Residues Following Treatment of Sugar Cane with Radioactive Diquat to Control Flowering

H. Wayne Hilton,[1] Quan H. Yuen,[1] Beverly V. Tucker,[2] Donald E. Pack,[2] and J. Nils Ospenson[2]

Diquat [6,7-dihydrodipyrido(1,2-*a*:2′,1′-c)pyrazidinium cation] can be used to control flowering of sugar cane. Residues were determined in sugar cane plants sprayed with ^{14}C-labeled diquat at the rate of 0.5 pound per acre (twice the recommended commercial dose). At times from 1 week to 8 months after treatment, entire plants, plus processed fractions, were analyzed for total radioactivity. Within 8 months the radioactivity in all usable fractions decreased to below the limit of detectability of 0.01 p.p.m. calculated as diquat. Since at least 8 months would elapse from the application of diquat for control of flowering to harvest, there would be no detectable residues of diquat in the harvested crop. Since ring-^{14}C-labeled material was used, no metabolites containing the pyridine ring or fragments thereof remain in the usable portions of the crop.

Photoinduction of floral primordia in sugar cane (*Saccharum* spp.) can be prevented by artificial lights at night during the critical inductive period, leaf desiccation with contact herbicides or withdrawal of irrigation water, or chemicals that inhibit photosynthesis (Burr *et al.*, 1957).

Flowering of sugar cane in Hawaii is initiated between September 1 and 20 in plants older than 6 months. Floral intensity, as numbers of flowering stalks (also called tassels or arrows) per unit area, varies with variety, temperature, elevation, sunlight, and probably soil and nutrients (Coleman, 1963). Flowering stalks lose apical dominance and shoots develop from lateral buds; the altered stalks are more easily broken by wind, and the quality of the juice from the lateral shoots is poor. The net result is a loss of sugar-storage capacity for the unit area.

Artificial lighting as a flowering inhibitor is uneconomical. To some extent, it has been possible to select hybrid varieties that have a relatively low flower intensity and, in irrigated areas, to regulate the applied water. However, better means have been sought to provide greater certainty in prevention of flowering.

Vegetative productivity may be increased up to 20%—about 2 tons of sugar per acre—if a period of at least 6 to 12 months follows the floral-control treatment. Thus, during Hawaii's 2-year crop cycle, only first-year treatment increases yield.

Two types of chemical treatment have been reasonably effective in control of flowering: contact herbicides (such as oils, pentachlorophenol, and Versene), and herbicides that are photosynthetic inhibitors (such as monuron and diuron). Only monuron at 4 pounds per acre has been used commercially. Chemical control of flowering has apparently not been investigated for other grass species.

The bipyridinium herbicides, diquat [6,7-dihydrodipyrido(1,2-a:2′,1′-c)pyrazidiinium cation] and paraquat (1,1′-dimethyl-4,4′-bipyridinium cation), are strong contact herbicides that also inhibit photosynthesis. They effectively control sugar cane flowering at rates of application as low as 1/8 pound per acre (Hawaiian Sugar Planters' Association, 1962; Nickell and Tanimoto, 1964; Tanimoto and Nickell, 1967). Treatment with these herbicides, by aircraft over the sugar cane fields, must be made between September 1 and 15 in Hawaii.

The present paper reports results from a test in which diquat, labeled in 1, 3, 4, 9, 10, 12, 13, and 14 positions with ^{14}C, was applied to sugar cane foliage. Samples of whole sugar cane, bagasse, sirup, and filter cake were analyzed at intervals up to 8 months for total radioactivity. The use of ^{14}C-labeled diquat made possible detection not only of residues of the parent compound but also residues of any metabolites. In a study of this type, it is necessary to label the most stable part of the molecule to ensure that the maximum number of metabolites, exclusive of small 1- or 2-carbon fragments, are detected. Since the most stable parts of the diquat molecule are the pyridinium rings, the labeled material used in this study was labeled in these rings.

EXPERIMENTAL

Treatment. Diquat dibromide monohydrate-1,3,4,9,-10,12,13,14-^{14}C, of specific activity 8.99 mc. per gram (3.26 mc. per mmole), was synthesized by Imperial Chemical Industries, Agricultural Division, Jealott's Hill Research Station, Bracknell, Berkshire, England. Tergitol NPX surfactant was obtained from the Union Carbide Chemical Co. All other chemicals were of reagent-grade quality.

Each plant (variety H 37-1933)—9 feet tall and consisting of five individual stalks—was covered with a plastic-film sheath and sprayed through a fitting at the top of the sheath to simulate aircraft application. The plants were sprayed with pure radioactive diquat at 0.5 pound per acre, double the expected commercial rate. Each plant received 5.21 mg. of the cation (10.24 mg. of the salt). The aqueous spray solution also contained 0.1% (v./v.) Tergitol NPX surfactant. Twelve test plants were treated at a single location at the Hawaiian Sugar Planters' Association Kunia Substation on Oahu, Hawaii. The plot also included untreated controls.

