heads, distilled whole tall oils, distilled tall oils with high rosin acid contents, and distilled tall oils with high fatty acid contents were tested. Of the materials tested, the most efficient and most economical stabilizers were: two distilled whole tall oils, Indusoil M-28 and Acintol D; and Acintol FA2, a distilled tall oil fatty acid. Stability data are presented in Table VI on 5% malathion dusts made with six different lots of LVM attapulgite using 5% of Indusoil M-28 as a stabilizing agent. Comparison of the stabilizing efficiency of Indusoil with diethylene glycol revealed that the lower-cost Indusoil was more efficient. However, as with glycols, the tall oil compounds did not completely inhibit the decomposition.

Treatment of LVM attapulgite with varying amounts (up to 8%) of Indusoil had no effect on the surface acidity as measured by the method of Walling (*13*). This would indicate that the mode of stabilization is other than the neutralization of surface acidity, as is indicated for the glycol deactivators (*6*, *12*).

In Figure 3, data are presented on the stabilizing effect of Indusoil M-28 on 5% malathion dusts made with two different samples of the same montmorillonite (carrier *E*). Clay samples were chosen having low and high activity as indicated in Figure 2. Data in Figure 3 indicate that, although Indusoil was effective in decreasing decomposition of malathion with montmorillonite, commercial use of this technique may not be feasible, since decomposition is unpredictable because of apparent variations in the montmorillonite activity. The activity of attapulgite is believed to be consistent enough to predict the extent of decomposition in a specified period. Overages of the insecticide can then be impregnated so that the product will meet the analysis guarantee.

Data are presented in Table VII on the stabilizing efficiency of Indusoil on 25% malathion-attapulgite dusts. Three different lots of attapulgite were used, and the samples were stored at room temperature (25° C.) for 6 and 12 months. Again, the data indicate that Indusoil is effective in reducing decomposition, and the extent of decomposition for a specified period of time is fairly consistent and predictable.

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INSECTICIDE-SYNERGIST ANALYSIS

Chromatographic Separation and Colorimetric Determination of Pyrethrins and Piperonyl Butoxide Mixtures

PAUL T. ALLEN, HERMAN F. BECKMAN,[1] and J. F. FUDGE

State Chemist Laboratory, Texas Agricultural Experiment Station, College Station, Tex.

THE INSECTICIDE referred to as pyrethrins is a mixture of four organic compounds (*4*), extracted from the dried flowers of *Chrysanthemum cineraefolium*. Piperonyl butoxide (3,4-methylenedioxy-6-propylbenzyl) (butyldiethylene glycol) ether is a synthetic organic compound used as a synergist for pyrethrins and similar compounds. Pyrethrins are used either alone or with piperonyl butoxide for the control of household and stored grain insects because of fast knock-down and relatively low toxicity to warm-blooded animals. The cost of pyrethrins sprays may be reduced with piperonyl butoxide since lower concentrations may be used without loss of over-all insecticidal potency.

In the colorimetric determination of pyrethrins, color formation is inhibited by piperonyl butoxide. The reverse is true when determining piperonyl butoxide. The quantitative determination of pyrethrins in samples containing piperonyl butoxide has not been possible. High boiling hydrocarbons also interfere with the development of a clear-colored solution in the determination of pyrethrins or piperonyl butoxide.

Information in the literature suggests that pyrethrins may be separated from piperonyl butoxide by partition chromatography, but no one has suggested this as a method for separate determination.

Jones *et al.* (*6*) investigated the color-forming characteristics of piperonyl butoxide as a methylene dioxyphenyl compound. Jones' method for the analysis of piperonyl butoxide, with some variations, was used in this experimental work. Other colorimetric methods available for the analysis of piperonyl butoxide are: Beroza's (*2*) method using chromotropic and sulfuric acids and Blum's (*3*) method using gallic and sulfuric acids. These can lead to charring and interferences when other organic compounds are present.

Piperonyl butoxide may be separated from certain other insecticides by partition chromatography (*7*). Samuel (*7*) used the Harris (*5*) procedure for the analysis of certain chlorinated hydrocarbon insecticides and found that only a few compounds would interfere. He used the Jones method for colorimetric measurement of piperonyl butoxide.

[1] Present address, Pesticide Residue Research, University of California, Davis, Calif.

