Table III. Bioassay Data for Carbinols

Compound No.[a]	ΣLog K_0'[b]	—Log LC_{50} *Metatetranychus citri*	—Log LC_{50} *Tetranychus bimaculatus*	—Log LC_{50} *Heliothrips haemorrhoidalis*	LC_{50}, P.P.M., *Culex quinquefasciatus*	Topical LD_{50}, γ/G., *Musca domestica*
4449	0.00	0.16	0.00⁻	0.05	10–100	>500
282	1.00	0.82	1.22	0.03	10	>500
4531	0.50	0.30	0.47	0.54	10–100	>500
4525	1.00	0.00⁻	0.00⁻	0.00⁻	10	>500
4526	1.50	0.70	0.70	0.55	1–10	>500
3295	2.00	0.82	1.05	0.85	1–10	>500
4532[c]	1.00	0.00⁻	0.00⁻	0.00⁻	10–100	>500
4527	1.39	0.05	0.00⁻	0.70	1–10	>500
4535	0.39	0.22	0.07	0.09	10–100	>500
6116	0.78	−0.08	0.17	0.29	10–100	>500
6117	1.11	0.22	0.22	0.09	10–100	>500
4539	1.11	0.27	0.17	−0.15	10	>500
6119	1.56	0.00⁻	0.00⁻	0.00⁻	1–10	>500
4536[d]	0.89	0.17	0.27	0.20	1–10	500
4538	1.39	0.75	1.05	−0.08	1–10	>500
6120	1.28	0.66	0.33	0.00⁻	1–10	>500
6118	1.78	0.60	0.62	0.00⁻	1–10	>500
p,p'-DDT	2.50	0.00⁻	0.00⁻	3.00	0.01–0.1	1.65
FW-293	2.50	1.70	1.80	0.30	...	...

[a] See Table I for coded structures of compounds.
[b] See (*5*); this value represents sum of logarithms of van der Waals attractive forces of five substituent groups, with H = 1.00.
[c] Compound 4532 ($R_1 = R_3 = R_4 = H$, $R_2 = R_5 = Cl$) may be of questionable purity; b.p. 140–4°/1.5 mm. (not listed in Table I).
[d] Compound 4536 ($R_1 = R_3 = R_4 = H$, $R_2 = Cl$, $R_5 = CH_3$) may be of questionable purity (not listed in Table I).

cuticle to the site of action (*5*) may be the limiting mechanism in this study.

Acknowledgment

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PESTICIDE FORMULATION

Hydroquinone and Its Derivatives as Stabilizers for Pyrethrum and Allethrin

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INSECTICIDAL PREPARATIONS CONTAINING PYRETHRUM deteriorate with age and deterioration is hastened by exposure to air, heat, and sunlight. For these reasons, a stabilizer is desired to maintain reasonably good keeping qualities. In this work, hydroquinone and four of its derivatives were incorporated into pyrethrum dusts and tested as stabilizers. Two of the compounds were also studied as stabilizers for allethrin, which is closely related to some of the components of pyrethrum. The insecticidal, active components of pyrethrum are pyrethrins I and II and cinerins I and II, to which allethrin is closely related.

The stability of pyrethrum, which has been the subject of numerous investigations, is dependent on the physical state of the formulation. Fine dust mixtures and ground or pulverized pyrethrum flowers lose insecticidal activity more rapidly than whole flowers (*22*).

Moreover, artificially prepared dusts consisting of pyrethrum extracts combined with inert diluents lose activity more rapidly than ground pyrethrum flowers (*19, 21*), partly because more surface is exposed. Gnadinger and others

Because insecticidal preparations containing pyrethrum deteriorate with age, it is desirable to incorporate a stabilizer to maintain good keeping qualities. Hydroquinone, 4-methoxy-2-propenylphenol, 2,5-di-*tert*-butylhydroquinone, *tert*-butylhydroquinone, and butylated hydroxyanisole were tested as stabilizers for pyrethrum. The last two compounds were also tested as stabilizers for allethrin. Dusts containing the insecticide and additives were aged for long periods of time and then tested biologically. Hydroquinone, *tert*-butylhydroquinone, butylated hydroxyanisole, and 4-methoxy-2-propenylphenol were effective in stabilizing pyrethrum, but *tert*-butylhydroquinone and butylated hydroxyanisole were ineffective in stabilizing allethrin.

have reported (*12*, *13*) that ground pyrethrum flowers and kerosine solutions of pyrethrum lose activity when stored in the dark and in the absence of oxygen.

The nature of the decomposition of pyrethrins is not clearly understood. Tattersfield and Martin (*22*) stated that the loss of activity involves reaction with oxygen other than oxidation and West (*26*) suggested that the change was due to polymerization involving the pentadienyl side chain.

