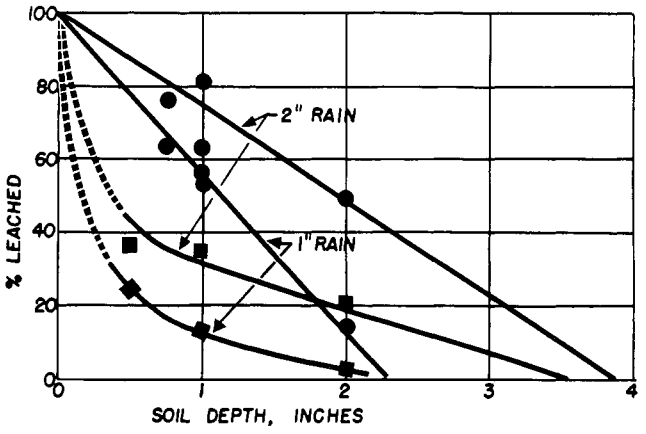

Figure 3. Effect of soil depth on leaching

● *N*-1-naphthylphthalamic acid
■ Experimental form with stearic acid

naphthylphthalamic acid and this experimental formulation have been compared on three soil types: the standard soil, a silt soil from Mississippi, and a black loam from Illinois. Under the standard test conditions, the experimental formulation with stearic acid retarded leaching on the soils tested (Table IV).

The incorporation of water-insoluble acids to control the soil mobility of *N*-1-naphthylphthalamic acid is a more versatile technique than the electrolyte method. Wettable powder and granular type formulations are being tested.

The authors believe that the effectiveness of stearic and similar insoluble organic acids, in decreasing the mobility of *N*-1-naphthylphthalamic acid in soil, is due to the following factors: The waxy additive tends to coat and protect the particles of herbicide from contact with the soil and water; the additive furnishes hydrogen ions, which react with the soil and decrease its tendency to ionize the herbicide; and one equivalent of a weak acid (such as stearic) is as effective as a manyfold excess of a much stronger acid (such as sulfuric). This is explained by the fact that when the organic acid additive and the herbicide are properly blended, each particle carries its own antileaching agent, the hydrogen-donating activity of the additive is confined to soil in the immediate vicinity of each particle. In the case of water-soluble acids the effect is dissipated over the entire soil surface.

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Table IV. Effect of Soil Variations

Soil	*N*-1-Naphthylphthalamic Acid, % Leached	Experimental Formulation	
		Leached, %	I.F.[a]
Standard sandy loam	56	14	4.0
Mississippi silt	68	24	2.8
Illinois black loam	55	20	2.8

[a] See footnote *a*, Table I.

REPELLENT ANALYSIS

Separation and Differentiation of Mixtures of 2,3,4,5-Bis(Δ²-butenylene)tetrahydrofurfural, Its Alcohol, and Its Acid, by Paper Chromatography

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2,3,4,5-Bis(Δ²-butenylene)tetrahydrofurfural has been proposed for use as a fly repellent. A method whereby it and its alcohol and acid derivatives may be readily separated by paper chromatography is given.

A paper chromatographic procedure for the separation and identification of 2,3,4,5-bis(Δ²-butenylene)tetrahydrofurfural, its alcohol, and its acid is outlined. The aldehyde, also known as R-11, has been proposed as a fly repellent for dairy cattle.

Apparatus

Tank and accessories, as described by Mitchell (*2–4*).

Filter paper, Whatman No. 1, 8 × 8-inch sheets.

Sprayer, for application of immobile solvent and chromogenic agents. (A suitable glass sprayer is supplied by University Apparatus Co., Berkeley, Calif., which also supplies glass troughs and clips.)

Reagents

1. Immobile Solvent. Dimethyl formamide (Matheson, Coleman, and Bell, Inc., East Rutherford, N. J.), 20% (v./v.) solution in ethyl ether, ACS grade.

2. Mobile Solvent. Mixed octanes, commercial grade (Phillips Petroleum Co., Bartlesville, Okla.).

3. Chromogenic Agents. (*a*) Dissolve 1 gram of potassium periodate in 100 ml. of water. (*b*) Dissolve 1 gram of potassium permanganate and 2 grams of sodium carbonate in 100 ml. of water. Immediately prior to spraying, mix four parts of (*a*) with one part (*b*) (*1*).

4. Standards. Dissolve 0.2 gram of each substance, and a mixture of 0.2 gram each of all three substances, in 10-ml. volumes of ethyl acetate. Dilute 0.5 ml. of each of the four solutions to 10-

ml. volumes with ethyl acetate (0.001 ml. is equivalent to 1 γ of each substance). Store solutions in small glass-stoppered containers.

