COMMUNICATIONS

Rotenone and Deguelin in *Tephrosia vogelii* at Several Stages of Maturity

Changes in rotenone (R) and deguelin (D) concentrations were followed by thin-layer densitometry in the leaflets and other parts of two lines of *Tephrosia vogelii* Hook. f. In one line, relatively free of R, total leaflet D increased from 1.6 to 3.2%. In the other line leaflet R increased with plant age from 0.3 to 1.1% and leaflet D increased from 1.1 to 1.9%. Rotenoid levels were higher in leaves formed later in the growing season but remained essentially constant in a given leaf throughout its life on the plant. Concentrations of rotenoids in the other plant parts were substantially lower and increased little or none as the plant matured. Abscission of lower leaves and increase in stem-to-leaf ratio combined to reduce the total rotenoid content of the whole plant late in the season.

Tephrosia vogelii Hook. f. is a possible domestic source of the natural insecticides rotenone (R) and deguelin (D). Although *T. vogelii* is native to the tropics, it has provided good yields of leaves and stems when grown in the southeastern continental United States. It fails to set seed in this area, however, and seed must be produced in more tropical regions where day length favors flowering; *e.g.*, Puerto Rico. In addition to its domestic adaptability, *T. vogelii* has the further advantage over present commercial sources—*Derris* and *Lonchocarpus* roots imported from the tropics—of concentrating rotenoids in leaflets, which are more amenable than roots to mechanical harvesting.

The development of a thin-layer densitometric (tld) procedure for rotenoids (Delfel and Tallent, 1969; Delfel *et al.*, 1970) permitted, for the first time, R and D to be conveniently measured independently. The distribution of R and D in the various parts of fresh *T. vogelii* plants was determined; the highest concentrations of R and D individually, as well as of the sum of the two, occurred in the leaflets. In this earlier work (Delfel *et al.*, 1970) all samples were from plants approximately 4 months old. We now report the use of tld to follow changes in R and D levels in leaflets, petioles, stems, and roots of *T. vogelii* as a function of plant maturity. For simplicity the sum of R and D concentrations is referred to as total rotenoids, although minor amounts of tephrosin, and perhaps of other rotenoids present, were not determined.

MATERIALS AND METHODS

Studies during 1970 Growing Season. Two *T. vogelii* lines were examined: B.L. 656, an R-free line available from the breeding program at the Federal Experiment Station, Plant Science Research Division, ARS, USDA, Mayaguez, Puerto Rico; and P.I. 257533, an unselected introduction. They were planted May 8, 1970, at Glenn Dale, Md., in randomized blocks with four replications, and emergence occurred May 19. Plants were thinned to one per 15 cm in rows 12.8-m long and 76-cm apart. Harvest times were determined by plant development stage based on number of leaf nodes on the main stem (Higgins *et al.*, 1964). The first of seven harvests was on July 6, 48 days after emergence, with succeeding harvests at 63, 76, 90, 99, 118, and 139 days; the last was on October 5. These harvests coincided with development of leaves at nodes 6, 10, 13, 16, 18, 22, and 25. There were approximately 14 days between the first four harvests, 21 days between the last two, and 9 days separated harvests 4 and 5. Six whole plants were taken from each plot in the first five harvests. In the last two harvests, only four plants were removed per plot. This reduction would have allowed an additional harvest if frost had been late enough. The plants were placed in insulated packing and, with one exception, were shipped by direct flight to Peoria the same day as harvested and held overnight at 1 °C before analysis. Harvest No. 4 was delayed 24 hr in transit. No severe wilting or leaf loss was observed on any of the shipments.

The plants of all harvests were hand-separated into leaflet, petiole, stem, and root fractions, which were composites of the four or six plants from each plot. In harvest No. 6 (118 days after emergence), random leaflet samples were taken from each individual plant before the composite samples were prepared. The roots of harvest No. 3 and all plant parts of succeeding harvests were chopped in a Wiley mill equipped with a $^1/_4$-in. screen. Parts of small plants from earlier harvests were cut with scissors into small pieces. Moisture content of each plant part was determined by heating 1- to 5-g samples at 80 °C for 3 hr. For the determination of rotenoids, 1- to 5-g samples were steeped in acetone (30 ml/g for leaflets and 15 ml/g for other parts) in the dark at room temperature for 2 days and the stored in a freezer (−18 °C) until analyzed. Experiments by Barnes and Freyre (1966, 1967) established the suitability of this procedure. Each extract was analyzed at least in duplicate by tld (Delfel and Tallent, 1969; Delfel *et al.*, 1970). Results are expressed as percent rotenoid in samples on a dry basis (DB).