Various attempts have been made to stabilize pyrethrum flowers, extracts, and formulated dust mixtures; and a number of patents have been issued covering the use of stabilizing materials combined with pyrethrum (*4*, *7–10*, *15*, *17*, *20*, *24*, *25*, *27*). Several workers have indicated that polyhydroxybenzenes, such as pyrocatechol (*16*) and hydroquinone (*3*, *11*), are effective in stabilizing the pyrethrins. Aminophenols (*5*), aromatic aldehydes (*23*) and certain dyestuffs (*18*) have also been found to retard the rate of decomposition of formulations containing pyrethrins.

In contrast to the relative instability of the pyrethrins, several investigators have found that allethrin is somewhat more stable. Blackith (*2*) has reported that allethrin is more stable to ultraviolet photolysis than the pyrethrins. Fales and others (*6*) observed no loss in effectiveness during storage of allethrin in Freon aerosols. According to Granett, Connola, and Lemback (*14*), allethrin is less subject to breakdown during storage and exposure to heat and light than the pyrethrins.

The present investigation was undertaken to determine the effectiveness of hydroquinone, *tert*-butylhydroquinone, 2,5-di-*tert*-butylhydroquinone, 4-methoxy-2-propenylphenol, and butylated hydroxyanisole in stabilizing pyrethrum. The work was primarily concerned with pyrethrum; but for comparison two of the compounds were also tested with allethrin. The various additives were mixed with pyrethrum extract in a mutual solvent and then deposited on Attaclay. The resulting dust formulations were aged and the rate at which pyrethrum deteriorated was determined biologically with adult male German cockroaches [*Blattella germanica* L.]. As allethrin is generally known to be a poor insecticide against cockroaches, the rate at which the formulations containing allethrin deteriorated was determined biologically with houseflies [*Musca domestica* L.].

Materials

Hydroquinone, *tert*-butylhydroquinone, 2,5-di-*tert*-butylhydroquinone, and butylated hydroxyanisole were commercial grade materials supplied by Tennessee Eastman Co., Kingsport, Tenn.

The sample of 4-methoxy-2-propenylphenol was prepared by a method described in the literature (*1*).

Attaclay is a commercial product supplied by the Attapulgus Clay Co., Philadelphia, Pa.

Unstabilized pyrethrum extract containing 2.5% pyrethrins by weight was supplied by McLaughlin Gormley King Co., Minneapolis, Minn.

Allethrin, 90% purity, was supplied by McLaughlin Gormley King Co., Minneapolis, Minn.

Preparation of Dusts Containing Pyrethrum

The dust formulations were made by preparing a solution of the additive in acetone and adding this to a solution of the pyrethrum extract in deodorized kerosine. This mixture was then sprayed onto Attaclay in a slowly rotating jar provided with a hole in the center of the lid for introduction of the pyrethrum additive-solvent combination. The dusts were prepared according to the following formula:

12.8 ml. of pyrethrum extract
200 mg. of additive
3.8 ml. of acetone
50 grams of Attaclay

Acetone was used as a solvent when it was necessary to dissolve the additive. The pyrethrum extract consisted of 2.0 grams of pyrethrins in 100 ml. of deodorized kerosine. The final dust preparation had a pyrethrins concentration of 0.5% and an additive content of 0.4%, since the acetone and kerosine were vaporized in a few days.

A second series of dusts was prepared in a similar manner using butylated hydroxyanisole and 4-methoxy-2-propenylphenol in 0.02 and 0.2% concentrations rather than 0.4%, the concentration used in the first series.

For aging, the various formulations were spread uniformly in borosilicate glass dishes, 8.5 inches in diameter, and exposed to air at 80° ± 5° F. and a relative humidity of 30 ± 10%. The mixtures were not exposed to sunlight. The dust mixtures were stirred at weekly intervals with a spatula.

Preparation of Dusts Containing Allethrin

Dusts were prepared according to the following formula:

3.3 grams of allethrin, 90% purity
0.8 gram of butylated hydroxyanisole or *tert*-butylhydroquinone as additive
25 ml. of acetone
196 grams of Attaclay (or 198 grams when no additive was used).

In the preparation of the dust mixture, the allethrin, additive, and acetone were sprayed onto 25 grams of Attaclay in a slowly rotating jar and the remaining portion of the Attaclay was blended with the primary mix in a ball mill. The resulting dust contained 1.48% allethrin and 0.4% additive.

Each of these dust formulations was placed in 12 × 20 inch trays and exposed to air at 80° ± 5° F.

