Occurrence of Rotenoids in Some Species of the Genus *Tephrosia*

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Leaves, stems, roots, seeds, and pods of 16 species of *Tephrosia* were successfully assayed for rotenoids, using a modification of the Gross-Smith red color test. While 14 species contained some rotenoids, only five contained rotenoids in their leaves. *T. vogelii* had the highest leaf rotenoid content (ranging from 0.65 to 4.25%), and appears to be a prospective commercial source of rotenone and rotenoids.

THE ROTENONE OF COMMERCE is now extracted solely from the roots of *Derris* and *Lonchocarpus* (*2*), which are grown in tropical regions of the world. The cultivation of these plants is difficult, because of the liana type of growth and the labor involved in harvesting the small, fibrous roots. These factors, and the distance involved in transportation lead to a high cost of production and an uncertain supply of the insecticidal material.

Rotenone and other rotenoids have been reported in 12 additional genera of leguminous plants, but the concentration was thought too low for commercial extraction. *Tephrosia* contains the largest number of rotenoid producing species, and several investigators (*6–8*) have suggested that some species of this genus might be possible sources of rotenone. Twenty-two species of *Tephrosia* have been reported as fish poisons (*6*), and 21 species have been listed as containing rotenone or related compounds (*3*). Few reports give quantitative results for these species, however.

A project for development of a commercial source of rotenone from among the species of the genus *Tephrosia* has been initiated at this station. As a part of this investigation, all available species of *Tephrosia* were screened with respect to the amount and distribution of rotenoids within the plant. Data resulting from a study of 16 species are presented in this paper.

Materials and Methods

Seeds of *Tephrosia* were obtained from 13 tropical countries and started in a greenhouse; 3 weeks after germination the seedlings were transferred to a field of Toa silty clay in a completely randomized design. All plants were fertilized with 9–10–5 commercial fertilizer at 6, 12, and 18 weeks after transplanting. Dusting sulfur was applied periodically for mite control.

Entire plants were taken as samples after 6 months, when the majority of species were flowering or fruiting. Additional seeds and pods were collected as these matured. Samples were separated into roots, stems, leaves, seeds, and pods for analysis.

The samples were dried for 24 hours at 80° C. in a forced-air oven, then ground in a Wiley mill to pass a 40-mesh screen. Portions (100 mg.) were extracted with acetone for 4 hours in Soxhlet vessels. The extract was made up to volume with acetone, and aliquots were taken for the colorimetric determination of rotenoids (*5*).

Histochemical studies of *T. vogelii*, *T. candida*, and *T. noctiflora* were performed by making freehand sections of fresh material from all parts of the plants and applying the Durham test for rotenoids (*4*). Examinations with a dissecting microscope were made to determine the location of any positive reaction to this test.

Table I. Rotenoid Content[a] of Plant Parts of 16 Species of *Tephrosia* Grown at the Federal Experiment Station, Mayaguez, P. R.

Species	Rotenoid Content, % Range			
	Leaves	Stems	Roots	Seeds
T. cathartica Urb.	0	0	0	0
T. sp.	0	0	0	0
T. grandiflora Pers.	0	0	0	0.65–0.85
T. maxima	0	0	0	0.90–1.05
T. candida DC.	0	0	0	0.90–1.15
T. subtriflora	0	0	0.05–0.10	0
T. villosa Pers.	0	0	0.10–0.15	0
T. wallichii Graham	0	0	0.20–0.25	0
T. holstii	0	0	0.05–0.10	0.90–1.00
T. brachydon	0	0	0.20–0.25	0.25–0.40
T. toxicaria (Sw.) Pers.	0	0	1.10–1.40	1.15–1.25
T. cinerea Pers.	0.50–0.70	0.25–0.35	1.00–1.25	1.00–1.10
T. purpurea Pers.	0.65–0.80	0.40–0.65	0.70–0.95	1.60–1.80
T. spaerospora	1.20–1.40	(Whole plant)		
T. noctiflora Boj.	1.30–1.60	0.20–0.35	0.40–0.50	1.65–2.00
T. vogelii Hook f.	0.65–4.25	0.40–0.90	0.30–0.45	0.90–1.40

[a] Figures on dry weight basis.

Results

The results of assays on 16 species of *Tephrosia* are presented in Table I. These species can be separated into groups according to the distribution of rotenoids within the plants. One group does not contain significant amounts of rotenoids (less than 0.1%, dry weight basis) in any plant part; a second group has rotenoids limited to the seeds; a third group has rotenoids limited to the roots; a fourth group has rotenoids both in the seeds and roots; and in a fifth group rotenoids are widely distributed throughout the plant.