Studies during 1971 Growing Season. Only leaflets of P.I. 257533 were examined. Plantings at Glenn Dale, Md., on May 20, 1971, were arranged in randomized blocks; plantings in Peoria, Ill., on May 14, 1971, were arranged in two rows and considered as approximating the more formal plantings at Glenn Dale. Six collections were made at Glenn Dale and five at Peoria. Sampling dates coincided with development of leaves at nodes 6, 10, 14, 18, 22, and 26. Four plants from Glenn Dale (one from each replication) and one from Peoria were removed on each of these dates and composited leaflets of each plant were analyzed. In addition, samples were taken from leaves at nodes 4 and 5, 8 and 9, 12 and 13, 16 and 17, 20 and 21, and 24 and 25. Leaflets from the same pair of leaves were sampled for as many harvests as possible before leaf abscission. For example, a leaflet was removed from leaf 4 in half of the plants and from leaf 5 in the other half in the first harvest. In succeeding harvests, the sampling was reversed to reduce the number of leaflets removed from a single leaf. Samples from Glenn Dale were chilled overnight in a cold room at 1 °C, packed in insulated cartons, and shipped by direct flight to Peoria. The samples arrived the day after harvest and were immediately analyzed. Samples in Peoria were analyzed a few hours after harvest.

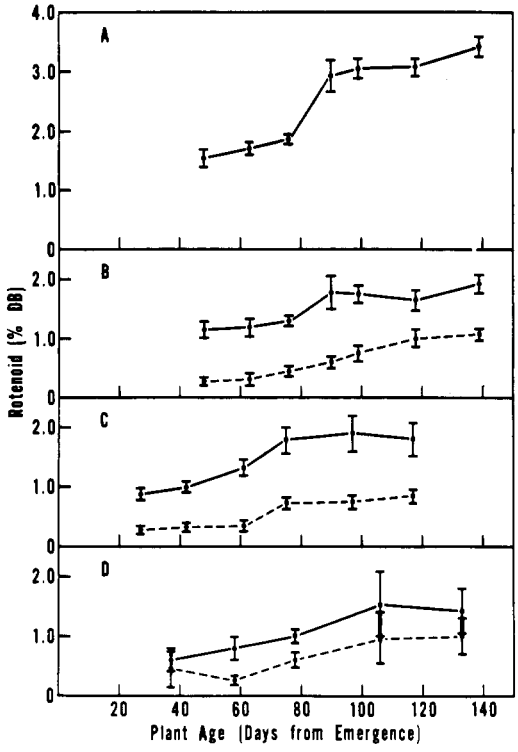

Figure 1. Rotenone (broken line) and deguelin (solid line) in leaflets of whole plants of *Tephrosia vogelii*. (A) B.L. 656, Glenn Dale, Md., 1970; (B) P.I. 257533, Glenn Dale, Md., 1970; (C) P.I. 257533, Glenn Dale, Md., 1971; (D) P.I. 257533, Peoria, Ill., 1971

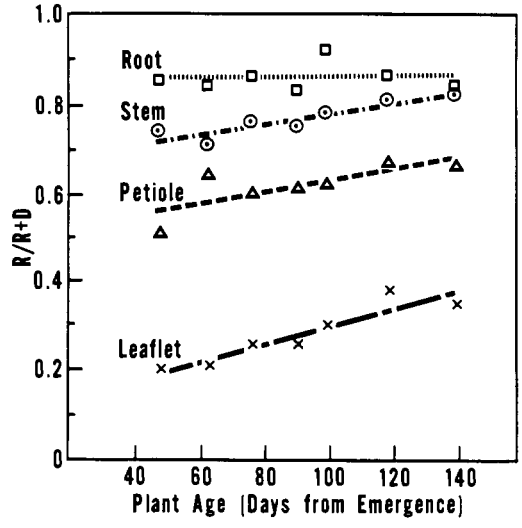

Figure 2. Regression lines for rotenone (R) to rotenone-plus-deguelin (R + D) ratios in P.I. 257533

