PICKING PYRETHRUM IN KENYA.
(KENYA INFORMATION OFFICE OFFICIAL PHOTOGRAPH)

PYRETHRUM FLOWERS

SUPPLEMENT TO SECOND EDITION

1936-1945

BY

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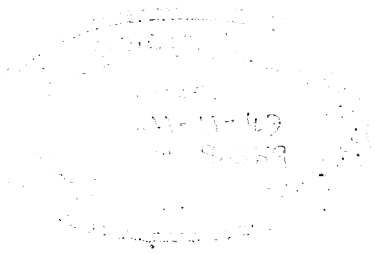

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TO

CORA C. GNADINGER

PREFACE

The first edition of "Pyrethrum Flowers" was published in September, 1933. The second edition was published in April, 1936. Since the second edition appeared, new sources of supply have been developed and technical improvements have greatly increased the uses of pyrethrum insecticides. The new material accumulated during the last nine years has been collected in this volume as a supplement to the second edition. This plan seems preferable to the publication of a third edition. Much of the early work included in the second edition is now only of historical interest; to include it in the present volume would merely confuse the reader seeking more recent information.

For convenience, the material in this supplement has, so far as possible, been arranged in the same order as in the second edition and the table of contents of the second edition has been included in the supplement.

The index to this supplement includes a completely revised index to the second edition. To facilitate use of the index, the pagination of the supplement has been made continuous with that of the second edition.

More than thirteen hundred references to the literature have been included in the supplement and these have been numbered continuously with the references in the second edition.

C. B. GNADINGER.

August, 1945.

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PYRETHRUM FLOWERS

SUPPLEMENT TO SECOND EDITION

1936 - 1945

CHAPTER XVII

COMMERCIAL SOURCES OF PYRETHRUM

The World War of 1914-1918 cut off supplies of pyrethrum from Dalmatia and enabled Japan to seize control of the American pyrethrum market. American importers of pyrethrum remained almost at the mercy of the Japanese until 1934, when a new source of supply began to develop. Importations from Japan ceased in 1941, but even before that, Kenya had become the principal supplier of pyrethrum to the United States.

KENYA PYRETHRUM

Pyrethrum cinerariaefolium was introduced into Kenya in 1928 by Gilbert Walker and by T. J. Anderson and V. A. Beckley *(1191)*.* Walker attempted to grow it on a commercial scale, but Anderson and Beckley raised it only for experimental purposes at the Scott Agricultural Laboratories of the Department of Agriculture at Nairobi. Fortunately for the development of the industry, a fair proportion of these plants flowered, the first record of pyrethrum blooming in the tropics. The pyrethrin I content of the first flowering (0.5 per cent) was sufficiently encouraging for the experimental growing to be continued, and the plot at the Scott Agricultural Laboratories was thereafter almost wholly devoted to the production of seed. Small quantities of the seed were distributed to a number of farms ranging in altitude from 5000 to 9500 feet above sea level. At the lower altitude either there was no flowering or an occasional plant bore one or two blooms. As the altitude increased, i.e., as the climate became cooler and cooler, the proportion of blooms increased until at about 8000 feet practically all of the plants bloomed.

*Numbers (italic) in parentheses refer to bibliography, page 594.

By 1931 it was evident that pyrethrum was suited to the cooler districts of Kenya. The government plots at 5800 feet altitude gave 4 hundredweight of dried flowers per acre; Walker's fields, at about 6500 feet, produced 6 hundredweight per acre. Upon these data it was decided to encourage pyrethrum growing, and a quantity of seed was obtained from the Plant Pathological Station of the Ministry of Agriculture and Fisheries, at Harpenden, Herts, England, where experiments on pyrethrum had been conducted for some time. A quantity of acclimatized seed was also available from Walker's fields. Further, in view of the high pyrethrin content of the pyrethrum grown in Kenya the importation of seed was prohibited, lest a poor strain be imported. Practically speaking, the whole of Kenya pyrethrum is derived from the two strains imported by Walker and the Kenya Department of Agriculture. A few small lots were imported before the prohibition, but these strains have almost disappeared.

The pyrethrum industry in Kenya, therefore, really dates from 1932, when expansion from the purely experimental stage to commercial production occurred. Further expansion was secured by the formation, early in 1933, of the Kenya Pyrethrum Growers' Association, now defunct, which consisted of the enthusiastic pioneers. Since then growth has been rapid, until today pyrethrum is one of Kenya's important crops in cash value and in margin of profit to the grower.

This expansion is in large measure due to the high toxicity of the Kenya flowers which has enabled them to secure a premium on the world's markets, together with high average yields per acre and efficient methods of preparation and marketing of the product.

When the industry was in its youth, many growers, attracted by the possibilities, planted pyrethrum under unsuitable conditions. While the world price was high, the low yields were profitable, but as soon as the price dropped, these fields became uneconomic. As a result, pyrethrum is today confined to a few districts which lie between 6500 and 9500 feet above sea level and where the soil is well drained. The principal pyrethrum districts are Molo, Mau Summit, Gilgil, and Limuru. Smaller quantities are grown in other places, and there is no reason why some of them should not increase considerably in importance.

According to Ball *(1161)*, "The crop will grow under a wide range of conditions in Kenya, but yields best at the higher altitudes over 7500 feet where the rainfall is fairly evenly distributed throughout the year, without a prolonged dry season. In

such areas the crop can be regarded as of permanent value occupying an important place in the economy of the farm, whereas at altitudes below 7500 feet, it is a valuable sideline, but it is doubtful whether its cultivation under 7000 feet is justified except during periods of exceptionally high prices or where other factors such as heavy rainfall and low temperatures may in part compensate for the other disadvantages of a low elevation. Unlike most other plantation crops, pyrethrum can be established at a comparatively low cost per acre and can easily be eradicated if prices or other factors cease to justify cultivation and for this reason there is always likely to be a considerable, fluctuating acreage of marginal land devoted to the crop, in addition to those areas at the higher elevations where it will occupy a permanent place in the economy of the farm."

Pyrethrum flourishes on a very wide range of soils throughout Kenya Colony. In most areas soils are well drained lateritic red loams of slightly acid reaction. Ball *(1161)* states, "On land where waterlogging is likely to occur due to hard pan close to the surface or its low-lying character, the plants will rapidly die out during the rainy season. Soils which are very loose in character and on which rooting is difficult, such as rich humus forest soils where the land has been recently cleared, do not give such high yields owing to the difficulty of maintaining a good stand of the plants and a tendency to grow vegetation rather than to produce flowers. Furthermore, the weed growth on such soils is likely to be very heavy and constant weeding tends also to reduce the stand of the plants.

"The highest yields in the colony have usually been obtained on the top of the open ridges in the high altitude areas in which the soil is a medium loam, free-draining and easy to cultivate and where the weed growth is not usually very heavy and in such areas yields as high as 1200 lbs. per acre of dry flowers per annum have been recorded over considerable acreages.

"The chief conditions necessary for the successful cultivation of pyrethrum may be summed up as follows:

1. High altitude — over 7500 feet, with evenly distributed rainfall of 40-45 inches.
2. Soils on which weed growth is not excessively heavy, and on which the plants can obtain a firm root-hold.
3. Absence of conditions likely to produce waterlogging.
4. Moderate fertility — excessive fertility in the soil is likely to produce heavy weed growth, and growth of leaf at the expense of flowers, but some growers have made the mis-

take of planting the crop on poor soils with the idea of reducing weeding costs. This is, of course, a mistake as it will lead to reduced yields.

5. Control of soil erosion. The crop is likely to suffer considerably from the effects of soil erosion and it is essential to take the necessary preventive measures such as contour ridging, etc., more particularly where the soil is shallow.
6. The establishment of a good stand in the field. An indifferent stand will never produce a full crop. When the stand becomes reduced, it is necessary to plough out the plantation and replant.
7. Clean weeding. This is absolutely essential both within the plants themselves and in the rows between the plants.

"Thorough cleaning and preparation of the land is essential, since it is not possible to perform thorough cultivation after the crop has been established and delayed attempts to eradicate couch grass usually involve the removal of a considerable number of plants. For this reason hand couching and burning should be performed if necessary, and several crops of couch grass may be removed by repeated ploughing, cultivation with spring-tined cultivators, collection and burning before the land is finally cleared. In the case of old wheat lands, germination of weed seeds by thorough cultivation should be effected. Deep cultivation, in excess of 6 in. to 8 in., does not appear to be necessary for the crop which is shallow rooted by nature, and a reasonably firm bed is desirable before the planting out so as to secure a good stand. When the land has been cleaned, it should be contour terraced prior to planting in order to prevent erosion of the soil. This is best effected by the construction of narrow base ridges which are on the usual variable grade recommended for arable crops, but in the case of exceptionally absorbent soils, level base terraces may be employed. The banks should be approximately 5 to 6 feet wide and about 18 to 24 inches high with a shallow drain 3 feet wide on the upper side. Pyrethrum can be planted over the banks and their shape should be maintained when cleaning by drawing the soil upwards. It is estimated that a native can contour 30 yards of such a bank daily by hand with a jembie and shovel, so that the cost of protection per acre is relatively small.

"It should again be emphasized that it is only advisable to construct level base terraces provided the soil is absorbent and heavy storms are not experienced. In an area of low rainfall

the level base terrace is an advantage since its construction ensures that all the rain will remain on the field.

"It is essential in the case of variable grade terraces to construct suitable outlets at the edge of the field to carry away the water which runs off, and in the construction of the terraces it is necessary to build them up well if they cross former gullies so that they may not break away during heavy rains.

"Planting should be carried out in the main general direction of the contour ridges, but provided the land has been adequately protected by ridges, there is no need to plant exactly on the contour. By following the general line of the contours, cultivation up and down slopes will be avoided.

"Experiments have been carried out in order to test the manurial requirements of the crop and up to date little beneficial effect has been derived from the use of either organic or inorganic manures. The effect of applications of phosphatic fertilizers, particularly in the form of super-phosphates, was to increase weed growth very appreciably with a consequent increase in weeding costs and rapid reduction of the stand of the plants due to these frequent weedings, and it was found that boma manure produced the same effect. A slight increase in yield of flowers was obtained in one year from the application of a top-dressing of sulphate of ammonia, but at the present time there are no indications to show that the application of manures to the crop is likely to be beneficial although it appears that it may be deleterious. It is proposed to continue these manurial experiments, until the effect has been determined under a fairly wide range cf conditions, although results to date are not in favor of the application of manures for the crop.

"The two types of planting material used are either seedlings or root divisions from existing plants. A considerable diversity of opinion exists as to which are the best to use, but it would appear that it is usually easier to establish a field with seedlings since they have a better root system and withstand transplanting better under unfavorable conditions than root divisions. At the same time, a considerable amount of labor and supervision is required for tending the seed beds and seedlings do not come into flower so quickly after transplanting as root divisions. Experiments are in progress to test which give the best ultimate yield and seedlings may be the most satisfactory in this respect. At the lower elevations, however, where a percentage of non-flowering or low producing plants is usually found in cultivations established from seedlings, it may be possible to eliminate these by

selection of material for root divisions which has shown its ability to flower and yield well under the particular conditions prevailing.