The method described for the determination of pyrethrins and piperonyl butoxide mixtures involves chromatographic separation of the insecticide from the synergist followed by colorimetric determination of each. There is also a separation of the pyrethrum components. A postulate is presented as to which part of the pyrethrin molecule is involved in the formation of color. The color formed follows Beer's law over a given range.

The colorimetric method by Williams *et al.* (*10*) for the analysis of pyrethrins consists of evaporating a carrier solvent from the pyrethrins, adding a color reagent, and reading the absorbance at 550 mμ.

Sweeney and Williams (*9*) investigated a colorimetric method for determining cyclethrin. They showed that cyclethrin could be separated from sulfoxide and piperonyl butoxide using an absorption chromatographic method. Their report indicated no attempt to measure the recovery of the synergists. The color reagent used for cyclethrin determination developed a similar color with pyrethrin and pyrethrolone. Cinerin I and allethrin failed to respond to the cyclethrin reagent.

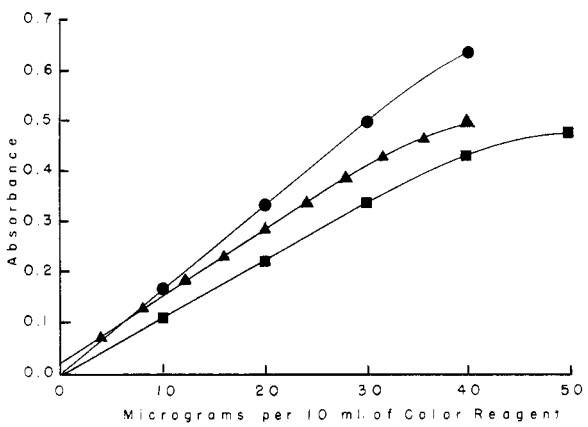

Figure 1. Standard curves for pyrethrins and piperonyl butoxide

- ● Pyrethrins not through chromatographic column
- ■ Pyrethrins through chromatographic column
- ▲ Piperonyl butoxide

Reagents

Mobile solvent, *n*-hexane saturated with nitromethane.

Dye solution, prepare 250 mg. of D. and C. violet No. 2 in 100 ml. of mobile solvent, filter, and store in a glass-stoppered bottle. (This dye may be purchased from Pylam Products Co., 779 Greenwich St., New York 14, N. Y.)

Pyrethrins color reagent, 200 ml. ethyl acetate mixed well with 800 ml. 85% orthophosphoric acid. This is the same reagent as given by Williams *et al.* (*10*).

Piperonyl butoxide color reagent, 0.25 gram of tannic acid was dissolved in 75 ml. of glacial acetic acid and added to 175 ml. of 85% orthophosphoric acid. (This color reagent must be prepared fresh daily.) This is somewhat of a departure from the Jones method in that only 0.025 gram of tannic acid is used to 20 ml. of glacial acetic acid and 80 ml. of orthophosphoric acid.

A standard solution of pyrethrins was prepared so that each milliliter would contain 100 μg. of pyrethrins (pyrethrins I and II plus cinerins I and II) and 25 μg. of a violet dye.

A standard solution of pyrethrins and piperonyl butoxide was prepared so that each milliliter would contain 100 μg. of pyrethrins, 1000 μg. of piperonyl butoxide, and 25 μg. of violet dye.

Silicic acid, Mallinckrodt No. 2844.

Apparatus

A means for providing pressure with a regulator and gage is necessary.

Blender, Waring Blendor—speed controlled by Variac.

Partition chromatographic column, Fischer and Porter Ultramax, $^3/_4 \times 18$ inch, fitted with Teflon connections and sintered glass filter.

Spectrophotometer, Beckman Model DK-2 or equivalent.

Method

Preparation of Column. The column is prepared as for the determination of aldrin and dieldrin by partition chromatography (*1*). This column has given good results in the separation of pyrethrins and piperonyl butoxide.

About 35 grams of silicic acid is placed in a Waring Blendor. Then 125 to 150 ml. of the mobile solvent is added and the two components are mixed well. To thicken the slurry slightly, a quantity of nitromethane (about 30 ml.) is added while the blender is running. If too much nitromethane is added, more silicic acid will thin out the slurry. The well blended slurry is quickly transferred to the chromatographic column by means of a large funnel. This quantity of reagent gives a full column without any waste or spilling. Bubbles trapped in the column are removed by stirring with a glass rod. Air pressure of 3 to 5 pounds is used to pack the silicic acid. The resulting column (about 20 cm. in length) has a rate of flow of about 2 drops per second.

