

ing at 11.8 μ , use is made of absorption bands beyond 12 μ both to correct for this interference and to estimate the amount of these additional pesticides.

ACKNOWLEDGMENT

The authors wish to thank Mr. John H. Jones of the Division of Cosmetics, Food and Drug Administration, for his assistance in the preparation of the manuscript. The authors also wish to thank Dr. Thomas H. Harris of the Pesticide Regulation Section, Plant Pest Control Branch, U. S. Department of Agriculture, Washington, D. C., for supplying several of the pesticide formulations.

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A NEW COLORIMETRIC METHOD FOR PYRETHRINS*

By H. L. WILLIAMS, W. E. DALE,† and J. P. SWEENEY‡ (Stored-Product Insects Laboratory, § Savannah, Ga.)

The extensive use of pyrethrum for the control of insects that attack stored-food products greatly emphasizes the need for a sensitive and specific quantitative analytical method for residues of this insecticide. A

* Presented at the Sixty-ninth Annual Meeting of the Association of Official Agricultural Chemists, Oct. 10-12, 1955, at Washington, D. C.

† Transferred April 25, 1955.

‡ Transferred April 23, 1956.

§ This is one of the field stations of the Stored-Product Insects Section, Biological Sciences Branch, Marketing Research Division, Agricultural Marketing Service, U. S. Department of Agriculture.

review of the methods published reveals that none are completely satisfactory with regard to both sensitivity and specificity. Both the A.O.A.C. mercury reduction method (1) and the Seil method (2) are time consuming and lacking in sensitivity. A hydroxylamine method developed by Edwards and Cueto (3) is not specific. Another procedure reported by Cueto and Dale (4) has proved useful in the analysis of paper coatings. This method, based on reaction with sodium sulfide, can be used for the analysis of allethrin, furethrin, and pyrethrum, but does not differentiate the three insecticides. A method reported by Levy and Estrada (5), which makes use of the reaction of the pyrethrins with sulfur and alkali, is likewise not specific. The spectrophotometric procedure of Shukis, *et al.* (6) requires that the sample be free of any ultraviolet-absorbing material that might interfere with the measurement of the pyrethrins.

In 1952, Jones, *et al.* (7) published a method for the determination of piperonyl butoxide. This method is based on the measurement of the blue color produced when piperonyl butoxide is heated with a reagent consisting of orthophosphoric acid, glacial acetic acid, and tannic acid. It was subsequently observed by Cueto (8) that the pyrethrins themselves when present in quantities larger than 1 mg reacted with the Jones reagent to produce a color, and attempts were made to utilize this reaction for a differential analysis of piperonyl butoxide and the pyrethrins. This approach was unsuccessful, however, because the colors developed for piperonyl butoxide and for the pyrethrins were not additive. Further work by the authors revealed that when the tannic acid was not included in the Jones reagent, a more intense pyrethrins color was obtained. Piperonyl butoxide, on the other hand, gave no color in the absence of tannic acid. By making use of this information, a new color reagent for the pyrethrins was developed. The reagent used in the method described here consists of 80 per cent orthophosphoric acid and 20 per cent ethyl acetate by volume.

METHOD

APPARATUS

- (a) *Spectrophotometer*.—Beckman Model B or equivalent.
- (b) *Test tubes*.—15×150 mm, matched.
- (c) *Shaking machine*.—Emerson Type S or equivalent.

REAGENTS

- (a) *Color reagents*.—80 ml 85% orthophosphoric acid + 20 ml reagent grade ethyl acetate.
- (b) *Skellysolve F*.—A hydrocarbon fraction with a boiling range of 30–60°C.
- (c) *Deobase*.—A purified grade of kerosene.
- (d) *Pyrethrum concentrate*.—20% pyrethrins concentrate, analyzed by the A.O.A.C. method.
- (e) *Primary standard*.—Dilute 100 mg pyrethrins (based on the A.O.A.C. analysis) to 1 l with Skellysolve F.
- (f) *Secondary standard*.—Dilute 25 ml of the primary standard to 100 ml with Skellysolve F. One ml of secondary standard contains 25 mmg pyrethrins.

PREPARATION OF STANDARD CURVE

Pipet 1, 2, and 3 ml portions of the secondary standard into 30×150 mm test tubes. Remove the solvent by careful evaporation on a water bath, taking care not to overheat the pyrethrins. (It is advisable to withdraw the tubes before the solutions have reached dryness and to complete the final stages of the evaporation by allowing the liquid to make contact with the hot side walls of the tubes.)