"It is important to select material for root divisions carefully from high yielding plants, and this applies particularly at the lower elevations. The common practice of removing every plant over a certain area for purposes of obtaining root divisions should not be encouraged, but more attention should be paid to the selection of planting material. Material to be used for splitting should have all flowering stems cut off before it is dug up and attempts should not be made to obtain too many root divisions from one plant. Small spindly divisions with little or no root system will be found difficult to establish and replanting and patching subsequently will be necessary. Care should be taken to ensure that every "split" (division) when planted has part of the root system of the parent plant and contains a due percentage of foliage. A large number of individual plants should be used to establish a plantation and attempts should not be made to establish a plantation from one or two high yielding plants propagated repeatedly by root divisions. The reason for this is that the pyrethrum plant is self sterile in Kenya and it is necessary to have a large number of individuals in the field so as to ensure adequate pollination which is necessary in order to obtain a high pyrethrin content in the flowers.

"A considerable number of farmers will continue to plant their plantations from seedlings on account of the greater ease with which they can be established in the field more particularly during unfavorable conditions and, also, the high cost of obtaining splits if it is necessary to buy when plants are not already available. It is of importance to use seed whose origin is known and which has been harvested from carefully selected material. Seed plots should be established from a large number of individual plants selected in the field for such characters as flower size, number of flowers, upright non-straggling habit and high pyrethrin content, and these should be planted all together in a plot by themselves and used for seed production. A large number of plants should be used for this purpose, so as to avoid any possibility of partial sterility through lack of sufficient numbers of individuals for cross-pollination.

"The seed should be harvested when the disk florets have died and tend to fall off the flower head exposing the greyish-brown seed. The seed should not be stored for long periods as it loses its viability fairly rapidly. One pound of seed is usually adequate to plant one to one and one-half acres in the field. The

seed does not usually germinate more than about 50 per cent in the seed bed and germination is slow and irregular taking about 12 to 14 days.

"The seed-beds should be made on well drained soil which is carefully and evenly leveled. Owing to the slow rate of germination of the pyrethrum seedlings, it is desirable to obtain a thorough germination of the weed seeds first before the pyrethrum seed is planted. Overhead shade should be provided for the seed-beds, and it is advisable to thatch the soil itself with a light covering of grass until the seedlings have germinated. The seed should be scattered lightly over the beds and covered with a very thin layer of soil and watered daily. The grass covering will be removed at about the 12th or 14th day when the majority of the seed has germinated. If any signs of damping off appear, the shade should be removed and the plants watered with water tinted with permanganate of potash. Pricking out of the seedlings about 3 in. x 3 in. apart is to be recommended, as this stimulates root development and will hasten the time at which they will come into bearing when transplanted into the field. Although this operation increases the labor costs, it is doubtless well worth while, since a return is secured much more quickly. It is important that the seedlings should be well grown before they are transplanted into the field and should have at least 10 or 12 leaves. If smaller seedlings are transplanted, they frequently become buried with soil when heavy rain falls at the time of transplanting into the field, and flowering will, of course, be delayed.

"A considerable amount of experimental work has been carried out on the correct spacing for pyrethrum and it would appear that in the high altitude areas a square spacing of 2 ft. x 2 ft. is likely to give the most satisfactory results. On rich forest soils, however, where rooting is loose and weeding expensive, it is advisable to adopt a somewhat wider spacing between the rows, spacing the plants closer together in the rows. In this manner more mechanical cultivation with oxen and donkeys can be performed, and there is less danger of loosening the roots of the plants. Under such conditions spacings of 2 ft. 6 in. or even 3 ft. between the rows and 16 in. to 18 in. between the plants in the rows are recommended.

"At the lower altitudes (7000 ft. to 7500 ft.) where growth is not so vigorous, a square spacing of 20 in. x 20 in. is recommended, except for rich forest soil conditions when the spacings adopted should be similar to those previously recommended.

"The fields should be carefully marked out prior to planting and it may be desirable to leave paths at intervals to facilitate handling the crop. If, however, wide spacings between the rows have been adopted, this will not be necessary, and even with a 2 ft. x 2 ft. spacing is not essential.

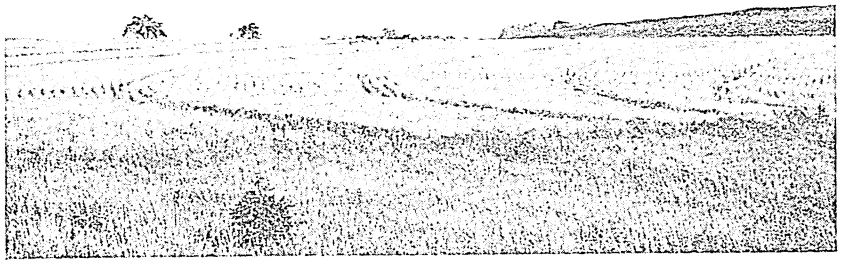
"If the field is being planted with splits, an entrenching tool should be used for digging the holes prior to planting out. Great care should be exercised in splitting up the plants to ensure that each split has a part of the parent root system and the plants should be firmly planted in the ground and the soil well tramped round them. Carelessness in firming the soil round the roots of the plant is a frequent cause for a poor stand subsequently due to high mortality. In the case of seedlings, the holes can be prepared with a pointed stick, but care should be taken to ensure that the roots point properly downwards and soil is well consolidated round the plants. Approximately 10 to 14 boys will plant 1 acre daily, but it is work that requires the closest supervision and should not be unduly hurried. Failure to secure a good stand will result in low yields and a short life of the plantation. A certain percentage of plants, more particularly on the looser soils is, however, likely to die due to defective root system or other causes and such gaps should be replanted as soon as possible before the rest of the plantation has commenced to flower. If attempts are made to patch up fields which have come fully into bearing, the results are never likely to be satisfactory owing to the difficulty of establishing the replanted material and the slow rate at which they will come into production, due to the competition from mature plants.

"Fields established from splits should, at the high altitudes, commence to flower approximately 14 weeks after they have been planted, while seedling material will probably take about 8 to 12 weeks longer. These periods are increased somewhat at the lower elevations where a percentage of the plants established from seedling material may even remain for a period of one year before commencing to flower, and the same may apply to a percentage of the root divisions unless these have been very carefully selected. There is a considerable difference of opinion as to the advisability of cutting off the flowering stems when the young plants begin to flower in order to stimulate their vegetative growth. Provided, however, good sized seedlings or splits have been used originally, there is no particular advantage in adopting this practice and picking of the flowers should commence as soon as possible.

"As previously mentioned, it is absolutely essential to keep the crop clean both from the weeds between the plants and in the plants themselves. A certain amount of mechanical cultivation can be performed with small ox cultivators pulled either by an ox or a donkey, the latter being more suitable for work between fairly close rows. While the plants are young, the majority of the weeding can be done in this manner, but care should be taken to remove all weeds growing in the plants themselves. The worst of such weeds is probably the sorrel, which grows up in the plant and eventually kills it, thus shortening the life of a plantation considerably and it is important to take care when planting from root divisions that these are free from the weed. Indigenous clovers also grow up in the plant and are extremely difficult to eradicate. On forest soils, where weed growth is heavy, it may be necessary to weed as frequently as every 14 to 17 days during the rains, but as a general rule, the period varies from a month to six weeks or more. The use of a Dutch or turnip hoe as opposed to the jembie for weeding is encouraged since it does not do so much damage and it is quite effective for the work provided weeds are not allowed to grow much beyond the seedling stage, which is essential if the plantation is to yield well. Some farmers use Planet Junior hoes pushed by hand for weeding. These are quite effective for performing the work, particularly in the early stages of the life of a plantation, but should not be used exclusively owing to their tendency to encourage the formation of a pan. It is desirable that an established plantation should be given at least one cultivation with ox cultivators annually to burst up the soil on the surface which has become packed owing to the frequent treading during picking, etc., and the operation is best performed immediately after the plantation has been cut back. The cultivators should penetrate about 2½ in. below the surface, but not deeper, otherwise they would tend to loosen the plants in the soil.

"Failure to keep pace with weed growth will sooner or later necessitate thorough deep weeding, which will remove a large percentage of the plants. The acreage of pyrethrum which will be grown on any farm is therefore likely to be limited by the amount of labor available for cleaning and picking. Under average conditions one boy is continuously employed in cleaning approximately 5 acres, although this figure will vary very widely.

"In Kenya, unlike other pyrethrum-producing countries, there is a continuous production of flowers, and a plant will carry every stage in the development of the flower head from the minutest



A KENYA PYRETHRUM FIELD IN FULL FLOWER.

button bud to fully mature heads or even seeds. Under such conditions it is impossible to harvest the crop with sickles and strip the flowers by machine; the flowers must be plucked individually in the field.

"It is important that flowers should be picked when there is no external moisture on them, either dew or rainfall, which is likely to cause heating before the flowers are dried. Such heating would be deleterious to the color and appearance of the dried product and also to its pyrethrin content. For these reasons picking should not be commenced early in the morning until the dew has dried off the flowers. The flowers should be picked into wicker-work baskets which permit of access of air at the sides so as to prevent heating and if they are not removed to the drying plant for some time, should be spread out on tarpaulins when the baskets have been filled. The average amount of flowers picked per day varies from 25 to 30 lbs. wet flowers per acre depending on the size of the crop and the nature of the labor employed and amounts considerably in excess of this may be picked during the periods of heavy flush. During the dry season the amount may fall to 12 to 15 lbs. daily.

"Observations carried out showed that pickings may range as low as 9.1 lbs. per acre of dried flowers per picking to 250 lbs. per acre dried flowers per picking during the heaviest flush periods in high altitude pyrethrum growing areas.

"Great care should be exercised over the picking operations so as to ensure that flowers only at the correct stage of maturity are harvested, i.e., when 3 to 4 rows of disk florets are fully open, and that the picking of immature flowers and buds, which would result in a lowering of the pyrethrin content, is avoided."

The increase in pyrethrin content of the flower head as it develops is about as follows *(1191)*:

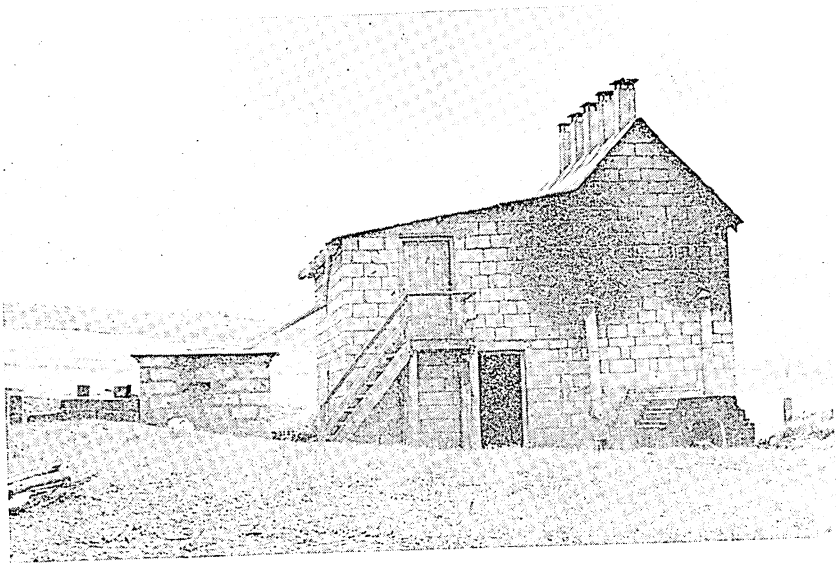
TABLE CI. WEIGHT AND PYRETHRIN CONTENT OF DEVELOPING FLOWER HEADS IN KENYA.