The surface of the silicic acid should be level to give a level band of dye.

Preparation of Standards and Standard Curve. A fresh standard solution must be used when the standard curves are prepared, since both the pyrethrins and piperonyl butoxide show decomposition when stored, especially when exposed to light.

Aliquots of 1, 2, 3, 4, and 5 ml. of the mixed standard solution are passed through silicic acid columns. The fractions which are collected in 50-ml. Erlenmeyer flasks for analysis of pyrethrins, are: 5 ml. before the violet dye band, all of the violet band, and 5 ml. after the violet band. After the three fractions for pyrethrins are collected, a 5-ml. portion is taken in a 125-ml. Erlenmeyer flask, an 80-ml. fraction in a 100-ml. volumetric flask, and a 10-ml. fraction in a 125-ml. Erlenmeyer flask last. The fraction in the volumetric flask is brought to volume with mobile solvent and a 10-ml. aliquot is transferred to a 125-ml. Erlenmeyer flask. The solvent is evaporated from all of the Erlenmeyer flasks. Ten milliliters of the pyrethrins color reagent are added to each of the three 50-ml. Erlenmeyer flasks, and 25 ml. of the piperonyl butoxide color reagent are added to each of the three 125-ml. Erlenmeyer flasks. After addition of the color reagent, the pyrethrins and piperonyl butoxide are swirled for exactly 1 minute and placed in a gently boiling water bath. The pyrethrins are heated for 5 minutes and the piperonyl butoxide for 12 minutes. The samples are then cooled to room temperature. A spectrophotometric determination is made at 550 mμ for pyrethrins and at 625 mμ for piperonyl butoxide. Standard curves of absorbance *vs.* concentration are made. Recovery of piperonyl butoxide from column is quantitative.

Analysis of Sample. Because pyrethrins–piperonyl butoxide mixtures are commonly marketed in 1 to 5 and 1 to 10

ratios, the curves were established using 1 to 10 ratio. Proper dilutions are made in the 1 to 5 ratio mixtures.

For samples containing 0.06% pyrethrins and 0.6% piperonyl butoxide, about 8 grams is weighed into a 50-ml. volumetric flask. After addition of 0.5 ml. of 2.5 mg. per ml. violet dye, the flask is made to volume with mobile solvent and mixed well. A 3-ml. aliquot is passed through a silicic acid column using mobile solvent for flow. Fractions are taken from the column and evaporated. The color is developed and analyzed spectrophotometrically. The absorbances are compared with those of the standard curve.

Results and Discussion

A commercial sample of a pyrethrins–piperonyl butoxide mixture that had failed to respond to the pyrethrins color test was placed in a silicic acid column using *n*-hexane saturated with nitromethane for elution. Fractions were collected and treated by the colorimetric method for pyrethrins. A positive test was obtained.

Twenty-five micrograms of violet dye, used in the location of the pyrethrins band, was treated with the color reagent for pyrethrins, and it did not interfere with color formation.

An aliquot of the pyrethrin standard solution was introduced into a column. The mobile solvent was used to elute the column. The fraction containing the violet dye and the 5-ml. fractions before and after the violet dye was evaporated and treated with the color reagent. This process was repeated with a graded series of aliquots. The resulting absorption-concentration curve for the chromatographed pyrethrins followed Beer's law (Figure 1) up to 30 μg. per ml. of color reagent. Data in Figure 1 show the recovery of pyrethrins before and after chromatographic separation. The recovery is consistent at about 65% (Table I).

When aliquots of the mixed standard solution were passed through the silicic acid column, the piperonyl butoxide was located in the gamma–benzene hexachloride region, which is immediately after the violet dye, but in a wider band. The colorimetric method of Jones for piperonyl butoxide was used with modifications.

To show that the separation of the pyrethrum extract is complete and quantitative in one pass through the column, the following procedure was followed. Five hundred micrograms of pyrethrins were placed in the column and recovered in the usual way. The solution was evaporated to dryness and 5 ml. of mobile solvent added. From this solution, 3 ml. was returned to a column and 2 ml. was treated with the color reagent to determine the recovery from the first column. Sixty-six per cent of the pyrethrins was recovered from the first column. Recovery from the second column was 100%.

