Transactions of the Illinois Academy of Science
(1989), Volume 82, 3 and 4, pp. 129-132

SEASONAL VARIATION OF CYANIDE PRODUCTION IN THREE ILLINOIS PLANT SPECIES

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ABSTRACT

Two populations each of *Phlox divaricata* L., *Mertensia virginica* (L.) Pers., and *Celtis occidentalis* L. from east-central Illinois were examined for cyanide production. The frequency and amount of cyanide produced was found to vary seasonally among the individuals tested within each population of each species. All three taxa peaked in cyanide production during the first two weeks in May.

INTRODUCTION

Many plants are capable of synthesizing compounds which liberate hydrogen cyanide when hydrolyzed. This phenomenon, known as cyanogenesis, has been reported from bacteria, lichens, fungi, ferns, fern allies, gymnosperms, and at least 2050 species, representing more than 110 families of angiosperms (Gibbs, 1974; Hegnauer, 1959; Seigler, 1976; Tjon Sie Fat, 1979).

Cyanogenesis is a result of the hydrolysis of cyanoglucosides or cyanolipids that yield one or more sugars or fatty acids, aldehydes or ketones and hydrogen cyanide (HCN). The amount of hydrogen cyanide liberated depends on several intrinsic (genetics, plant organ, age and even sex of the plant) and extrinsic factors (climate, available moisture, soil fertility, and frost damage) (Seigler, 1976; Hegnauer, 1959). Although numerous plant species have been reported as cyanogenic, little information is available regarding the extent of cyanogenesis in natural populations. Several previously published works indicate a high degree of cyanogenic variability in populations of *Trifolium repens* L. (Daday, 1965), *Lotus corniculatus* L. (Jones, 1977), and *Lotus alpinus* L. (Urbanska, 1982), and *Ranunculus montanus* Willd. (Dickenmann, 1982).

The present study was initiated to determine the extent of cyanogenic variability in populations of *Phlox divaricata* L. (common phlox), *Mertensia virginica* (L.) Pers. (bluebells), and *Celtis occidentalis* L. (hackberry) to see if individual plants were consistently cyanogenic or if cyanogenesis was transitory during growth.

MATERIALS AND METHODS

The plants utilized in this study were taken from natural populations at Burgner Acres Natural Area located in the Grand Prairie Division of Illinois (Schwegman, 1973) three miles north of the Shelbyville Moraine in Coles County, Illinois (Sec 1 T12N R8E). Burgner Acres is a ten acre woodlot with a maximum difference in elevation of 6 m. The dominant upland species are *Quercus alba* L., *Carya ovata* (Mill.) K. Koch, and *Fraxinus americana* L. The dominant lowland species include *Celtis occidentalis* L. and *Fraxinus americana* (Lehnen and Ebinger, 1984).

One upland and one lowland population each of *Celtis occidentalis*, *Mertensia virginica*, and *Phlox divaricata* were studied for cyanogenesis. In each population 30 individuals were randomly selected and marked with numbered plastic nursery tags. A leaf from each numbered individual was tested on a weekly basis for the presence of hydrogen cyanide, the woodland phlox and bluebell populations from spring emergence until dormancy in early summer, the hackberry populations from leaf development until the first hard frost in the fall. In hackberry, the leaves were chosen from the branch which was tagged.

Individuals were tested for cyanide production using the Feigl-Anger technique (Feigl and Anger, 1966; Tantisewie *et al.*, 1969). A small amount of each leaf blade (about 200 mg) was crushed and placed in a small vial with 4-5 drops of distilled water and a drop of antibiotic solution (0.5 mM streptomycin sulfate). A strip of filter paper impregnated with copper ethylacetoacetate and tetra base (4,4'tetramethyldiaminodiphenylmethane) was suspended with a dry cork over the sample, taking care to avoid contact with the leaf-water mixture. The test samples were read and recorded after 24 hours at room temperature. Although a quantitative determination of cyanide was not made, the color changes give an indication of the amount of hydrogen cyanide released (Dickenmann, 1982). A light blue color on the lower part of the test strip indicates a weak reaction (about 2-20 mg HCN per kg fresh weight); a moderate reaction is indicated when most of the paper turns light blue (about 21-50 mg hch per kg fresh weight), and a strong reaction when the paper turns a dark blue throughout (more than 50 mg HCN per kg fresh weight).

RESULTS AND DISCUSSION

All of the species examined were polymorphic for cyanide production (Table 1). In addition, the ability of any one individual to be cyanogenic varied throughout the growing season. Overall, it was common for an individual to be cyanogenic one week and test negative for cyanogenesis the following week.

Phlox divaricata had the lowest frequency of cyanogenesis with only weakly positive tests recorded. Throughout the populations no individual tested positive during the early part of the growing season although a few individuals were found to be cyanogenic during flowering and seed set. In most instances an individual

tested positive for cyanogenesis only once during the growing season, although two individuals in the upland population tested positive on consecutive weeks.

The results for *Mertensia virginica* were similar to those found in woodland phlox. Like phlox, no positive tests were recorded during the early part of the growing season, and with the exception of two moderate reactions, the tests were all weakly positive. Cyanogenesis was also more frequent during flowering and seed set. Most individuals gave a positive test only once during the growing season with only one individual from the lowland population testing positive for cyanide production for two consecutive weeks.

The highest cyanogenic frequency was found in the *Celtis occidentalis* populations. About 80% of the individuals from the upland population and 77% of the individuals in the lowland population were positive for HCN production at some time during the study. Positive tests were obtained only through the first weeks of the growing season with no positive tests recorded between June 10 and leaf abscission. In hackberry, 38% of the individuals tested positive for two or more consecutive weeks. Moderate and strong reactions were recorded for 38% of the individuals tested during the study.

Individuals within populations of each of the species tested varied in the frequency and amount of HCN produced. All three taxa peaked in cyanide production during the first two weeks in May, indicated by the number and strength of reactions which occurred during this period

It is possible that the cyanogenic compounds may serve as storage structures for nitrogen accumulated under conditions favorable for rapid nitrogen cycling, such as the warm, wet conditions in early spring. Nitrogen stored in these compounds might be utilized by the plants when nitrogen becomes limiting (Dement and Mooney, 1974). Cyanogenic compounds may also afford some protection against generalist herbivores (Jones, 1972; 1978) especially in young tissues (Rhoades and Gates, 1976) and during reproductive stages (Janzen, 1971).

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Table 1. Percent of cyanogenic individuals in upland and lowland populations of *Phlox divaricata* (common phlox), *Mertensia virginica* (bluebells), and *Celtis occidentalis* (hackberry) at Burgner Acres Natural Area, Coles County, Illinois.

WEEK	UPLAND			LOWLAND		
(month/day)	phlox	bluebells	hackberry	phlox	bluebells	hackberry
4/11	—	—	—	—	—	—
4/18	10	—	—	—	—	—
4/28	3	—	—	3	7	17
5/03	3	13	23	10	13	67
5/12	10	3	63	3	20	50
5/20	—	—	17	—	—	17
5/28	—	—	—	—	—	3
6/03	—	—	—	—	—	3
6/10-10/12	—	—	—	—	—	—