Stage of Development	Av. Dry Weight of Single Head Mgm.	Total Pyrethrins %	Av. Total Pyrethrins per Head Mgm.
Closed buds	46.8	0.84	0.39
Rays vertical	106.1	1.29	1.37
First row disk florets open	132.2	1.60	2.12
Second and third row disk florets open	157.5	1.63	2.57
Nearly the whole disk open	188.8	1.83	3.46
Fully open	209.1	1.67	3.50
Ripening	300.2	1.21	3.63

The flowers should not be packed tightly in the receptacles into which they are picked, but allowed to fall in loosely. Payment for picking is usually made on the basis of weight and varies from ¾ to 1 cent per pound of wet flowers (equivalent to about ¼ U. S. cent), although the work can often be performed slightly more cheaply by the employment of monthly labor at a fixed wage who are given a minimum task of 25 lbs. of wet flowers daily, picking in excess of this amount being paid extra depending on the amount picked. Picking is performed roughly at fortnightly to three weekly intervals throughout the flowering season which may continue for 9 to 10 months of the year. It is important, however, always to examine the flowers carefully in the field before commencing to pick rather than to attempt to follow any definite time interval.

In the early days of the industry when individual plantings were small, pyrethrum flowers were dried in the sun and in sheds. Now artificial drying is the practice, both on account of climatic conditions and of the quantities to be treated. The work of Beckley and McNaughtan *(1192)* showed that quick drying in the sun does not cause loss of pyrethrins and gives a product of good appearance. Prolonged drying in sun or shade causes loss of pyrethrins and poor color.

Artificial drying at 50° C. does not affect pyrethrin content or color but at 60° C. there is a slight loss of pyrethrins and some discoloration. At higher temperatures the loss of pyrethrins increases. Covello *(1320)* found that the pyrethrin content in pyrethrum dried in the shade, in the sun and artificially at 60° C.



A KENYA PYRETHRUM DRIER.
(KENYA INFORMATION OFFICE OFFICIAL PHOTOGRAPH)

decreased in that order. Treatment with 0.5 to 1.0 per cent of sulfur dioxide inhibited the activity of the oxidase and prevented loss of pyrethrins.

Several types of driers of varying efficiency have been in use, but a type described by Beckley and McNaughtan *(1192)*, after some modification, has been accepted as standard. It is cheap to build and to run, is simple, and is efficient. This Ainabkoi drier is a tall building divided into two parts, the charging and packing room and the drier proper. At the bottom of the drier proper is a system of flues where air, entering the chamber through properly distributed ports, is heated. The upper part is divided into five boxes, each carrying five to eight sets of trays in a vertical stack through which the hot air must pass before it escapes through the vent at the top of the building. The air is heated to 130° F. (54.4° C.), a temperature found by Beckley and later by Jary and others *(1706)* to be safe.

The wet flowers enter the drier proper in the top trays; it has been observed that, if the initial stages of drying are rapid, the soft achenes of immature flowers, which are always present in commercial pickings, are liable to caseharden and dry very slowly. When the flowers in the bottom trays are dry, i.e., when they break under a rolling squeeze with about 10 per cent moisture present, the trays are removed, all trays are moved down

one step, and fresh flowers are introduced at the top. The drier is thus continuous in its action, and flowers are exposed to the heat for as short a time as possible.

The dry flowers are at once removed from the trays and poured into kraft paper bags which hold about 50 pounds of dry flowers. The paper bags are cheap, light, and durable, and, above all, do not contaminate the flowers as would burlap. When a bag is full, a paper cover is pasted over it. Two or three times weekly on the large plantations the bags are sent by rail to Nakuru.

At Nakuru the pyrethrum passes almost immediately to the warehouses of the Kenya Farmers' Association where it is graded. The grades, as revised in February, 1938, are *(1190)*:

Grade I (Export) Flowers of good color. Not more than 33 per cent immature flowers and/or 8 per cent buds, and/or 10 per cent discolored flowers. Free from foreign matter.

Grade II (Local) Flowers of fair color containing 33 to 50 per cent immature flowers, and/or 12 per cent buds, and/or between 10 and 25 per cent discolored flowers. Free from foreign matter.

Grade III (Local) Flowers of reasonable color containing 50 to 75 per cent of immature flowers and 25 per cent buds, and/or 25 to 50 per cent discolored flowers. Free from foreign matter.

Flowers below Grade III are rejected. They contain less than 1 per cent pyrethrins and are powdered and used locally as dusts for protecting coffee and other crops.

Pyrethrum for export must be Grade I. The enforcement of this grade has resulted in the maintenance of high quality, notwithstanding excessive rainfall. The color and general appearance have improved considerably, and it is doubtful if any sun- or shade-dried pyrethrum surpasses the Kenya product in appearance. For a short while the pyrethrin content of Kenya flowers during the production year, 1937-38, dropped below the normal level, solely as a result of the excessive rainfall; with better weather conditions the normal content was again reached.

After being graded, about a ton of pyrethrum is heaped up and well mixed, and then transferred to the loading trough where the flowers are mixed again. They then pass to the hopper of the hydraulic baling press, and on the way a sample of definite weight is drawn.

The cage of the baling press is lined with burlap. Four hundredweight (448 pounds) of the mixed flowers are run in, and the bale is compressed to the standard dimensions of 22 x 20.5

x 37 inches under pressures varying from 4200 to 5800 pounds per square inch. The drier the flowers, the greater the pressure needed. During very dry weather it is essential to spray the flowers with water from an atomizer before baling; overdry flowers break down to a powder and will not bale properly. During the banding process the bale slowly expands to the shipping dimensions of 24 x 22 x 37 inches. After they are banded, the bales receive another burlap covering.

The samples drawn from the individual bales are bulked when 125 have been drawn, and from the bulk a sample is drawn, by quartering, for analysis at the Scott Agricultural Laboratories in Nairobi. The residue is baled normally and forms the test bale. The sample taken by quartering is representative of the 125 bales or 25 tons, and upon the results of the analysis is based the guarantee.

Continuing Ball's description *(1161)*, "At the end of the flowering season the pyrethrum plants have a large number of dead flowering stems and it is desirable to cut these back. The level to which the plants should be cut back has been a matter for dispute and for this reason experiments have been carried out to determine the correct method. The treatments adopted were as follows:

1. Control — uncut.
2. Cut high — flowering stems only removed.
3. Intermediate — half foliage cut in case of tufted plants and remainder of plants cut half through.
4. Cut to ground.

"Half of the area was also again cut in the middle of the flowering season to test whether it was possible to alter the time of flowering by cutting at periods other than before the onset of the rains. Results of this indicated that it is definitely inadvisable to try to alter the time of flowering by cutting at seasons other than the normal dry or dormant season. Results of the trials of different methods of cutting back are appended below:

	Cut once in dry season. lbs. dry flowers per acre	Cut high again during rains. lbs. dry flowers per acre
Control	1,155.5	417.0
Cut high	1,612.6	521.2
Intermediate	1,213.7	424.6
Cut to ground	877.1	294.0

"The plants which were cut to the ground have never grown to the same size and it would appear that this treatment would only be desirable if it was intended to rejuvenate an old plantation in order to permit of more thorough cultivation.

"Yields vary greatly throughout the pyrethrum growing areas but are influenced largely by altitude and the nature of the soil, the highest yields being obtained at altitudes over 8500 feet on soils which are of a medium loam character. Under these conditions, yields of 900 to 1000 lbs. of dry flowers per acre per annum are obtained while at lower elevation down to 7000 feet, yields in neighborhood of 500 to 600 lbs. of dry flowers may be expected. On rich forest soils with a very high humus content, these yields will be lowered and probably an average for such soils at 8000 feet will be in the neighborhood of 550 lbs. per acre.

"The useful bearing life of a plantation will vary considerably with soil, altitude, rainfall and other factors. There are indications, however, that the pyrethrin content of the flowers may fall after about the fifth year since establishment, which would necessitate ploughing up and replanting. Except, however, in the highest altitudes, the life of the plantation will probably be shorter than this, since considerations of weeding, reduction in stand, compaction of the soil will probably render it desirable to replant before this age is reached. The chief indication of a need for re-establishment is a reduced stand with consequent severe reductions in yield and increased weeding costs. It is essential, therefore, that a definite replanting program should be adopted every year, the percentage to be replanted being based on the average useful life of a field for the particular conditions obtaining. Thus on a loose forest soil where the useful life may be only three years, it will be necessary to replant about 33 per cent of the acreage annually, whereas in the high altitude areas on the tops of the ridges, 15 to 20 per cent will be sufficient for annual replanting."

Pyrethrum plantings in Kenya are sometimes severely damaged by *Thrips tabacci (2325)*. This insect pest is said to cause a marked reduction in yield, occasionally as high as 150 pounds per acre; nicotine sulfate and lime-sulfur are used for its control.

Browning or discoloring of the flowers is said to be caused by the fungus *Alternaria gossypina (2325)*.

The cost of production of Kenya flowers on land previously cultivated, estimated by Ball *(1161)* in 1938, was as follows:

Cost of establishment:	Per acre
Preparation of land and marking out	$ 4.09
Seed	1.27
Nursery work, labor and materials	1.34
Planting out seedlings	1.64
Weeding and cleaning	5.75
Cutting back and cleaning	.48
Total cost, four years life	$14.57
Cost per acre, per year	$ 3.64
Annual costs at 600 lbs. per acre, dry flowers:	
Establishment (see above)	$ 3.64
Cleaning	5.75
Picking	5.52
Cutting back and cleaning	.48
Depreciation on drier	2.88
Depreciation on trays	.40
Fuel for drying	.58
Labor for drying	.23
Labor for bagging	.16
Bags	2.76
Delivering	.23
Transport	.92
Total	$23.55

To this cost of about 4 cents a pound of dry flowers must be added the cost of supervision and interest on the capital investment. It should be noticed, also, that yields per acre do not yet average 600 lbs. for the country as a whole

A more complete and more recent estimate of the cost of producing Kenya pyrethrum indicated that on the basis of an average yield of 490 lbs. per acre, the cost at Nakuru is between 11.5 and 12 cents per pound, including all production costs. To this cost, of course, must be added freight to Mombassa, ocean freight, insurance, selling commissions, costs of analyses, etc.