Formation of the pink color by pyrethrins is inhibited by the presence of rotenone which causes an opaque peach-colored solution. When a solution containing pure rotenone and pyrethrins is passed through a silicic acid column, the pyrethrins are quantitatively recovered. Interference is encountered when rotenone-related resins are present. Since activated charcoal removes rotenone-related resins in the analysis for rotenone, removal of the related compounds of rotenone by activated charcoal and the rotenone by partition chromatography was attempted in that order. When this was tried, however, the charcoal also adsorbed the pyrethrins.

Schechter (*8*) estimated the per cent ratio of pyrethrins I and II to cinerins I and II to be about 70 to 30%, which is approximately the same as the recovery from the silicic acid column. Pyrethrins and cinerins differ in their side chains; pyrethrins have a conjugated side chain and the cinerins do not. The cinerins may be retained by the silicic acid column. Allethrin, the allyl analog of cinerin I, when treated with the pyrethrin color reagent, produced only a faint yellow color. The side chain may be involved in the formation of color, since allethrin does not have a nonterminal double bond or a conjugated side chain. This has also been suggested by Sweeney and Williams.

A time-of-heating absorption curve (Figure 2) was established for piperonyl butoxide using 16 and 120 μg. per ml. of color reagent. Both concentrations reached a peak absorbance and leveled in 10 to 12 minutes. The heating period of 5 minutes suggested by Jones *et al.* apparently caused inaccurate results. This is true for both technical and purified tannic acid. In making the absorbance measurements in the time-of-heating curve, the maximum absorbance at 4 and 6 minutes was 650 mμ; at 8 minutes, 645 mμ; at 10 minutes, 635 mμ; and at 12 and 14 minutes, 625 mμ (Figure 3). A

Table I. Recovery of Pyrethrins after Chromatographic Separation

Added, μg.	Recovered, μg.	Recovery, %
200	125	62.5
	137	68.5
	140	70.0
	137	68.5
	128	64.0
	Av. 133	Av. 66.7
300	203	67.7
	192	64.0
	195	65.0
	200	66.7
	198	66.0
	195	65.0
	Av. 197	Av. 65.7
400	263	67.8
	249	62.3
	255	63.8
	257	64.3
	250	62.5
	250	62.5
	Av. 254	Av. 63.9

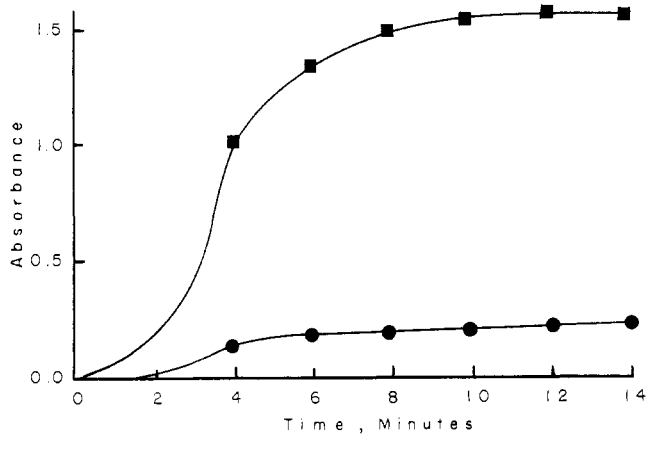

Figure 2. Effect of time on formation of color at 100° C.

■ 120 μg. piperonyl butoxide per ml. color reagent
● 16 μg. piperonyl butoxide per ml. color reagent

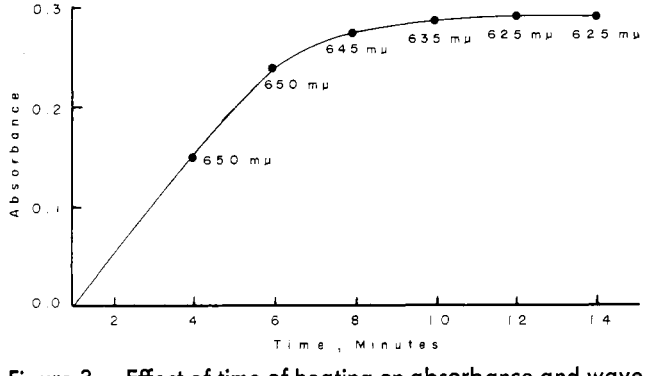

Figure 3. Effect of time of heating on absorbance and wave length of absorbance of piperonyl butoxide at 12 μg. per ml.

higher absorbance value was obtained using the purified tannic acid than the technical tannic acid, but only when the color reagent as described here was used —0.1 g. tannic acid to 30 ml. of glacial acetic acid and 70 ml. of orthophosphoric acid.