Analysis. On day 7, 14, 28, 56, 84, 140, 213, and 234 after treatment, one entire plant was cut at ground level; all adhering green and dry leaves were retained. Single plant weights ranged from 5 to 14 kg. depending on age and number of stalks per plant. Duplicate plants were taken for the 7-, 14-, 28-, and 234-day harvests, and untreated plants were processed as controls at 7 and 234

[1] Experiment Station, Hawaiian Sugar Planters' Association, Honolulu, Hawaii 96822
[2] Ortho Division, Chevron Chemical Co., Richmond, Calif.

days. Suckers from treated plots were also sampled at 234 days. The total material from each plant was chopped [first in a silage chopper (Silver Manufacturing Co., Salem, Ore.) and then in a Buffalo chopper (J. E. Smiths' Sons' Co., Buffalo, N. Y.)] to a consistency of fine chopped fodder, subsampled to 5000 grams, and frozen in plastic bags.

Samples were processed into various fractions representative of normal sugar-mill processing: chopped whole sugar cane, extracted fiber (bagasse), clarified concentrated juice (sirup), and the dried filter cake from calcium hydroxide clarification of the juice. The juice was extracted from whole chopped sugar cane at 10,000 p.s.i. in a Carver laboratory press, heated to boiling, and adjusted to pH 8.0 with calcium hydroxide. The limed juice was cooled and centrifuged to precipitate the heat- and lime-coagulated solids which constituted the filter cake. This cake was dried at 103° C. and ground to a powder. The clarified juice was concentrated to one fifth of its original volume, or to about 40 to 70% sucrose as determined with a sugar refractometer.

At Chevron Chemical Co. laboratories, representative 0.25- to 1.0-gram samples were wet-combusted using combustion reagents described by Van Slyke *et al.* (1951). The combustion gases were absorbed in a solution of ethanolamine and methyl Cellosolve (1 to 4 v./v.). An aliquot of the absorption solution was diluted with methyl Cellosolve and added to the scintillation solution, which consisted of 2,5-diphenyloxazole (PPO) in toluene. Samples were counted with a Nuclear-Chicago liquid scintillation counter, Model 725. The counting efficiency was approximately 62%. The limit of detection calculated as diquat is 0.01 p.p.m. for the whole sugar cane, bagasse, and sirup, and 0.02 p.p.m. for the filter cake. Counts were made so as to yield 25% confidence limits at the 95% level. Fortified knowns gave recoveries in the range of 93 to 103%.

RESULTS AND DISCUSSION

Diquat applied by aircraft over sugar cane produces a rapid leaf-contact and upper (wrapped)-stalk scorching with minimum penetration of the dense leaf canopy to the soil or lower portions of the stalks. The yellow, desiccated appearance of the treated field changes to the normal green color in 2 to 3 weeks as emerging new leaves without symptoms cover the treated ones. The burn persists on the contacted portions until the leaves fall. Since the lowest sugar cane leaves abscise one at a time at intervals of 10 to 12 days as new ones unroll at the apex, a complete turnover of leaves occurs in about 6 months.

Assuming diquat to be strongly adsorbed to the plant surfaces, and not translocated to new growth, total residues would be expected to diminish as new tissue replaced that burned by the chemical. It was also necessary to establish that little or no translocation occurred, and that little or no radioactive diquat or its radioactive metabolites persisted in the fiber or in the processed products. Diquat is expected to be used at least 8 months before harvest in the 2-year crop, as the beneficial effects result only from control of flowering in the first year.

The data in Table I show that no significant radioactivity was found in the whole sugar cane, bagasse, or sirup 234 days after treatment. Thus, 8 months after treatment with diquat at twice the recommended commercial rate, there were no detectable residues of diquat or metabolites in any components that might be consumed by humans or animals. The residues are actually low after even 140 days, varying from 0.01 to 0.03 p.p.m. calculated as diquat. Also, 8 months after spraying, whole sugar cane, bagasse, and sirup samples from suckers in treated plots did not contain detectable traces of radioactivity, the limit of detection being 0.01 p.p.m. calculated as diquat. Untreated controls showed no radioactivity above background levels.

Table I. Residue of ^{14}C in Sugar Cane after Treatment with Radioactive Diquat[a]

Days after Treatment	Residue Calculated as P.P.M. Diquat			
	Total chopped sugar cane	Bagasse fiber	Filter cake	Clarified concentrated juice
7	0.15	0.46	7.83	0.13
14	0.24	0.66	7.11	0.23
28	0.18	0.50	6.60	0.11
56	0.10	0.18	3.09	0.05
84	0.08	0.17	2.72	0.05
140	0.01	0.03	0.38	0.01
213	0.01	0.04	0.80	0.01
234	0.00	0.00	...	0.00

[a] 1 kg. of chopped sugar cane yields approximately 120 grams of bagasse fiber (dry weight), 3.0 grams of dry filter cake, and 170 grams of clarified concentrated juice.

The filter cake contained the greatest activity per unit weight, being approximately 15 times greater than that found in the bagasse. Although the filter cake contained the greatest quantity of radioactivity per unit weight, the bagasse—the major product—contained the major portion of the total radioactivity. The proportion of radioactivity in each of the three processed fractions remained remarkably constant throughout the test as long as significant radioactivity remained. An average of 76% of the total radioactivity was found in the bagasse samples, 12% in the filter cake, and 12% in the sirup. A separate assay at 84 days of fiber from leaves and stalks showed that 91% of the radioactivity present at that time was contained in the leaf portion.

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