The Kenya Pyrethrum Growers' Association, a purely voluntary organization, was founded in 1933 in order to promote the growing of pyrethrum and to assist growers in disposing of their product. They approached the Kenya Farmers' Association (Co-operative) Ltd., an organization of widespread and large dealings, to undertake the marketing and export. In turn, the Kenya Farmers' Association appointed an agent in London to undertake world sales. The London agent appointed an agent

in New York City. Under this arrangement the early exports were made. However, at the request of the industry, the "Sale of Pyrethrum Ordinance" was enacted in 1935, whereby all pyrethrum produced in Kenya has to be sold to a single buying and selling agency. The control of the industry was also invested in the Pyrethrum Board, elected by the growers. The agency appointed under the ordinance is the Kenya Farmers' Association.

The system evolved under the ordinance, in which all pyrethrum produced must be sold to a single central agency, ensures proper and full inspection and grading, and thus maintenance of quality. There is no confusion of grades, and the buyer knows that he is getting the best material available. The appointment of a sole agency also prevents unnecessary competition between agents. The producer, too, is assisted by the method of purchase. The individual grower does not have to export his pyrethrum to an agent overseas for sale and wait for the proceeds; he is at once credited with his delivery and receives payment monthly. This early and regular payment has been an important factor in the development of the industry. In order to keep the pyrethrum industry of Kenya completely under control, a planter must secure a license from the Department of Agriculture.

To show the seasonal yield of pyrethrum in Kenya, Smith *(2154)* gives the following yields for the crop year from April 1, 1938 to March 31, 1939.

1938	Tons
April	46.9
May	37.7
June	64.9
July	86.3
August	135.9
September	212.4
October	298.0
November	328.6
December	268.0
1939	
January	250.8
February	173.1
March	98.9

Production of pyrethrum in Kenya for twelve years has been:

Year	Pounds	Year	Pounds
1933	5,000	1939	6,427,000
1934	128,000	1940	13,126,000
1935	721,000	1941	12,909,000
1936	2,415,000	1942	12,251,000
1937	2,215,000	1943	9,200,000
1938	4,175,000	1944	14,665,000

In 1945 about 55,000 acres were devoted to the cultivation of pyrethrum in Kenya, a potential production of about 18,000,000 pounds.

The first Kenya pyrethrum flowers imported into the United States were received in 1934. A few additional small lots were received in the early part of 1935. The price paid for them was the current market price for Japanese flowers, about 18½ cents per pound, c.i.f. New York. During the spring and summer of 1935, the Japanese market broke and by autumn new crop Japanese flowers were selling for less than 8 cents per pound, c.i.f. New York; they eventually reached an all-time low of 7 cents per pound. In 1936, about 750 tons of Kenya flowers reached the United States. In spite of the fact that they were known to contain about 1.4 per cent pyrethrins, these Kenya flowers commanded a price of only 9 to 9½ cents per pound, or 1 to 1½ cents more than Japanese flowers containing 0.9 per cent pyrethrins. It was extremely unfortunate that flowers from so promising a source of supply should arrive on the market when prices were the lowest on record.

In 1936 an investigation was begun by the author, in collaboration with Mr. V. A. Beckley of the Kenya Department of Agriculture, for the purpose of establishing a more equitable basis for the sale of Kenya flowers in the United States *(1191)*.

There is normally an interval of 2 or 3 months between the time the flowers are baled at Nakuru and the time they reach the buyer in the United States; the ocean voyage alone takes 45 to 65 days. Before Kenya pyrethrum could be satisfactorily sold on the basis of a guaranteed pyrethrin content, it was necessary to ascertain whether there was an appreciable loss in pyrethrin content during this interval. For this purpose certain bales of whole flowers were carefully sampled at the time of baling at Nakuru and analyzed without delay at Nairobi. They were then included in commercial shipments and on arrival at Minneapolis were ground individually, sampled, and assayed by the Gnadinger method. Moisture was determined by drying for 5 hours at 100° C., and the pyrethrin content was calculated to the moisture-free basis (Table CII).

These comparative analyses show an average loss in transit of 1.4 per cent in the moisture content and 6.3 per cent of the pyrethrin content, on the moisture-free basis. The average pyrethrin content when assayed in Minneapolis was 1.54 per cent on the dry basis, corresponding to 1.42 per cent in flowers con-

TABLE CII. COMPARATIVE ANALYSES OF KENYA PYRETHRUM FLOWERS

	Analysis in Nairobi			Analysis in Minneapolis				
Bale No.	Date	Moisture %	Pyrethrins, dry basis %	Date	Moisture %	Pyrethrins, dry basis %	Interval *Days*	Pyrethrins lost %
678	9/8/36	8.3	1.73	12/7/36	8.0	1.50	89	13.3
714	9/9/36	7.6	1.71	12/7/36	7.1	1.51	88	11.7
729	9/10/36	9.2	1.67	12/8/36	7.4	1.57	88	6.0
753	9/15/36	8.6	1.72	12/8/36	7.2	1.57	83	8.7
777	9/16/36	9.9	1.60	12/8/36	7.4	1.63	82	..
801	9/23/36	8.1	1.84	12/8/36	7.3	1.70	75	7.6
825	9/24/36	9.2	1.89	12/8/36	7.9	1.56	74	17.4
850	9/24/36	8.5	1.67	12/8/36	7.5	1.52	74	8.9
875	9/25/36	9.6	1.65	12/8/36	6.8	1.63	73	1.2
897	9/29/36	9.2	1.55	1/12/37	8.6	1.57	104	..
922	9/30/36	10.9	1.53	1/12/37	7.8	1.59	103	..
947	10/1/36	10.3	1.67	1/12/37	7.7	1.54	102	7.8
22	10/9/36	10.0	1.70	1/12/37	7.0	1.57	94	7.6
47	10/14/36	9.7	1.69	1/12/37	8.5	1.66	89	1.8
647	12/2/36	10.0	1.66	2/17/37	9.3	1.53	76	7.8
747	12/3/36	10.1	1.64	2/17/37	7.4	1.52	75	7.3
947	12/8/36	9.3	1.56	3/23/37	8.7	1.50	104	3.8
47	12/15/36	9.6	1.59	3/23/37	7.4	1.54	97	3.1
47A	2/3/37	9.3	1.58	4/20/37	8.0	1.63	75	..
747	1/13/37	7.9	1.58	5/5/37	8.4	1.55	111	1.9
847	1/20/37	9.0	1.67	5/5/37	9.1	1.52	94	9.0
247	12/21/36	9.8	1.65	5/5/37	7.8	1.56	134	5.4
347	12/21/36	11.0	1.75	6/18/37	9.5	1.56	178	10.8
447	1/5/37	9.1	1.69	6/18/37	8.9	1.46	163	13.6
147A	2/4/37	8.6	1.49	6/18/37	7.8	1.46	133	2.0
247A	2/9/37	8.0	1.56	6/18/37	10.1	1.52	128	2.5
347A	2/16/37	8.3	1.59	6/18/37	8.7	1.41	121	11.3
T1	2/23/37	8.4	1.60	6/18/37	9.7	1.51	114	5.6
T2	3/9/37	8.8	1.66	6/18/37	7.0	1.52	100	8.4
T3	4/27/37	12.4	1.43	7/28/37	8.5	1.43	91	..
T4	5/26/37	11.9	1.69	7/28/37	6.5	1.43	62	15.4
Av.		9.4	1.65		8.0	1.54	99	6.3

taining 8 per cent moisture. More than a hundred sample bales, in addition to those reported in Table CII, were also assayed.

In defining a minimum guarantee for the pyrethrin content of Kenya flowers, the following points were taken into consideration: The guarantee should be set as high as possible to yield the greatest return to the Kenya growers. It should not be so high as to result in large numbers of borderline cases, which lead to friction and argument between seller and buyer.

Allowance must be made for the loss in transit so that the goods arriving in the United States are at least equal to the

guarantee; otherwise claims and disagreements will arise. New crop Japanese flowers, sold on a guarantee of 0.90 per cent, nearly always contain at least that amount when they arrive in the United States. The sampling of shipments is just as important as the chemical analysis.

Finally, on the basis of the foregoing analyses and these considerations, the following tentative standard was adopted:

1. Samples are to be taken by the pyrethrum grader in Nakuru under direction of an officer of the Chemical Section of the Scott Agricultural Laboratories. Each ton lot of flowers is to be well mixed in a heap before baling, and five samples of 4 pounds each are to be drawn as the flowers pass to the baling press. One hundred and twenty-five such samples, representing a 25-ton shipment, shall be well mixed, and a representative sample of 4 pounds drawn for analysis.
2. A minimum pyrethrin content of 1.3 per cent shall be guaranteed. A certificate of analysis issued by the Scott Agricultural Laboratories showing 1.4 per cent of pyrethrins at the time of shipment shall be final proof that the goods comply with the guarantee.
3. The pyrethrin content shall be determined by the Gnadinger copper reduction method.

On such a basis the buyer could afford to pay for Kenya flowers thirteen-ninths of the current market price of Japanese pyrethrum. This tentative standard was put into effect at the beginning of the 1937 season and immediately resulted in greatly improved prices to the Kenya growers. It remained in effect until the Japanese were driven from the market.

The proposal to pay the Kenya growers a premium of 44.4 per cent above the Japanese price was made by McLaughlin Gormley King Company, Minneapolis, in December, 1936. The offer was officially accepted by the Kenya Pyrethrum Board in March, 1937, and was immediately put into effect. Coming at a critical time, when prices received by Kenya growers were at, or below, cost of production, it had far-reaching effects on the future of pyrethrum in Kenya Colony. Other importers were compelled to fall into line, and prices of Kenya pyrethrum rose from a low of 9 cents, c.i.f. New York, in 1936 to a high of 36 cents per pound in 1940. The correspondence that led to the establishing of the standard for Kenya pyrethrum is reproduced on the following pages.

McLAUGHLIN GORMLEY KING CO.
INCORPORATED
MANUFACTURING CHEMISTS · IMPORTERS AND MILLERS

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MGK
MINNEAPOLIS
MINNESOTA
U.S.A.

Dec. 14, 1936

Mc Kenzie & Company
80 Wall Street
New York, New York

Attention: Mr. Mc Kenzie

Dear Sir:

When I had the pleasure of seeing you recently, we discussed the possibility of buying and selling Kenya Pyrethrum Flowers on the basis of a guaranteed pyrethrin content.

This Company originated the practice of selling Japanese Pyrethrum on a guaranteed pyrethrin content in 1929. We first sold our customers on a guarantee of 0.75% pyrethrins, but by the end of 1931 we had raised the guarantee to 0.90%. At first we were not able to buy from the Japanese with any guarantee on their part, consequently, we put our own man in Japan, in 1930, to select high test lots for us. This man still passes on most of the lots we buy, but the Japanese will now sell on a guaranteed minimum content of 0.90% pyrethrins, furnishing a certificate of analysis by the Hokkaido Institute or the Imperial Institute, both Government laboratories.