RESULTS AND DISCUSSION

The concentration of R and D in leaflets from the whole plants is shown for both growing seasons in Figure 1. For each R and D point, 95% confidence intervals are indicated. These confidence intervals were calculated using separate standard deviations for R and D for each harvest. The interval bars for the Peoria plants are somewhat larger because only one whole plant was analyzed per harvest. The results in Figure 1 for the two lines studied generally confirm and extend those of Barnes *et al.* (1967), namely, that leaflet rotenoid concentration in *T. vogelii* plants first increases and then levels off as the plant matures. Our earlier samplings made it possible further to show that the buildup in rotenoid

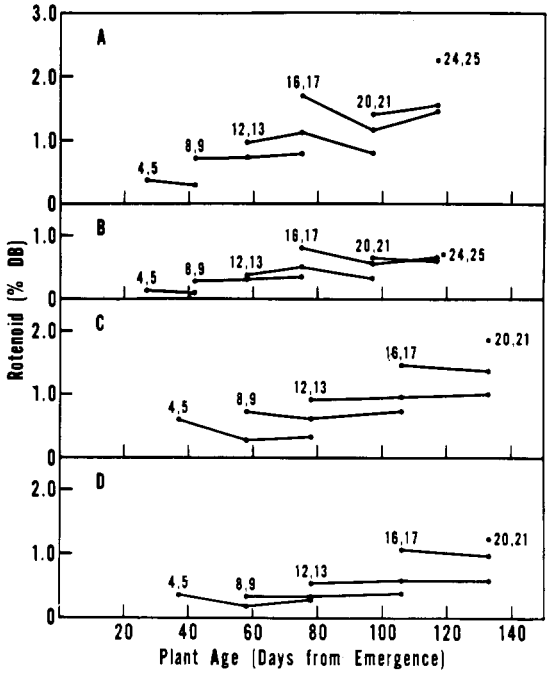

Figure 3. Rotenoids in individual leaves of *T. vogelii*, 1971 plantings. The pairs of numbers refer to the nodes corresponding to the leaves on the main stem from which samples were taken and the higher numbers represent later appearing nodes. (A) Deguelin, Glenn Dale, Md.; (B) rotenone, Glenn Dale, Md.; (C) deguelin, Peoria, Ill.; (D) rotenone, Peoria, Ill.

concentration follows a sigmoidal pattern, with the most rapid increase occurring in the middle of the growing season. Moreover, as a result of the independent rotenone and deguelin determinations, it is evident that these substances follow individually the same general pattern as total rotenoids. However, the contribution of each to the total pattern does not necessarily remain constant throughout the life of the plant. In P.I. 257533, 1970 planting, the ratio of R to total rotenoids (R + D) in the leaflets increased with plant age from approximately 0.20 to 0.35 (Figure 2) and results for 1971 were in good agreement. A similar but slightly smaller increase in the R/(R + D) ratio occurred in petioles and stems from the 1970 planting, but no significant change in ratio occurred in roots.

To further explore the nature of the change in rotenoid content with plant maturity, we analyzed leaflets from individual leaves. Confidence intervals for the resulting data were similar to those given in Figure 1 but are omitted in Figure 3 to permit relevant results from all plantings to be presented without excessive congestion. Barnes and Freyre (1967) reported higher rotenoid concentrations in leaves nearer the plant apex. Our data explain this observation by revealing that leaves formed earlier in the life of the plant generally have lower concentrations of rotenoids and that, at least over a period corresponding to three harvests and within the expected variability range, this concentration remains essentially constant in a given leaf. Thus, taken as a whole, Figure 3 supports the following generalization: the increase in total leaflet rotenoid concentration with plant age is not due to changes within individual leaves; instead it is a result of the appearance of leaves with higher rotenoid levels as the plant reaches later stages of growth.

The concentration of rotenoids in petioles, stems, and roots (Figure 4) was considerably lower than in leaflets and increased only slightly or perhaps not at all as the plants matured. Again, the confidence bars are omitted to avoid cluttering.

