor principles derived from *Vinca rosea* Linn. I. Vincaleukoblastine and leurosine. Cancer Research 20(7):1016-1022.

Neuss, N., M. Gorman, G. H. Svoboda, G. Maciak, and C. T. Beer. 1959. Vinca alkaloids. III. Characterization of leurosine and vincaleukoblastine, new alkaloids from *Vinca rosea* Linn. J. Am. Chem. Soc. 81:4754.

Noble, R. L., C. T. Beer, and J. H. Cutts. 1958. Role of chance observations in chemotherapy: *Vinca rosea*. Ann. N. Y. Acad. Sci. 76(3):882-894.

Noble, R. L., C. T. Beer, and J. H. Cutts. 1958. Further biological activities of vincaleukoblastine—an alkaloid isolated from *Vinca rosea* (L.). Biochem. Pharmacol. 1:347-348.

Svoboda, G. H. 1958. A note on several new alkaloids from *Vinca rosea* Linn. I: Leurosine, virosine, perivine. J: Am. Pharm. Assoc., Sci. Ed. 47(11): 834.

Svoboda, G. H., Norbert Neuss, and Marvin Gorman. 1959. Alkaloids of *Vinca rosea* Linn. (*Catharanthus roseus* G. Don.) V. Preparation and characterization of alkaloids. J. Am. Pharm. Assoc., Sci. Ed. 48(11):659-666.

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