Add 5 ml of the color reagent, using a pipet with a wide orifice. Place the tubes on a shaking machine for 1 min. and then immerse them in a boiling water bath for exactly 3 min. The rose-red color which develops is stable indefinitely. Remove the tubes from the bath and transfer the contents to the matched 15×150 mm test tubes. Centrifuge at medium speed for 15 min. or until no bubbles remain in the solutions.

Allow the solution to cool to room temperature and read the absorbance in a spectrophotometer at 550 mμ against a blank of 5 ml of the color reagent.

ANALYSIS OF SAMPLE

Grind the grains and extract them with Skellysolve F for 1 hr in a tumble-action mechanical extractor. (It is advisable to use at least 2 ml of the Skellysolve F for each g sample.) Extract materials such as cotton, wool, and paper in a Soxhlet extractor.

Evaporate an aliquot of the extract, containing preferably 25–100 mmg pyrethrins, on a water bath to 25–35 ml. Transfer to a 30×150 mm test tube and complete the evaporation. (In the case of samples containing appreciable amounts of oil or color, such as grains, it is advisable to add 0.2 ml deobase.) Add 5 ml of the color reagent and develop the color as described in PREPARATION OF THE STANDARD CURVE.

Prepare a sample blank by carrying a sample of untreated material, equivalent in weight to that of the sample being tested, through the analytical procedure.

Determine the pyrethrins content of materials such as kraft paper, wool, and cotton cloth by direct comparison of the color developed with that developed when an external standard is similarly treated.

NOTE.—Materials such as grains, burlap, and cardboard contain substances that suppress the pyrethrum color, and consequently internal standards must be used. They are most conveniently prepared by the addition of a known amount of pyrethrins standard to extracts of untreated samples.

Calculation.—Mmg pyrethrins in aliquot of test sample = mmg pyrethrins added to aliquot of untreated sample $\times (c-a)/(b-a)$, where a = absorbance of sample blank; b = absorbance of untreated sample + standard; and c = absorbance of test sample.

DISCUSSION

Development of the Color Reagent.—The initial step in the improvement of the Jones reagent was the testing of a number of combinations of ingredients. In each case, 100 mmg of pyrethrins was heated with 5 ml of the color reagent being tested. The results, as shown in Table 1, indicated that the most promising results were given by combinations of orthophosphoric acid with either acetyl triethyl citrate, glacial acetic acid, or ethyl acetate. Further work revealed that the maximum color was produced by a mixture consisting of 80 parts by volume of orthophosphoric acid and 20 parts of ethyl acetate.

TABLE 1.—*Effect of composition of reagent on color produced*

EXPT. NO.	REAGENT (PER CENTS BY VOLUME)	COLOR
1	20% acetyl tributyl citrate + 80% orthophosphoric acid	light pink
2	20% acetyl triethyl citrate + 80% orthophosphoric acid	rose red
3	20% isoamyl acetate + 80% orthophosphoric acid	none
4	20% acetic anhydride + 80% orthophosphoric acid	pink
5	20% glacial acetic acid + 80% orthophosphoric acid	rose red
6	20% ethyl acetate + 80% orthophosphoric acid	rose red
7	100% orthophosphoric acid	light pink
8	100% phenylethyl acetate	pink
9	100% carbolic acid	none

Characteristics of the Color Reaction.—The red color produced by the reaction of the pyrethrins with the orthophosphoric acid-ethyl acetate reagent has an absorption maximum at 550 m μ (Fig. 1). Beer's law is obeyed up to a concentration of 100 mmg and as little as 7 mmg can be accurately measured (Fig. 2).

A study of the effect of heat on the development of the pyrethrins color indicated that the peak of the color intensity was obtained by heating for periods of from 1 to 3 min. at 100°C. At temperatures below 100°C. the color developed more slowly, and its maximum intensity was less than that obtained when the reaction was carried out at 100°C. Prolonged heating, even at temperatures as low as 60°C., resulted in gradual decomposition of the color complex.