Procedure

On a line 1 inch from the bottom of an 8 × 8 inch sheet of filter paper, mark equal intervals, with a hard pencil, beginning about 1 inch from either side. By means of a capillary pipet, spot 0.001-ml. portions of solutions at the marked intervals. If use of a larger volume of sample is desired, allow spot to dry, and respot with a similar volume on the same location.

Add about a 0.5-inch layer (about 50 ml.) of mobile solvent (reagent 2) to each trough.

Clip top edge of paper to the rod, which will be used to suspend it in the tank; invert and clip bottom edge to an auxiliary glass rod suitably supported in a well ventilated hood. Impregnate the paper with immobile solvent (reagent 1) by spraying rapidly and uniformly in horizontal passes, beginning at the spotted base line and progressing down to the opposite edge of the paper. Immediately invert the sheet, unclip it from auxiliary glass rod, and transfer the paper to tank so that lower edge dips into the mobile solvent. Seal glass cover on the tank with cellophane tape. Allow it to stand while the mobile solvent front ascends the paper. Five minutes is approximately minimum, at which sensitivity is best (about 1 γ) but separation is poorest. One hour, or until solvent front closely approaches (but does not reach) the top of the sheet, is approximately maximum, at which separation is best but sensitivity is poorest—i.e., for the aldehyde, 3 to 4 γ; for the alcohol, 2 to 3 γ; and for the acid, 1 to 2 γ. From solutions of the pure compounds, separation is relatively good; this makes it practicable to take advantage of the better sensitivity of a comparatively short development period.

Remove the paper from the tank, mark solvent front, and hang it from a rod in the hood until dry (15 to 30 minutes). Spray the paper with mixed chromogenic agents (reagent 3). Allow it to stand for 3 to 4 minutes, place a pan underneath, and wash the paper with a gentle stream of water from a wash bottle to remove excess chromogenic agent.

Table I shows R_f values for the three compounds.

Table I. R_f Values for 2,3,4,5-Bis (Δ^2 - butenylene)tetrahydrofurfural, Its Alcohol, and Its Acid

Substance[a]	R_f Values Average	Range[b]
2,3,4,5-Bis(Δ^2-butenylene)tetrahydrofuroic acid	0.07	0.03–0.13
2,3,4,5-Bis(Δ^2 - butenylene) tetrahydrofurfuryl alcohol	0.44	0.25–0.57
2,3,4,5-Bis(Δ^2 - butenylene) tetrahydrofurfural	0.83	0.65–0.94

[a] 98 observations for each substance, temperature 23° C.

[b] Range of separation of substances in 14 chromatograms appears wide when tabulated or graphed; however, when chromatograms are viewed individually, range is not particularly noticeable. Average R_f values best illustrate order of separation and distance of substances from one another, as viewed in any chromatogram. Uncontrollable variables, which cause the high or low values of the range, affect all compounds in chromatogram similarly.

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REPELLENT RESIDUES

Determination of Small Quantities of 2,3,4,5-Bis(Δ^2-butenylene)tetrahydrofurfural (Repellent R-11) in Milk

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The insect repellent 2,3,4,5-bis(Δ^2-butenylene)tetrahydrofurfural, (R-11), was tested for its appearance in milk, when used as a spray for dairy cattle. It was administered daily for 5 weeks to dairy cows at a level 10 to 20 times that needed for effective fly control. Analysis of milk samples throughout the treatment period failed to show the presence of any of the repellent within the sensitivity of the method, which was 0.1 p.p.m. The analysis was carried out by separating the butterfat from the milk, reacting an extract of this fat with 2,4-dinitrophenylhydrazine, and measuring the resulting product spectrophotometrically. The validity of determinng total R-11 content of milk by analysis of the fat only was demonstrated by measurement of the partition of R-11 between butterfat and aqueous phases.

THE BUTADIENE-FURFURAL condensation product, 2,3,4,5-bis(Δ^2-butenylene)tetrahydrofural, hereafter referred to as R-11, has been shown to be an effective insect repellent providing protection against houseflies and stableflies (*3*), particularly when added to synergized pyrethrins (*5*), and offers promise as a repellent for use in dairy barns. Before R-11 could be used as an ingredient in dairy sprays, however, additional information was required on its possible appearance in milk following application to lactating animals. Milk samples from animals sprayed with R-11 were analyzed to determine if the repellent is excreted in the milk, and if so, to evaluate the amount which might be present.

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Testing Program

Treatment of Animals Five purebred Holstein cows were selected from a production herd, which was hand milked three times each 24 hours. The cows were kept on sudan grass and native grass pastures except for periods when they were brought into the barn for milking and supplemental feeding.