Biological Tests

Tests with Dusts Containing Pyrethrum. The rate of deterioration of pyrethrum was determined biologically with adult male German cockroaches. At the termination of any given aging period, 10 cockroaches were placed in Petri dishes 100 mm. in diameter, containing 20 mg. of the insecticidal dust, and left for 24 hours. Mortality was then checked. Each test was replicated four times with 10 cockroaches per replication, or a total of 40 insects per test. This amount of dust containing 0.5% pyrethrins was determined by a series of tests as the minimum required to

Table I. Effectiveness of Hydroquinone and Its Derivatives as Stabilizers for Pyrethrum

Additive		% Mortality of Cockroaches after Indicated Weeks' Aging of Dusts										
Compound	Concn., %	1	2	3	4	5	6	8	10	12	16	24
None	...	100	100	93	58	75	..	55	60	63	55	55
Butylated hydroxyanisole	0.4	100	100	100	95	100	..	95	98	93	98	98
4-Methoxy-2-propenylphenol	0.4	100	100	100	90	100	..	100	98	100	93	93
Hydroquinone	0.4	100	100	100	95	98	..	90	98	98	95	80
tert-Butylhydroquinone	0.4	100	100	100	95	100	..	85	98	98	95	95
2,5-Di-*tert*-butylhydroquinone	0.4	100	100	88	93	83	..	73	75	75	80	65
None	...	...	100	...	60	...	54	54	..	...	..	..
Butylated hydroxyanisole	0.02	...	100	...	84	...	74	66	..	...	..	..
Butylated hydroxyanisole	0.2	...	100	...	100	...	98	100	..	...	..	..
4-Methoxy-2-propenylphenol	0.02	...	100	...	86	...	74	48	..	...	..	..
4-Methoxy-2-propenylphenol	0.2	...	100	...	98	...	98	96	..	...	..	..

obtain complete mortality of the cockroaches in 24 hours. It was assumed then that any decrease in effectiveness of a dust was due to deterioration of the pyrethrins. The results obtained are shown in Table I.

Tests with Dusts Containing Allethrin. Houseflies of the CSMA '51 (Chemical Specialties Manufacturers Association) strain were used as test insects. A 1.5% concentration of allethrin was determined as the minimum necessary to obtain a mortality of 90 to 100% in 24 hours. A drop in mortality as the materials aged was assumed to correspond to the loss in insecticidal activity of allethrin.

The dusts were tested as follows: Fifty 3-day-old housefly adults were anesthetized with carbon dioxide and placed in Petri dishes 100 mm. in diameter, containing 20 mg. of insecticidal dust. The amount of dust used was determined by preliminary trials as the minimum required for the primary concentration to give 100% mortality with freshly prepared dusts. The flies were swirled within the dishes and coated with dust and then transferred immediately into stainless steel cages 2 × 5 inches in diameter and faced on top and bottom with 14-mesh screens. The flies were provided with a 5% solution of sugar for food. Mortality was checked after 24 hours. Each test was replicated four times using four cages of 50 flies per replication or a total of 200 flies per test. The results obtained are shown in Table II.

Statistical Evaluation of Data

The data in Tables I and II were analyzed statistically. This analysis indicated that the mortality counts based on 10 cockroaches (Table I) have overlapping 95% confidence limits (binomial distribution) in all sets of four or five counts with three exceptions out of 80 sets. In the tests with flies (Table II) the 95% confidence limits of the extreme values in each set failed to overlap. This difference from the cases in which cockroaches were used is probably due to the difference in sample sizes.

Discussion of Results

The mortality response with pyrethrum alone, as illustrated in Table I, seemed to confirm previous findings (*21*, *23*). A marked drop in toxicity occurred between 3 and 4 weeks of aging. The insecticidal activity of the pyrethrum then appeared to remain at a fairly constant level for the duration of the test, which was 24 weeks. Hydroquinone, butylated hydroxyanisole, *tert*-butylhydroquinone, and 4-methoxy-2-propenylphenol in 0.4% concentration gave stability to pyrethrum for this period. The least efficient was 2,5-di-*tert*-butylhydroquinone, which had relatively little effect. In 0.02% concentration, butylated hydroxyanisole and 4-methoxy-2-propenylphenol slowed the deterioration rate of the pyrethrum; but in 0.2% concentration, both materials were very effective, as the insecticidal efficiency of the dusts containing them decreased very little over 8 weeks, when the test was terminated.

The data obtained with pyrethrum dusts cannot be compared directly with those obtained using allethrin, because different test insect species were used and the tests were conducted at different times. The investigation was primarily concerned with the stabilization of pyrethrum. Cockroaches were used in this study because of the ease of handling. At the completion of this work, it was decided to investigate the effect of the two best pyrethrum stabilizers on the stabilization of allethrin. In this case houseflies were used rather than cockroaches, because the latter respond rather poorly to allethrin.