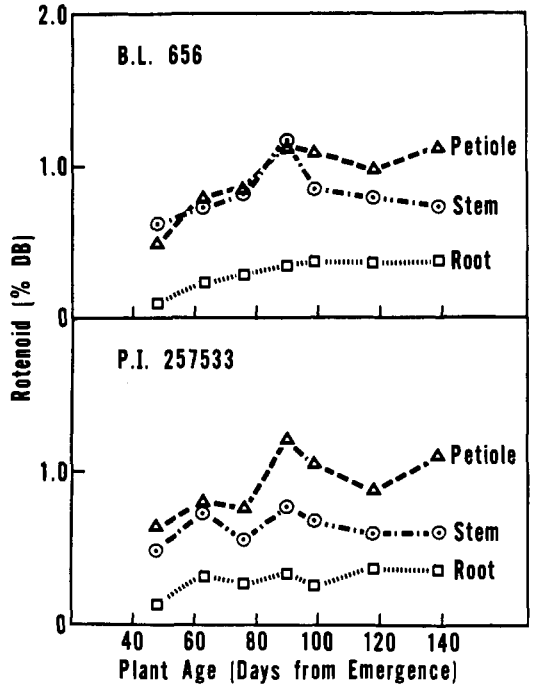

Figure 4. Total rotenoid content in petioles, stems, and roots of *T. vogelii*, 1970 plantings

Table I. Rotenoids in Leaflets from Individual Plants[a]

Replication and plant	B.L. 656		P.I. 257533	
	Rotenone	Deguelin	Rotenone	Deguelin
A1	1.0	2.2	0.9	1.6
A2	0.9	2.3	0.7	1.2
A3	0.0	2.2	1.0	1.2
A4	0.0	2.8	1.1	1.6
B1	0.0	2.8	1.1	1.8
B2	0.0	3.3	0.8	1.3
B3	0.0	3.2	0.9	1.3
B4	0.0	1.8	1.0	1.3
C1	0.0	2.2	0.7	0.6
C2	0.0	2.2	0.9	1.2
C3	0.0	2.5	0.8	1.4
C4	0.0	2.3	1.2	1.7
D1	1.0	2.0	0.8	1.2
D2	0.0	2.6	0.9	1.5
D3	0.0	3.2	1.1	1.6
D4	0.0	2.7	0.9	1.5

[a] Harvested 118 days after emergence.

Previous analysis of B.L. 656 showed it to be R-free (Delfel *et al.*, 1970). In the present study, R was found in approximately half of the composite samples (four to six plants) of this line. Analysis of the leaflets of individual plants from 1970 harvest No. 6 (Table I) showed that three of the 16 plants contained R. Since *T. vogelii* tends to be self-pollinating (Martin and Cabanillas, 1970), the R contamination is more likely from an admixture of seeds from other lines than from an outcrossing of B.L. 656. Although the R-bearing plants of B.L. 656 have essentially the same amount of R as those of P.I. 257533, their D contents are higher, so the contaminating seeds are probably not P.I. 257533. Analyses of the leaflet samples from individual plants clearly show sufficient variability in both lines to promise improvement by further breeding work.

Although the concentration of rotenoids in total leaflets from plants of both lines increased initially and then leveled off, the rotenoid concentration based on the whole plant decreased slightly during late stages of growth (Figure 5). This change

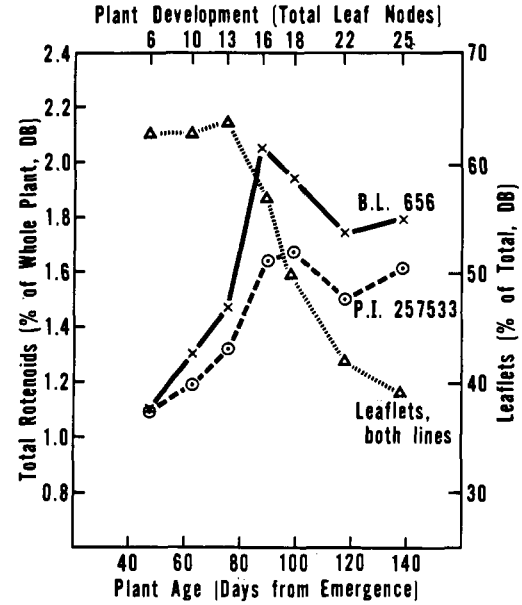

Figure 5. Total rotenoid concentration, proportion of leaflets in whole plants and plant development, 1970 plantings

reflects a decrease in the percentage of leaflets in the whole plant from about 65 to 40% and an increase in stem percentage from 15 to 44%. A major factor in this change is abscission of lower leaves. The increase in total leaflet rotenoid concentration indicates that harvest should be late in the growing season. However, in southern areas with long growing seasons, leaf abscission eventually equals leaf production and further delay of harvest would be of no advantage (Higgins and Decker, 1971).

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