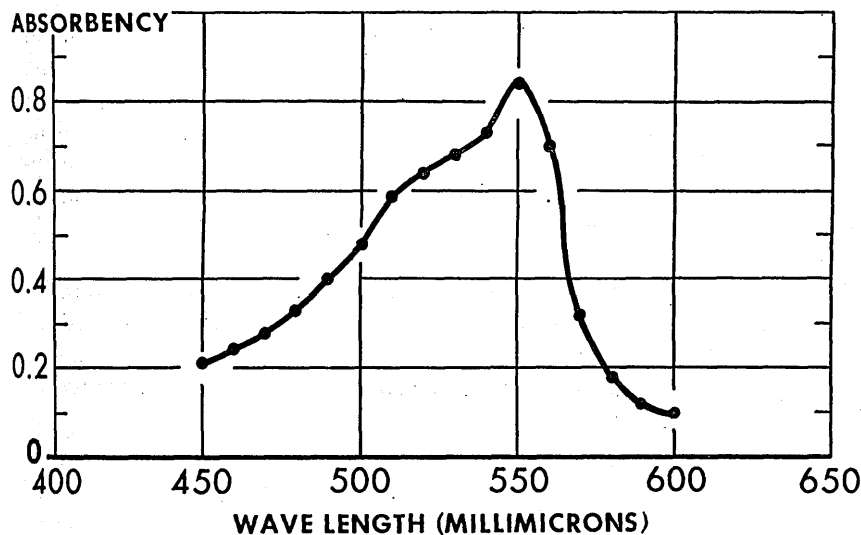


Fig. 1.—Absorption spectra of pyrethrins color developed with orthophosphoric acid and ethyl acetate.

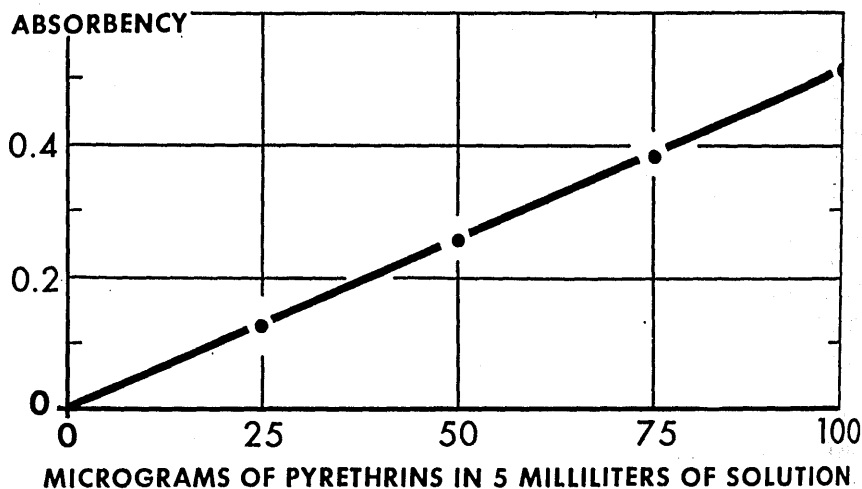


FIG. 2.—Relationship of absorbance at 550 $m\mu$ to concentration of pyrethrins.

The specificity of the reaction was determined by conducting a number of tests with different compounds. As shown in Table 2, of the compounds tested, the pyrethrins cyclothrin and pyrethrolone produced a red color.

Color development was suppressed, however, by compounds frequently employed as synergists for the pyrethrins. No color developed when piperonyl butoxide was present in sufficient excess, such as at a ratio of 10 parts to 1 part pyrethrins. Sulfoxide, if present in a 5 to 1 ratio, reduced color development by 30 per cent.

When the pyrethrins were separated from synergist compounds, normal color reactions were obtained. Tests were conducted in which piperonyl butoxide was separated from the pyrethrins by chromatography, and good recoveries of the pyrethrins were obtained. Work is still being done on the separation of the pyrethrins from other synergists and on the application of chromatography to the determination of the pyrethrins in samples treated with synergized pyrethrins. Results of these studies will be presented at a later date.

To determine whether the color reaction was given by both pyrethrins I and pyrethrins II, two concentrates of known pyrethrins composition were analyzed. As shown in the following tabulation, two mixtures containing pyrethrins I and II in two different proportions reacted to produce the same color absorbance:

<i>Ratio I:II</i>	<i>Absorbance of 100 mmg</i>
1:1.02	0.51
1:1.54	0.51

The color reactions of pyrethrins I and II were investigated further by separating the two forms of pyrethrins on a chromatographic column

TABLE 2.—*Color reaction of various compounds with the orthophosphoric acid-ethyl acetate reagent*

COMPOUND	COLOR OBTAINED
Pyrethrins	red
Cyathrin	red
Pyrethrolone ^a	red
Furethrin	yellow, turning to green
Ryanodine	brown
Toxaphene	turbidity
Allethrin	none
Natural chrysanthemum monocarboxylic acid	none
Natural chrysanthemum dicarboxylic acid	none
Synthetic Cinerin I	none
Cinerolone	none
Technical allethrolone	none
Technical chrysanthemum monocarboxylic acid	none
Rotenone ^a	none
Lindane	none
Methoxychlor	none
DDT	none
Chlordane	none
Dieldrin	none
Aldrin	none
Sulfoxide	none
Piperonyl butoxide	none