Over 500 plants of *T. vogelii* were sampled, and the rotenoid content of the leaves was found to vary from 0.65 to 4.25%. These plants consisted of 12 separate introductions, and the mean rotenoid content for the introductions varied from 1.10 to 3.37%.

The parts of *T. vogelii*, *T. candida*, and *T. noctiflora* were examined histochemically, to determine the location of rotenoids within the plant organs. Rotenoids were found to be restricted to large isodiametrical cells which occur at random in the parenchymatous tissue in all parts of the three species examined. In general, a positive reaction to the Durham test for rotenoids occurred only in organs which yielded rotenoids on extraction. Thus a positive Durham

test was obtained with seeds of *T. candida*, but with no other plant part of this species. The roots, stems, leaves, and seeds of *T. vogelii* all gave a positive response with both histochemical and extraction methods. The pods of *T. vogelii* (and all other species examined) failed to show the presence of rotenoids when assayed by the extraction method, but when tested by the Durham method they gave an ephemeral, but definitely positive, reaction in the few idioblasts which were present.

Discussion

The quantitative results presented are based on production of red color in the presence of rotenone and the necessary reagents. Other rotenoids, such as elliptone, deguellin, and dihydrodeguellin, give a red color under the conditions of the test. Mixtures of rotenone and other rotenoids are known to occur in several genera (*6*, *8*), and the ratio of the mixtures may vary. Because the results presented are based on a standard curve of pure rotenone, and the toxic principles may vary in proportions in different species, these results should be taken as an approximation of actual rotenoid content.

Eight of the 16 species studied have been reported to contain rotenone or rotenoids in some plant part (*3*). The remaining eight species have not been previously reported to contain rotenoids, although *T. grandiflora* has been reported to be of insecticidal value (*6*). Of the five species found to contain rotenoids in the leaves, only *T. vogelii* had been previously reported (*1*).

The amount of rotenoids and the range of variation in the rotenoid content of leaves of *T. vogelii* indicate that this species is the most promising as a new source of rotenone. Preliminary trials have shown that this species can be grown in the continental United States and is readily adaptable to mechanized cultivation.

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FERTILIZER-INSECTICIDE MIXTURES

Stability of Certain Insecticides in Mixtures with Fertilizers

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Relatively few data are available on the stability of insecticides in mixtures with fertilizers. Therefore the stability of a number of fertilizer-insecticide mixtures was studied at 30° and 100° C. using a chromatographic procedure to detect the appearance of degradation products and organic chlorine analyses to determine decreases in organic chlorine contents. Most mixtures had good stability for at least 1 month at 30° C. with losses of organic chlorine varying from negligible to more than 50% on aging for 8 months. At 100° C. nearly all mixtures were unstable, and lost approximately 20% of their organic chlorine in the first 24 hours. Instability of insecticides in fertilizer mixtures was related to minor characteristics of the fertilizer, such as iron content and surface acidity.

COMMERCIAL PRODUCTION and use of fertilizer-pesticide mixtures in the United States have increased rapidly since 1950. Consumption amounted to nearly 109,000 tons in the year ended June 30, 1956 (*14*). Such mixtures contain almost exclusively the recently developed chlorinated hydrocarbon insecticides and are used mainly for the control of soil insects and pests. Berry (*2*) showed that such mixtures were used in 34 of 43 states and were approved by state agricultural authorities in 20 states. Because the rate of insecticide application is usually not more than a few pounds per acre, utilization of fertilizer as a carrier has the advantage of convenience, uniformity of distribution, and potential economy in the over-all cost of insecticide and fertilizer application.

The literature contains little experimental evidence relative to the compatibility of insecticides with fertilizers and their stability in fertilizer mixtures (*7*). Certain data, however, are available on DDT (*4*) and aldrin (*5*). The present work was initiated to expand the data on compatibility and stability. An effort also was made to evaluate some of the causes of the instability of insecticides in the presence of fertilizer salts.

Materials and Methods

Technical grades of the insecticides listed in Table I were used in this study. The listed materials include those reported to have been the most frequently used in the formulation of commercial fertilizer-pesticide mixtures in 1956 (*2*). The test fertilizers are described in Table II. The 5–20–20, 8–16–16, and 10–20–0 grades were experimental products formulated from ordinary and triple superphosphate, potassium chloride, ammonium nitrate, and aqua ammonia. The other materials and mixtures were commercial products of unknown formulation.

Fertilizer-pesticide mixtures containing 0.5 to 1% pesticide were prepared by adding 10% solutions of pesticide in a volatile solvent such as acetone to 900-gram samples of fertilizer in a continuously rotating laboratory mixer. Any residual solvent was removed by sub-