That the guarantee of 0.90% pyrethrins is a fair one for Japanese Flowers is shown by the analyses of seven crops of flowers on page 153 "Pyrethrum Flowers," Second Edition. The average for the seven crop years is 0.97% for flowers assayed within six months of harvesting. The minimum guarantee of 0.90% pyrethrins has proved satisfactory to both sellers and buyers. We mention these facts merely to show how the guarantee of 0.90% on Japanese Flowers was established.

We believe it is now possible to establish a guaranteed minimum pyrethrin content for Kenya Flowers. Conditions are much more favorable for setting up a guarantee for Kenya flowers than they were when the Japanese guarantee was established.

We now have analyses of shipments received at all seasons of the year. Each of the following analyses represents a lot of from 5 to 50 tons; analyses were made on arrival of the goods at Minneapolis.

Date of analysis		Pyrethrins, %
Jan.,	1936	1.47
Feb.,	1936	1.47
Mar.,	1936	1.48
May,	1936	1.32
June,	1936	1.32
June,	1936	1.39
July,	1936	1.46
July,	1936	1.46
July,	1936	1.37
Sept.,	1936	1.51
Oct.,	1936	1.57
Nov.,	1936	1.46
Average		1.44

In my opinion it is not possible to buy and sell pyrethrum on the exact pyrethrin content of the flowers. This is because:

1. The limit of error of the assay methods is about 3%.
2. There is a considerable variation in the pyrethrin content of individual bales in a shipment and accurate sampling of large shipments of whole flowers is difficult.
3. The pyrethrins are subject to decomposition during storage and shipping. The extent of the decomposition varies with the maturity of the flowers, temperature and other conditions during storage and shipping. These conditions are usually beyond the control of seller and buyer.
4. Different lots of flowers do not decompose at the same rate.

The variation in the pyrethrin content of a twenty ton lot of Kenya Flowers is shown by the following analyses. The entire car was ground and packed in 100 lb. bags. As the flowers came from the mill, each bag was sampled with a grain-trier and the samples from 50 bags were made into a composite sample which was thoroughly mixed and assayed. Each analysis, therefore, represents 50 bags or 5000 lbs. of flowers. The time between the analyses of the first and last samples was about a week.

Sample	Pyrethrins %
1	1.63
2	1.57
3	1.67
4	1.43
5	1.35
6	1.49
7	1.45
Average	1.51

The loss in pyrethrin content during shipment from Kenya to Minneapolis has not been determined for all seasons of the year. A shipment of flowers left Kenya September 30, 1936, reached New York, Novem-

ber 11th, and arrived at Minneapolis, November 23rd. This 45 ton shipment contained 9 bales, each of which had been sampled and assayed before leaving Kenya about the middle of September. These same bales were sampled and assayed in Minneapolis, December 7, 1936. The analyses are given below:

Bale No.	Kenya Analyses Moisture %	Pyrethrins as rec'd %	dry basis %	Minneapolis Analyses Moisture %	Pyrethrins as rec'd %	dry basis %	Difference in pyrethrin content - dry basis %
678	8.3	1.59	1.73	8.0	1.38	1.50	- 13.3
714	7.6	1.58	1.71	7.1	1.40	1.51	- 11.7
729	9.2	1.52	1.67	7.4	1.45	1.57	- 6.0
753	8.6	1.57	1.72	7.2	1.46	1.57	- 8.7
777	9.9	1.45	1.60	7.4	1.52	1.63	- 1.9
801	8.1	1.69	1.84	7.3	1.58	1.70	- 7.5
825	9.2	1.72	1.89	7.9	1.44	1.56	- 17.4
850	8.5	1.53	1.67	7.5	1.41	1.52	- 9.0
875	9.6	1.49	1.65	6.8	1.52	1.63	- 1.2
Avg.	8.8	1.57	1.72	7.4	1.46	1.58	- 8.1

The average pyrethrin content of the nine bales determined at Minneapolis was 8.1% lower than when determined in Kenya, about 85 days earlier. This difference of 8.1% includes loss of pyrethrins due to decomposition as well as differences due to unavoidable errors in sampling and analyzing. Pending further work, a difference of 8% can be taken as representing the loss between Kenya and the United States for the fall and winter months, with perhaps higher losses during the warmer months.

The period of 85 days is longer than would normally be required for shipment due to the seamen's strike at New York and the fact that the writer wished to analyze these samples personally. The normal period would be 50 to 60 days.

In setting a tentative guaranteed minimum pyrethrin content for Kenya Flowers, the following points should be kept in mind:

1. The guarantee should be set as high as possible to yield the greatest return to the Kenya growers.

2. It should not be so high as to exclude a large proportion of the Kenya crop.

3. It should not be so high as to result in large numbers of border-line cases, which lead to friction and argument between seller and buyer.

4. Allowance must be made for the loss in transit so that goods arriving in the United States are at least equal to the guarantee, otherwise claims and disagreements will arise. New crop Japanese flowers, sold on a guarantee of 0.90%, nearly always contain at least that amount when they ar-

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McKENZIE & COMPANY
COMMISSION MERCHANTS
80 WALL STREET

CODES
BENTLEY'S
A.B.C. 5TH EDITION
A.B.C. 5TH ED. IMP.
A.B.C. 6TH EDITION
SCHOFIELD'S
ACME

NEW YORK. December 16, 1936.

DR. C. B. GNADINGER, GENL. MGR.,
McLAUGHLIN GORMLEY KING CO.,
Minneapolis,
Minn.

Dear Sir:-

We are in receipt of your favor of 14th inst. and thank you for the detailed information you give; also for your fair treatment of the question of price. This is going to make a very good impression on The Kenya Farmers' Association.

You will remember we discussed the above question when we had the pleasure of meeting you here; if this industry is to continue, there must be some reasonable profit for the growers, and the prices they have been receiving for the past year have not been satisfactory. This is the reason, in the lower altitudes, why the farmers have been grubbing their flowers and planting other crops- on account of unsatisfactory yield. If they received better prices they no doubt would have continued to raise Pyrethrum Flowers. The general tone of your letter carries out your understanding of this condition, and for this we wish to express our deep appreciation.

We believe the time will come when Kenya Flowers will be sold on guarantee, and we do not think that time is far off.

We are sending the copy of your letter which you so kindly enclosed to Messrs. R. O. Treatt & Co. Ltd., London, and we know they will appreciate it quite as much as we.

As the Christmas Season is upon us, we wish to express our appreciation of the courtesy and fair dealing extended us by your house during the past year, and we hope we may retain your confidence and good will during the coming year, and wish you all Health and Prosperity.

Respectfully yours,

McKenzie & Co

MM/C

rive in the United States.

5. The sampling of shipments of flowers is just as important as the chemical analysis.

In order to assist in establishing a more favorable basis for marketing Kenya Flowers, this Company suggests the following:

1. We will buy Kenya Flowers on a guaranteed minimum pyrethrin content of 1.30%, paying a price proportionally higher than the current market price for Japanese Flowers. That is, if the current market price for Japanese Flowers guaranteed to contain 0.90% pyrethrins were 8.5 cents per lb., c.i.f. New York, we would pay 12.28 cents per lb., $(8.5 \times \frac{1.3}{.9})$ for Kenya Flowers guaranteed to contain 1.30% pyrethrins.

2. We will accept a certificate of analysis issued by Scott Agricultural Laboratories, Nairobi, showing that the flowers contain 1.40% pyrethrins or more, as conclusive and final proof that the goods comply with the guarantee. This would allow 7% for loss in transit and errors in sampling and analyzing.

3. Samples are to be drawn by a qualified person, approved by the Scott Laboratories, in either of two ways.

   a. By thoroughly mixing the flowers in a pile, before baling, and drawing representative portions from the pile.

   b. By boring cores from the bales with a one inch auger, the number of bales to be sampled to equal 15% of the number shipped.

4. Pyrethrin content is to be determined by the copper-reduction method of Gnadinger and Corl.

This plan is suggested as a temporary working agreement; if it is found that the standard is too low or too high or if the plan is otherwise unfair to either seller or buyer, it can be modified accordingly.

We should like to have your comments on this suggested plan. A copy of this letter has been sent to Dr. V. A. Beckley of the Scott Agricultural Laboratories, Nairobi.

Yours very truly,

MC LAUGHLIN GORMLEY KING COMPANY

C B Gnadinger
General Manager

C B Gnadinger
J

Code Addresses
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"COTONWASTE"

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McKENZIE & COMPANY
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ACME

NEW YORK April 19, 1937.

MESSRS. McLAUGHLIN GORMLEY KING CO.,
Minneapolis,
Minn.

Gentlemen:

You will be interestedto know The Kenya Farmers' Association (Co-operative) Ltd., at Nakuru, through the Pyrethrum Board, have decided on a selling policy.

1 - In future, Pyrethrum Flowers will be sold with a guaranteed Pyrethrin Content of 1.30%.

2 - A certificate from the Government Chemist will be issued with each consignment of flowers certifying that the flowers contain at the time of analysis 1.40% or more of pyrethrins. This will allow for an error of .03% and a loss of .07% in transit.

3 - Samples to be drawn by a person approved by the Chief Government Chemist. Flowers to be thoroughly mixed in one ton lots - representative sample to be drawn from each ton lot - these samples to be thoroughly mixed, quartered and analyzed. The Pyrethrin content to be determined by the copper reduction method of Gnadinger and Corl.

They further state the experience they have had shows, although they will sell at a minimum of 1.30% pyrethrin content, the flowers invariably contain a much higher content, and they close the letter by stating they would like us to convey to you the great appreciation of the Board and Pyrethrum Growers of the Colony generally, for the valuable assistance you have rendered them in arriving at a figure estimate of the Pyrethrin content of their flowers on arrival on the American market.

We regret exceedingly the tonnage seems to be so small, caused very largely we suppose by the low price ruling last year together with a continued drought of months: And also, we note, there seems to be developing in South Africa and the Belgian Congo, a market for their flowers; this, of course, has an influence on restricting the tonnage for America.

We also think you might be interested in the fact that Messrs. R. C. Treatt & Co. Ltd., London, are selling Pyrethrum Flowers in Europe on the same basis they receive from the American sales.

Respectfully yours,

EL/C

SCOTT AGRICULTURAL LABORATORIES

TELEGRAMS: "AGRICOLA"
TELEPHONE: No. 222.
In reply please quote } No Insecti/2/3/120.

DEPARTMENT OF AGRICULTURE,
P. O. BOX ~~329~~ 338,
NAIROBI,
KENYA.

24th. March, 1937.

Dr.C.B.Gnadinger,
Mc Laughlin Gormley King Co.,
Minneapolis, Minnesota,
United States of America.

Dear Sir,

Sale of Pyrethrum under Guarantee.

I am at last in a position to answer your letter of December 14th. with reference to the enclosure to Mc Kenzie and Co..

It will be possible to arrange for the sampling and the analysis of pyrethrum being exported and so to sell on a guarantuee of a minimum of 1.30% pyrethrins on receipt, i.e., we will supply a certificate to the effect that a certain hundred bales, 20 tons, contain an average of at least 1.40% pyrethrins on despatch.