^a Tested by Pesticide Chemicals Section, Entomology Research Branch, ARS, U.S.D.A.

of Celite and eluting pyrethrins I with *n*-hexane and pyrethrins II with ethyl ether according to the procedure of Lord, *et al.* (9). Both fractions produced the rose-red color with the absorption spectrum characteristic of the total pyrethrins. As a further check, after the two forms of pyrethrins were separated according to the method of LaForge and Haller (10), both of the forms produced colors which had identical absorption spectra.

Characteristics of the Color Complex.—If, after the development of the pyrethrins color, the solution was made alkaline by the addition of pellets of sodium hydroxide, the red color complex was converted to a yellow oil-like substance. If the solution was then extracted with ethyl ether, the color complex was found in the ether layer. If the ether was evaporated and the residue made acid by the addition of a mineral acid, the red color reappeared. This indicated that the pyrethrins color complex acts like an indicator: it is red in the presence of an excess of hydrogen ions and yellow if the OH ions are predominant.

Application of the Method.—Table 3 gives the results of recovery studies in which the method was used on samples treated with known quantities of the pyrethrins. Good recoveries were obtained in all cases.

TABLE 3.—*Recovery of added pyrethrins*

TEST SAMPLE	PYRETHRINS ADDED	PYRETHRINS RECOVERY	RECOVERY	AV. RECOVERY	ERROR
	<i>mg</i>	<i>mg</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Wool	23.5	25.0	106	105	6.69
	47.0	52.5	112		
	70.5	68.0	98		
Kraft paper	23.5	23.0	98	97	5.19
	47.0	48.0	102		
	70.5	64.5	92		
Botany wool	23.5	24.0	102	102	4.42
	47.0	50.5	107		
	70.5	69.0	98		
Muslin cloth	23.5	22.5	96	100	4.04
	47.0	47.5	101		
	70.5	73.0	104		
Raisins	23.5	22.5	96	97	3.14
	47.0	47.0	100		
	70.5	66.5	94		
Lima beans	104	86	83	—	—
Cow peas	104	82	79	—	—

The method should prove useful for the analysis of pyrethrum concentrates because of its simplicity, sensitivity, and reproducibility. Duplicate results vary by no more than 2 per cent.

Although cyclothrin gives a positive test, it can be differentiated from pyrethrins because of the longer period of heating required to produce the maximum color. One hundred mg of pyrethrins will react with the color reagent to produce the maximum color after a heating period of 1 minute. An equivalent quantity of cyclothrin requires a much longer heating period for peak color development.

Since allethrin gives a negative reaction, it can be differentiated from both pyrethrins and cyclothrin by this method.

SUMMARY

A new colorimetric method has been developed for the determination of pyrethrins. It is based on the measurement of the red color produced when pyrethrins are heated with a reagent consisting of 80 per cent orthophosphoric acid and 20 per cent ethyl acetate.

The color is stable indefinitely and appears to be fairly specific for pyrethrins.

Piperonyl butoxide suppresses the pyrethrins color; however, it can be separated from pyrethrins by chromatography.

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IDENTIFICATION OF STORED PRODUCTS INSECTS BY THE MICROMORPHOLOGY OF THE EXOSKELETON

II. ADULT ANTENNAL CHARACTERS

By MANION M. JACKSON, ALDRICH F. RATAY, and EDWARD J. WOZNICKI
(Food and Drug Administration, Department of Health,
Education, and Welfare, Philadelphia, Pa.)

This paper represents a completed unit in a series of micromorphological observations of common insect contaminants encountered in cereal grains, grain products, and stored products in general. Specifically, this paper deals with characters of the adult insect antennae which have diagnostic value in fragment identification. The general organization and background of these coordinated fragment studies have been given by Heuermann and Kurtz;¹ the application of this information has been discussed by Kurtz and Harris.²

The antennae, like other segmented appendages, may be recovered as intact structures from coarsely ground products, or when finely comminuted products become reinfested. Because of the variation in the number of joints or segments, and particularly, the wide variation in form, the antennae are frequently used by the taxonomist in classification. Many of these characteristics have value in fragment identification, especially

¹ HEUERMAN, R. F., and KURTZ, O. L., *This Journal*, 38, 766 (1955).

² KURTZ, O. L., and HARRIS, K. L., *ibid.*, 38, 1010 (1955).