The data shown in Table II indicate no significant difference in activity of the freshly prepared allethrin dusts and those aged for 24 days. After a 60-day aging period all materials were practically inactive against houseflies when the dust dosage was 20 mg., although considerable activity still existed when 40 mg. was used. Data from the table indicate that neither of the materials tested stabilized allethrin.

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Table II. Effectiveness of Hydroquinone Derivatives as Stabilizers for Allethrin

Additive	Dust Dosage, Mg.	% Mortality of Housefly Adults after Indicated Days' Aging of Dusts		
		1	24	60
None	20	88.5	94.0	0
	40	...	...	57.5
Butylated hydroxyanisole	20	89.0	88.5	0
	40	...	...	38.5
tert-Butylhydroquinone	20	85.5	85.5	0.5
	40	...	...	48.5

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PESTICIDE RESIDUES

Determination of Cycloheximide (Acti-dione) Residues in Cherries

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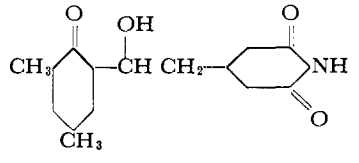
The antibiotic cycloheximide has been found very effective in the control of cherry leaf spot. Because preharvest sprays are usually required, a method for determining cycloheximide residues on the fruit was desired. Chloroform extraction of the macerated fruit, followed by S. *pastorianus* bioassays of the extracts, detects as little as 0.04 p.p.m. in the fruit. When the ripe fruit on the tree was sprayed with cycloheximide, the residues had a half-life period of about 24 hours after the first day. This rate of inactivation is much greater than the rate in aqueous solutions of about the same pH and temperature and suggests that an enzyme system of the fruit may be involved.

CYCLOHEXIMIDE (Acti-dione), an antibiotic which was isolated in 1946 from streptomycin-producing strains of *Streptomyces griseus* (*3, 7, 13*), has been shown (*6*) to have the following structure:



It has been reported effective for control of cherry leaf spot at concentrations of 0.5 to 2.0 p.p.m. (*1, 2, 5, 8–11*). Because preharvest sprays are usually required in order to obtain satisfactory control of this disease, a method for determination of cycloheximide residues on the fruit was desired.

A bioassay method for cycloheximide which employs a yeast, *Saccharomyces pastorianus*, as the test organism has been described by Whiffen (*14*). As this method has a working range from about 1.5 to 8 p.p.m., a preliminary concentration step was required. It was found that a preliminary separation and concentration could be effected by extracting the cycloheximide from the macerated fruit with chloroform, evaporating the solvent from the extract, and preparing an alcohol-water suspension of the residue for the bioassay.

Procedure for Total Amount on Outside and Inside of Fruit

Grind a 500-gram sample of cherries (including pits and juice, when present) in a Waring Blendor for 2 to 3 minutes. Transfer the macerated product to a 2-liter flask containing 300 ml. of chloroform and boil the mixture under reflux for 45 minutes. Cool to room temperature and transfer to a separatory funnel. Draw off 250 ml. of the chloroform layer and evaporate to 10 to 20 ml. on a steam bath. Remove the last of the solvent by blowing air on it at room temperature. Suspend the dry residue in 1 ml. of S.D. 3A ethyl alcohol and dilute with 9 ml. of water. Bioassay the solution against *S. pastorianus* with a sufficient number of replicate assays to obtain the desired confidence limits.

Calculation of Results

The percentage of cycloheximide which theoretically could be recovered by this method, if the entire 300 ml. of chloroform extract were recovered, would be 92.6%. The calculation is as follows:

$$\frac{X}{100 - X} = \frac{21 \times 300}{500}$$

$$X = 92.6$$

where 21 = distribution coefficient of cycloheximide between chloroform and water

300 = volume of chloroform, ml.

500 = approximate volume of cherries, grams or ml.

As only 250 ml. of the chloroform extract is actually used, the theoretical recovery is $92.6 \times \frac{250}{300}$ or 77.2%. As the actual recovery is only 75% of theoretical (Table I), a further correction factor of 0.75 is added. In verifying the procedure, the actual recovery from macerated cherries was found to be from 60 to 84% of the theoretical recovery, with an average of about 75% (see Table I). This probably means that the distribution coefficient of cycloheximide between chloroform and macerated cherries is somewhat less than the distribution coefficient between chloroform and water. As the 10 ml. of solution used for the bioassay represents 500 grams of cherries, the cycloheximide content of the fruit is calculated by means of the following expression:

$$\text{Cycloheximide content, p.p.m.} = \frac{\text{bioassay result, } \gamma\text{/ml. (or p.p.m.)}}{50 \times 0.772 \times 0.75}$$