The method of sampling will be as close as possible to your suggested method (a). Ton lots of flowers will be well mixed in a pile before baling, and a sample of a definite weight will be drawn. Twenty such samples will be well mixed together and a single representative sample will be drawn for analysis. This system will give us as representative a sample as it is possible to draw.

For this year analyses will be made by me personally. After this year an officer will be specially engaged to work on pyrethrum solely and analyses will be made by him. It is possible that it may eventually be found desirable to situate him at Nakuru where he will be in a position to maintain close control. In this case duplicate samples will be sent to me and check analyses made at intervals.

Now that arrangements have been made for the proper sampling of the flowers before baling and the industry is in a position to supply flowers of a guaranteed content, I wish to offer you my thanks for the great assistance you have been to the industry in suggesting the new marketing basis. Apparently already the market has responded.

Yours faithfully,

V. A. Beckley

SENIOR AGRICULTURAL CHEMIST.

VAB/LOHJ.

The pendulum had now swung the other way; the 36 cent price was too high and synthetic chemicals began to replace pyrethrum on a large scale. Furthermore, pyrethrum was now too high-priced to be used for horticultural purposes. Displaying their usual foresight, the Kenya authorities decided to stabilize the price at a reasonable level. Beginning in the spring of 1940, the import price was gradually lowered, a cent or two at a time, until the stabilized price of 18½ cents per pound, c.i.f. New York, was announced to the import trade on July 14, 1941. This stabilized price remained in effect until January, 1944, and had a beneficial effect on the entire pyrethrum industry. At this level, certain improved pyrethrum insecticides could compete with almost any other horticultural insecticide; the sale of synthetic insecticides, as substitutes for pyrethrum, declined.

The original agreement with the Kenya Farmers' Association specified that the pyrethrin content of the flowers should be determined by the Gnadinger copper reduction method. However, some importers wished to buy on the basis of assays made by the Seil method and sales were finally made providing for analysis by either the Gnadinger method or the Seil method, at the option of the buyer. Since the Seil method gives higher results than the Gnadinger method, the willingness of Kenya to sell on assays by the Seil method is easily understood.

The Gnadinger method was used almost exclusively in Kenya until about 1941. The reasons for this have been stated by Beckley *(1190)*, as follows:

"In the two most widely accepted 'acid' methods the determination of pyrethrin II involves extraction with ether. The altitude of the Scott Agricultural Laboratories is just under 6000 feet and ordinary ethyl ether boils at about 28.5° C.; air and water temperatures of over 25° C. are common. Continuous extraction, as in the Tattersfield method, is expensive in ether on account of inefficient cooling, whilst shaking with ether as needed in the Seil method is apt to be dangerous on account of the high pressures developed in the separating funnel after shaking. In order to employ either of these methods special equipment would be needed, which, up to the present, it has not appeared necessary to install." Apparently the necessary equipment was later installed.

In 1943, all pyrethrum brought into the United States was imported by the Federal Government and allocated to American processors. At that time, the Seil method was specified as the basis of payment. This action, adopting the Seil method, was taken by officials, with no experience in buying pyrethrum, without consulting the industry. The old basis of purchase had been on a specified price for flowers testing 1.3 per cent pyrethrins, with no allowance for higher pyrethrin content, but with a deduction of 10 per cent in price for each 0.1 per cent deficiency in pyrethrin content; no claim unless assay on arrival in the United States showed less than 1.28 per cent pyrethrins. The new basis provided for the same penalty for deficiency in pyrethrin content and also provided for a premium of 10 per cent in price for each 0.1 per cent excess pyrethrin content above 1.3 per cent, no premium unless more pyrethrins present than 1.32 per cent. There could be no valid objection to the payment of such a premium. However, both the adoption of the Seil method and the payment of the premium increased the price to American processors of pyrethrum.

The establishing of premium payment for pyrethrin content in excess of 1.3 per cent is significant in view of the fact that it is feasible to produce flowers in commercial quantities in Kenya testing as high as 1.8 per cent pyrethrins.

The deduction of 10 per cent for each 0.1 per cent deficiency and the premium of 10 per cent for each 0.1 per cent excess pyrethrin content were based, in part, on overhead costs of manufacturing extracts and were not, therefore, directly proportional to pyrethrin content. In July, 1944, however, the United States Government began writing its contracts with American processors on the basis of 25 cents a pound for flowers containing 1 per cent pyrethrins, with pro rata adjustment in price for lower or higher pyrethrin content.

So long as the price of 25 cents a pound per one per cent pyrethrins, with pro rata adjustment, was applied only to Kenya flowers, the increased cost to American processors could be absorbed. When, however, the same price basis, with pro rata adjustment, was applied to Brazilian flowers testing 0.7 to 0.9 per cent pyrethrins, the increased costs to processors were too great to be absorbed. This is best shown by the following examples:

Cost of pyrethrum flowers, basis 32½ cents per pound for 1.3 per cent pyrethrin content, with 10 per cent discount for each 0.1 per cent deficiency in pyrethrin content:

Pyrethrin content, per cent.....	1.30	1.20	1.10	1.00	0.90	0.80	0.70
Cost, ex dock, New York, cents..	32.50	29.25	26.00	22.75	19.50	16.25	13.00

Cost of pyrethrum flowers, basis 25 cents per pound for 1.0 per cent pyrethrin content, with pro rata adjustment for higher or lower content:

Pyrethrin content, per cent.....	1.30	1.20	1.10	1.00	0.90	0.80	0.70
Cost, ex dock, New York, cents..	32.50	30.00	27.50	25.00	22.50	20.00	17.50

The Government had failed to consider that it costs as much to process a ton of Brazilian flowers testing 0.7 per cent pyrethrins as it does to process a ton of Kenya flowers testing 1.3 per cent, but the yield of extract from the Brazilian flowers is only about half that from the Kenya flowers.

The stabilized price of 18½ cents per pound, c.i.f. New York, remained in effect until January, 1944, but increased war-risk insurance and handling charges, for the account of the buyer, had increased the cost to 22¼ cents per pound. The original contracts provided for payment of war-risk insurance by the Kenya shipper up to ½ per cent. The commercial war-risk insurance rate rose to 20 per cent in October, 1942, but eventually declined to 4 per cent when the United States Government took over the writing of such insurance in 1942.

The British Ministry of Supply had taken over the distribution and sale of all Kenya pyrethrum on January 1, 1943. In June, 1944, the British Government increased the price so that American processors paid 32½ cents per pound, ex dock New York, for flowers testing 1.3 per cent pyrethrins. All contracts outstanding when the British Government took over the distribution of Kenya pyrethrum were eventually filled at the original contract price of 18½ cents, although the British Government had, in the meantime, paid higher prices to the Kenya growers to stimulate production.

Analyses of shipments of Grade I Kenya pyrethrum for nine crop years are given in Table CIII. The crop year in Kenya extends from April 1 to the following March 31. Analyses for 1936-1942 are by the Gnadinger method; analyses for 1943-1944 are by the Seil method. Each analysis represents a lot of 25 tons or more.

TABLE CIII. PYRETHRIN CONTENT OF GRADE I KENYA PYRETHRUM FLOWERS BY CROP YEARS.

1936 %	1937 %	1938 %	1939 %	1940 %	1941 %	1942 %	1943 %	1944 %
1.32	1.13	1.42	1.32	1.27	1.29	1.20	1.12	1.30
1.39	1.39	1.35	1.18	1.25	1.22	1.28	1.35	1.19
1.33	1.49	1.33	1.14	1.25	1.33	1.28	1.37	1.18
1.45	1.53	1.26	1.42	1.34	1.29	1.32	1.37	1.19
1.67	1.45	1.27	1.38	1.28	1.18	1.25	1.47	1.30
1.41	1.24	1.43	1.33	1.34	1.34	1.23	1.45	1.24
1.44	1.44	1.37	1.31	1.31	1.27	1.26	1.33	1.41
1.43	1.40	1.35	1.41	1.33	1.25	1.26	...	1.34
1.43	1.31	1.42	1.42	1.35	1.12	1.21	...	1.30
1.38	1.28	1.43	1.47	1.19	1.22	...	...	1.35
1.38	...	1.48	1.41	1.11	1.24	...	...	1.30
...	...	1.39	1.32	1.12	1.28	...	...	...
...	...	1.49	1.32	...	1.19	...	...	...
...	...	1.53	1.25	...	1.28	...	...	...
...	...	1.38	...	...	...	...	...	...
...	...	1.30	...	...	...	...	...	...
...	...	1.30	...	...	...	...	...	...
...	...	1.40	...	...	...	...	...	...
...	...	1.37	...	...	...	...	...	...
Avg. 1.42	1.37	1.38	1.33	1.26	1.25	1.25	1.35	1.28

The following are analyses of commercial shipments of Grade II Kenya flowers by the Gnadinger method:

Crop Year	Pyrethrins, %	Crop Year	Pyrethrins, %
1940	1.08	1941	1.04
1940	1.07	1941	1.09
1941	1.04	1941	1.08
1941	1.05	1941	0.99

TANGANYIKA PYRETHRUM

Experiments on the production of pyrethrum in Tanganyika were begun in 1932 at Iheme Experiment Station and later at Dabaga and Mufindi. There was a small surplus for export in 1937, and 25 tons were exported in 1938 *(1651)*. In 1939 Smith *(2154)* reported that the yield in the Iringa district of the Southern Highlands was about 10 tons a month.

In the pyrethrum producing areas of Tanganyika the altitude is 5500 to 7000 feet. The soil is forest land or grass land of granitic origin. Temperatures vary from 50° to 70° F. and rainfall is 50 to 80 inches a year. Seed are sowed in beds in September, and the seedlings are set in the fields in April. Harvesting continues from September until June. The crop is artificially dried. A grading station and packing plant have been established at Iringa. Shipments are made from Dar-es-Salaam. It

has been estimated that Tanganyika can produce 2000 tons of pyrethrum a year *(1651)*.

At the suggestion of the Director of Agriculture of Kenya, the Tanganyika growers decided to hand over their export activities to the Kenya Farmers' Association, and now that organization controls the export and sale of the Tanganyika product as it does for Kenya.

Imports of Tanganyika pyrethrum into the United States have been:

Year	Pounds
1941	56,000
1942	224,000
1943	209,000
1944	81,000

In 1945 about 5000 acres were devoted to the cultivation of pyrethrum.