However, the color intensity was essentially the same for the technical piperonyl butoxide (80%) as for the 100% pure material when the color reagent contained purified tannic acid.

An absorption-concentration curve (Figure 1) was then established for piperonyl butoxide, using the mixed standard solution and a heating period of 12 minutes. Beer's law was obeyed up to 28 μg. of piperonyl butoxide per ml. in the final aliquot.

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INSECTICIDE EFFECTS ON PLANT GROWTH

Effect of Various Insecticides on Growth and Respiration of Plants

E. P. LICHTENSTEIN, W. F. MILLINGTON,[1] and G. T. COWLEY

Departments of Entomology and Botany, University of Wisconsin, Madison, Wis.

The effects of insecticidal soil residues on the plant growth, morphology (corn and peas), and respiration of root tips of corn, oats, peas, and cucumbers have been investigated. The plants were grown in a "soil" of minimum sorptivity (quartz sand) which had been treated with 18 different insecticides at 30 p.p.m. Although the conditions under which the plants grew were extreme, many insecticides either had no inhibitory effect on plant growth or caused a growth increase. In general, insecticides of the chlorinated hydrocarbon group inhibited plant growth less than the organophosphates and Sevin. Insecticides, which inhibited growth, affected corn more than peas. A significant reduction in the rate of respiration of root tips was caused by lindane (corn, oats), dieldrin (corn, oats), *p,p'*-DDT (oats, peas, cucumbers), methoxychlor (peas), Systox (oats, peas), and Sevin (peas, cucumbers). Parathion significantly increased the respiration of corn root tips.

IN MOST CASES the concern about insecticidal residues has been limited to the problem of food contamination: residues on or in crops and insecticidal contamination of meat and milk. However, the effect of pesticidal residues on the biological complex of soils in which our food supply originates also presents a problem of primary importance. Many authors during the past 15 years have investigated the effect of insecticidal residues in soils on crop yield as well as on growth and phytotoxicity (*1–3, 7*). The respiration of root tips of tree seedlings, as affected by various pesticides, has been investigated, to some extent, by Voigt (*9*). As a result of direct applications of insecticides to soils or to crop spraying, many of our soils contain insecticidal residues. It is, therefore, important to find out if and to what an extent crops growing in these soils might be affected.

[1] Present address, Department of Biology, Marquette University, Milwaukee, Wis.

An attempt has been made to evaluate the effect of various insecticides on the growth and respiration of corn, oats, peas, and cucumbers. These plants were grown in quartz sand which had been treated with unusually high concentrations of insecticides. If no effect was noticeable under these conditions, probably no harm would be done to crops growing in soils to which insecticides had been applied at "normal" rates. However, if considerable damage to the plant tissue occurred, further investigations would be necessary. Conversely, certain chemicals might affect plants in a beneficial way.

Procedure

Effect of Insecticides on Plant Growth

1. To test the effect of insecticides on plant growth, quartz sand was treated at 30 p.p.m. with various toxicants. Eleven chlorinated hydrocarbon insecticides were used [lindane, chlordan, aldrin, dieldrin, heptachlor, heptachlor epoxide, Shell–4402 (1,3,4,5,6,7,8,8-octachloro - 3a,4,7,7a - tetrahydro - 4,7-methanophthalan), toxaphene, endrin, *p,p'*-DDT, and methoxychlor], five organophosphate insecticides [parathion, methyl parathion, phorate, malathion, and Systox (*O,O*-diethyl *O*-ethyl-2-mercaptoethyl phosphorothioate)], and two carbamates [Sevin (*N* - methyl - 1-naphthyl carbamate) and Isolan], as well as the hydrolysis product of Sevin (1-naphthol). All the insecticides (analytical grade) were applied in an acetone solution to the quartz sand. Control sand was treated with acetone only (*4*). After the acetone had been removed by a gentle air stream on a specially designed apparatus (*5*), the sand was further mixed on paper and then placed in 6-inch wide, nonglazed clay pots. For each insecticidal treatment, two pots were filled with quartz sand, which was then wetted with a complete plant nutrient solution.