The price of Tanganyika flowers has been the same as the price of Kenya pyrethrum. The pyrethrin content of Tanganyika flowers has been about the same as that of Kenya flowers, as the following analyses of commercial lots show:

Crop Year	Pyrethrin, %	Method of Analysis
1939	1.30	Gnadinger
1940	1.25	Gnadinger
1941	1.18	Gnadinger
1942	1.07	Gnadinger
1943	1.27	Seil
1943	1.25	Seil

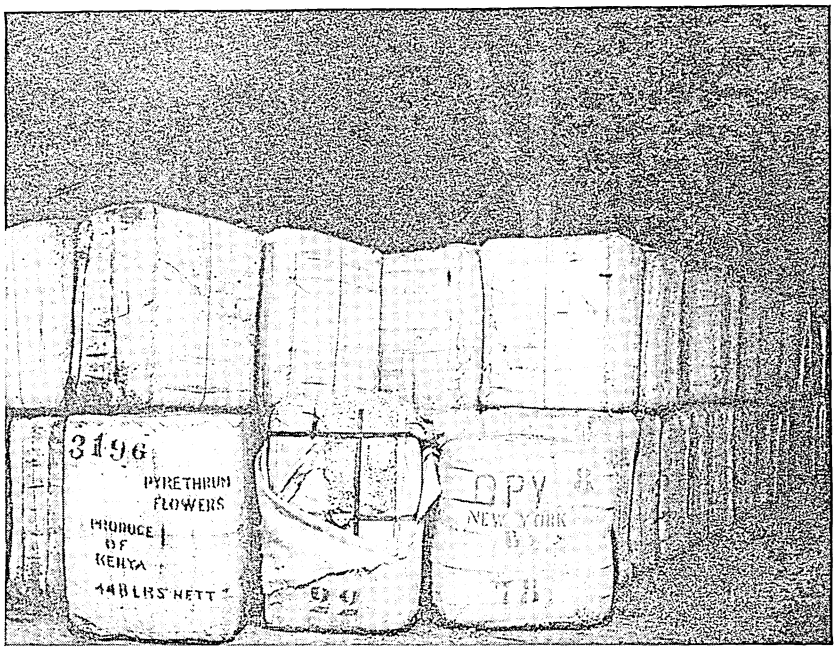
Uganda Pyrethrum

A small amount of pyrethrum was produced in Uganda in 1943. Like Tanganyika flowers, Uganda pyrethrum has been marketed by the Kenya Farmers' Association. The pyrethrin content of one commercial lot from the 1943 crop was 1.49 per cent. The 1945 crop was about 800 tons.

Belgian Congo Pyrethrum

Pyrethrum is grown in Belgian Congo in the Kivu and Ruanda-Urundi districts at altitudes of 4500 to 6000 feet, on well drained volcanic soil. In 1937, about one ton was produced. Newman *(1948)* says the production was 3 tons for 1938 and 22 tons for 1939. Small quantities were exported to the United States in 1939. Since then production has increased steadily, the quantities imported into this country being:

Year	Pounds	Year	Pounds
1939	2,000	1942	223,000
1940	17,000	1943	200,000
1941	179,000	1944	770,000



BALES OF KENYA AND BELGIAN CONGO PYRETHRUM.

The area devoted to the cultivation of pyrethrum in 1945 was about 4000 acres.

The following analyses of commercial shipments to the United States have shown pyrethrin contents about the same as Kenya flowers, and the price has been the same.

Crop Year	Pyrethrins, %	Method of Analysis
1939	1.44	Gnadinger
1943	1.24	Seil
1944	1.21	Seil
1944	1.23	Seil
1944	1.11	Seil
1944	1.30	Seil

Castagne *(1282)* considers pyrethrum of the Belgian Congo comparable to Kenya pyrethrum. He states that the ratio of pyrethrin I to pyrethrin II varies according to the development of the flower. This is confirmed by Covello *(1319)*, who found the ratio of pyrethrin I to pyrethrin II to be 2.4 in the buds and 1.1 in the fully open flowers. He suggests that pyrethrin II may be formed from pyrethrin I.

Brazilian Pyrethrum

Production of pyrethrum in Brazil was begun on a commercial basis in 1933. The small quantity produced was used locally until 1934 when trial shipments were made to the United States followed by similar small shipments in 1937. Brazilian pyrethrum was first grown in small areas within 100 miles of Port Alegre and near Pelotas in Rio Grande do Sul *(1034)*. There are also some plantings in the State of Sao Paulo *(1651)*. The climate is mild, temperatures varying from 50° to 76° F. Rainfall is about 50 inches a year. Plantings are made at elevations below 1000 feet, on calcareous soil. The principal harvest occurs in December and January.

Prior to 1940, official statistics of the crop were not compiled, but estimates placed production in that year at less than 300 tons *(1061)*.

Braddock *(1243)* estimated production in Brazil as follows:

Year	Metric Tons
1938	300 to 400
1939	300 to 400
1940	600 to 650
1941	600 to 1000

This estimate of the 1941 crop has been confirmed by others *(1933)*.

Cultivation in Brazil is by small landowners who dry their own crops and sell them to middlemen. This accounts for the lack of uniformity usually found in shipments of Brazilian flowers. The dried flowers are packed in bags of 70 kilos and sent to Porto Alegre and Rio Grande, from which they are exported.

Mohr *(1899)* has described the production of pyrethrum in the higher altitudes of southern Brazil. He states that most Brazilian flowers contain more than 0.95 per cent pyrethrins by the Seil method.

Imports of Brazilian pyrethrum into the United States have been:

Year	Pounds	Year	Pounds
1934	5,000	1941	11,000
1937	1,000	1942	397,000
1938	497,000	1943	593,000
1939	80,000	1944	2,203,000
1940	78,000		

The quality of Brazilian pyrethrum has been inferior. The pyrethrin content is about as high as that of Japanese pyrethrum and is far less than that of Kenya pyrethrum.

The following analyses are from commercial lots, with the exception of the samples of 1937 crop.

Crop Year	Pyrethrins, %	Method of Analysis
1937	1.19	Gnadinger
1937	0.85	Gnadinger
1937	0.79	Gnadinger
1938	1.15	Gnadinger
1938	0.91	Gnadinger
1940	0.85	Gnadinger
1943	0.87	Gnadinger
1943	0.81	Gnadinger
1943	0.73	Gnadinger
1944	0.86	Seil
1944	0.73	Seil

The price of Brazilian pyrethrum has usually been higher, per unit of pyrethrin content, than that of Kenya pyrethrum.

Japanese Pyrethrum

Until 1940, Japan was the principal supplier of pyrethrum for the United States. In that year Kenya growers shipped five times as much pyrethrum to this country as the Japanese. Production in Japan is said to have remained at a high level until war began, as the following unofficial statistics indicate *(1651)*:

Year	Production, Pounds	Year	Production, Pounds
1936	24,367,000	1938	24,192,000
1937	24,084,000	1939	22,566,000

Table CIV. Pyrethrin Content of Japanese Pyrethrum by Crop Years.

1936 %	1937 %	1938 %	1939 %	1940 %
1.10	0.98	1.04	0.96	0.81
1.05	1.06	0.93	0.92	...
0.95	0.93	0.96	1.01	...
0.89	0.92	0.98	0.97	...
0.87	0.81	1.00	0.91	...
0.96	0.77	0.91	0.93	...
0.86	0.82	0.97	...	...
0.90	0.85	0.92	...	...
0.88	0.79	0.90	...	...
0.87	0.88	0.90	...	...
0.76	0.80	0.98	...	...
...	0.78	...	...	...
...	0.84	...	...	...
...	0.83	...	...	...
...	0.82	...	...	...
Avg. 0.92	0.86	0.95	0.95	0.81

Analyses by the Gnadinger method of commercial lots of Japanese flowers from 1936 to 1941 are appended in Table CIV.

Reference to Table CV shows that Japanese exports to this country dropped sharply in 1939, 1940 and 1941.

Dalmatian Pyrethrum

Although efforts were made in 1933 and 1934 to improve the quality of Dalmatian pyrethrum, little progress has been made. Production of pyrethrum in Yugoslavia in the last ten years has rarely exceeded a thousand tons a year. Nearly all of this production has been sold in Europe. Imports into the United States since 1934 have not exceeded 230 tons a year.

Other Foreign Sources

Attempts to grow pyrethrum have been made in almost every country in the world. Some progress has been made in India. Lahiri, Ghosh and Chopra *(1777)* report that pyrethrum has been grown in Kashmir, Muree Hills, Kulu Valley, North Western Frontier Province, Kurrum Valley, United Provinces and other places. Their analyses of samples from 1938, 1939 and 1940 crops show pyrethrin contents of 0.49 per cent to 1.3 per cent. Small acreages were planted in 1944 in Ceylon.

Bal *(1159)* has reviewed pyrethrum production in India. According to Puntambekar *(2013)*, Indian pyrethrum grows best where rainfall is 40 to 60 inches per year, evenly distributed. The best flowers, 1.41 per cent pyrethrins, were grown at Assam, altitude 4900 feet, rainfall 84 inches.

Shafik and Hindi *(2114)* report that pyrethrum grows well in Egypt except in the extreme south and in soil with high water level.

Plantings of pyrethrum were made in Southern Rhodesia in 1931. The plants did not thrive under dry land farming but results were more promising on irrigated land. Under irrigation, yields varied from 400 to 1200 pounds per acre *(1134)*.

Unpublished reports by Kroll, Moore and Drain have indicated that pyrethrum may be successfully grown in Haiti.

According to Holman *(1651)* and others, experimental or small commercial plantings have also been made in Albania, Algeria, Angola, Argentina, Australia, Bermuda, Bulgaria, Canada, Chile, China, Cyprus, Ecuador, Egypt, Eire, England, Fiji Islands, France, Greece, Guatemala, Italy, Jamaica, Madagascar, Mexico, Morocco, Netherlands East Indies, New Zealand, Nigeria,

PYRETHRUM PRODUCING AREAS OF AFRICA

1. Kenya
2. Tanganyika
3. Belgian Congo
4. Uganda

Northern Rhodesia, Nyasaland, Palestine, Persia, Peru, Philippines, Puerto Rico, St. Helena, Spain, Sudan, Sweden, Switzerland, Trinidad, Turkey, Russia, Union of South Africa, Virgin Islands and other localities.

None of these places can be considered a commercial source of supply at present, although some are capable of future development.

Leyton and Ubilla *(1799)* state that pyrethrum grown in Chile in 1939 averaged 1.63 per cent pyrethrins.

Analyses of samples or small lots received in the United States from several of these sources are given herewith.

Source	Crop Year	Pyrethrins, %	Method of Analysis
Bulgaria	1938	1.19	Gnadinger
China	1940	1.07	Seil
India	1940	1.27	Gnadinger
Peru	1938	0.75	Gnadinger
Peru	1939	0.79	Gnadinger
Southern Rhodesia	1940	1.03	Gnadinger
Haiti	1945	1.87	Seil
Ethiopia	1945	0.94	Seil

Consumption of Pyrethrum in the United States

The rapidity with which Kenya replaced Japan as the principal supplier of pyrethrum to the United States is illustrated in Table CV, which shows importations from all sources for the eleven years 1934-1944. Imports from Kenya, Tanganyika and Uganda are not given separately but are combined under "British East Africa." Importations from Japan were prohibited by Executive Order No. 8832 on July 26, 1941.

Estimated importations for 1945 are:

British East Africa	13,000,000	pounds
Belgian Congo	2,800,000	pounds
Brazil	2,200,000	pounds
Total	18,000,000	pounds

Government Regulations Affecting Pyrethrum

For many years there has been a duty of twenty per cent on importations of pyrethrum extracts entering the United States. The duty on ground flowers is five per cent (Treasury Decision 50670, July 29, 1942). There have also been regulations of the U. S. Department of Agriculture prohibiting more than two per cent of sand and five per cent of stems in importations of pyrethrum flowers. The Federal and State regulations on the labelling of pyrethrum insecticides are discussed in Chapters IX, XII, XIII, XIV and XXIII.

The first of the war-time regulations seriously affecting importations of pyrethrum was the Executive Order of July 26, 1941, previously mentioned, which prohibited importations from Japan (Federal Register, vol. 6, p. 3715).

On June 13, 1942 the War Production Board issued General Preference Order M-179 *(1742)*. This order prohibited deliveries of pyrethrum insecticides by producers without first obtaining approval by WPB. It also prohibited acceptance by any person of more than thirty days' supply of pyrethrum insecticides.

TABLE CV. IMPORTATIONS OF PYRETHRUM INTO THE UNITED STATES (POUNDS)*

Source	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944
British East Africa**	.....	.....	1,614,000	1,423,000	2,864,000	5,404,000	10,387,000	10,068,000	8,830,000	5,985,000	7,685,000
Belgian Congo	.....	.....	.....	.....	.....	2,000	17,000	179,000	223,000	200,000	770,000
Brazil	5,000	.....	.....	1,000	497,000	80,000	78,000	11,000	397,000	593,000	2,203,000
Japan	10,094,000	15,203,000	9,934,000	17,850,000	10,896,000	7,586,000	2,031,000	762,000	.....	.....	.....
Yugoslavia	299,000	154,000	119,000	519,000	218,000	388,000	66,000	.....	.....	.....	.....
Italy	113,000	66,000	61,000	277,000	28,000	78,000	11,000	.....	.....	.....	.....
United Kingdom	42,000	147,000	29,000	22,000	34,000	11,000	.....	.....	.....	.....	.....
Russia	38,000	5,000	.....	.....	.....	.....	.....	.....	.....	.....	.....
China	.....	2,000	.....	.....	.....	.....	.....	.....	.....	.....	.....
Greece	.....	.....	.....	1,000	.....	.....	.....	.....	.....	.....	.....
India	.....	.....	.....	.....	.....	20,000	.....	.....	.....	.....	.....
Peru	.....	.....	.....	.....	.....	.....	1,000	.....	2,000	.....	.....
Chile	.....	.....	.....	.....	.....	.....	.....	.....	.....	18,000	.....
Total	10,591,000	15,577,000	11,757,000	20,093,000	14,537,000	13,569,000	12,591,000	11,020,000	9,452,000	6,796,000	10,658,000
Average value, cents per pound	.195	.131	.080	.109	.171	.234	.234	.167	.166	.169	.231

*From U. S. Dept. of Commerce. **Includes flowers from Kenya, Tanganyika and Uganda.

General Preference Order M-179 was amended on August 30, 1943 to "Allocation Order M-179" *(2306)*. The amended order prohibited the manufacture of pyrethrum insecticides containing rotenone. It permitted the manufacture of only those kinds of pyrethrum insecticides approved by WPB. It forbade producers to use pyrethrum without specific approval of WPB. The use and sale of pyrethrum insecticides was brought completely under Government control by this order. The only type of pyrethrum insecticide whose manufacture was approved by WPB was highly concentrated extract for use by the armed forces; the by-products of the manufacture of this extract were allocated for agricultural uses. Only a few producers were equipped to manufacture concentrated extract to meet the specifications, therefore it became necessary to take flowers from some importers and divert them to other processors. Allocations of pyrethrum for civilian uses practically ceased.

Allocation Order M-179 was revoked on September 13, 1944 and simultaneously pyrethrum was placed under allocation as an Appendix A material under General Allocation Order M-300, Schedule 48, issued by WPB *(2307, 2308)*. This order remained in effect until September 30, 1945. The War Production Board Orders provided heavy penalties for violators.

The official in direct charge of pyrethrum for WPB was Mr. Melvin Goldberg, of Washington, D. C. The pyrethrum industry was indeed fortunate in having Mr. Goldberg in that position. He handled the difficult task with great ability and his fairness and integrity won the respect even of those who were sometimes adversely affected by his decisions. His services to the Government were invaluable.

The control of the use of pyrethrum insecticides allocated by WPB for agricultural purposes was delegated to the War Food Administrator in 1943 *(2328, 1942)*. Under this delegation of authority, the War Food Administrator issued Food Production Order 11 *(1350)* on April 29, 1943. This order restricted the use of pyrethrum to certain crops and to the dairy industry; it was amended on February 28, 1944 *(2327)*. Food Production Order 11 was changed to War Food Order 46 on April 22, 1944 *(2109)* and the latter was amended on July 13, 1944 *(2110)*. War Food Order 46 was revoked November 27, 1944 *(2111)*.

In the winter of 1941-1942, after the entry of the United States into the war, the civilian demand for pyrethrum insecticides increased rapidly because of the fear of shortages caused by war conditions. Domestic prices increased somewhat and this

was fortunate, because prices of pyrethrum insecticides were frozen at March, 1942, levels under General Maximum Price Regulation issued April 28, 1942 by the Office of Price Administration (Federal Register, vol. 7, p. 3153).

This increase in domestic prices prior to April, 1942, enabled processors to absorb the large increase in war-risk insurance without further increasing prices after March, 1942, until the Kenya price was raised in June, 1944.

When the price of Kenya flowers was raised, by agreement between the British and United States Governments, to 32½ cents per pound, ex dock New York, the Office of Price Administration established maximum prices for pyrethrum insecticides in Revised Maximum Price Regulation 298, issued June 30, 1944 *(1238)*.

The British Ministry of Supply became the sole purchaser of pyrethrum in British East Africa on January 1, 1943 and all outstanding contracts, between the Kenya Farmers' Association and American importers unshipped on that date, were cancelled. The U. S. Board of Economic Warfare, later called the Foreign Economic Administration, negotiated an agreement which was signed on July 13, 1943 by the British Raw Materials Mission and the U. S. Commodity Credit Corporation. By this agreement the Commodity Credit Corporation acquired the contracts which the British Ministry of Supply had cancelled and these contracts were eventually filled at the original price. Commodity Credit Corporation continued to handle the importation of pyrethrum into the United States until January 1, 1944, when this function was transferred to the U. S. Commercial Company, a government agency.

On October 30, 1943 the U. S. Government entered into a contract with the Brazilian Government covering importations of pyrethrum from Brazil until December 31, 1945. A contract was negotiated on November 16, 1943 between the Governments of the United States and the Belgian Congo for importation of pyrethrum until December 31, 1946.

The United States Government encouraged the cultivation of pyrethrum in Chile, Peru, Mexico and Central America by supplying seed and technical advice and, in some cases, by supervising the plantings. Development contracts were negotiated in some of these countries, whereby the United States agreed to purchase the crops for three years. At the beginning of 1945, about 500 acres were under such contract in Mexico and about 500 acres in Guatemala.

Other Species of Pyrethrum*

Pyrethrum roseum has been grown commercially in Spain and in Russia. The cultivation and use of this species in Spain is described by Obdulio and Capdevila *(1955)*.

The pyrethrin content of *Pyrethrum roseum* and 14 other species of pyrethrum, growing wild in Armenia, has been reported by Mardzhanyan *(1841)*, who also tested the toxicity of powders made from these species on cabbage worms (Table CVI).

Table CVI. Pyrethrin Content and Toxicity of Various Pyrethrum Species (Mardzhanyan)

Botanical Name	Total Pyrethrins (Ripert Method) %	Pyrethrin I (Tattersfield Method) %	Cabbage Worms Killed After 48 Hours %
Pyrethrum cinerariaefolium	0.94–1.20	0.23–0.76	100
Pyrethrum roseum	0.43–0.75	0.21–0.60	100
Pyrethrum carneum	0.32–0.71	0.27–0.87	100
Pyrethrum tamrutense	0.51	0.50	100
Pyrethrum szowitssi	0.26	0.21	0
Pyrethrum balsamita	0.42	0.11	0
Pyrethrum canescens	0.13	0.11	0
Pyrethrum myriophyllum	0.27	0.12	0
Pyrethrum sosnowskyanum	0.09	...	0
Pyrethrum parthenifolium	...	0.18	0
Pyrethrum macrophyllum	...	0.15–0.32	0
Pyrethrum punctatum	...	0.21–0.29	0
Pyrethrum chyliophyllum	0.18	0.05	0
Pyrethrum pulverilentum	...	0.10	0
Pyrethrum vulgarae	...	0.05	0

Pyrethrum tamrutense is highly resistant to drought.

A sample of *Chrysanthemum frutescens* from Tanganyika assayed 0.12 per cent pyrethrins, but biological tests gave negative results *(1651)*.

Acree and LaForge *(993)* investigated the pyrethrin content of the field daisy, *Chrysanthemum leucanthemum*. They concluded no pyrethrins are present in daisy flowers, confirming the earlier work of Gnadinger and Corl (page 63).

*Throughout this book the term pyrethrum, unmodified, is applied to *Pyrethrum cinerariaefolium*.

CHAPTER XVIII

ACTIVE PRINCIPLES OF PYRETHRUM

The work of Staudinger and Ruzicka which resulted in the isolation and identification of the pyrethrins was published in 1924, after it became apparent that their primary objective, the synthesis of the active principles of pyrethrum, could not be attained. These gifted men then turned their attention to other fields, Staudinger to the structure of rubber and Ruzicka to the chemistry of hormones. Their work on pyrethrum was accepted virtually without question by Tattersfield, Gnadinger, their associates and others who pioneered the industrial and technical development of pyrethrum insecticides.

In 1935, however, the scientists of the U. S. Department of Agriculture began the publication of a long series of papers describing a comprehensive investigation of the pyrethrins and related compounds. Some idea of the difficulty of the work involved can be gained from the fact that even today the exact structure of the pyrethrins is not definitely known.

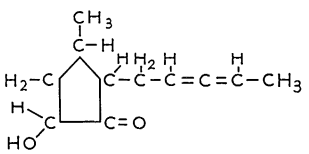
The Pyrethrins and Their Derivatives

Haller and LaForge *(1573)* prepared the semicarbazone of pyrethrin II from a concentrate containing 80 per cent pyrethrin II (page 37). The semicarbazone melts at 165°. The pure pyrethrin II which LaForge and Haller claimed to have prepared by distillation in a molecular still *(538)* apparently was not pure, but was altered by distillation, since it did not yield a crystalline semicarbazone. Ripert *(2048)* states that pyrethrin II is not identical with the product described by Haller and LaForge. The latter obtained a nearly pure semicarbazone of pyrethrin I, m. p. 118°, from concentrates rich in pyrethrin I. Pyrethrin I was also altered by molecular distillation. The semicarbazone of pyrethrolone was prepared by the method of Staudinger and Ruzicka (page 26). Its melting point was 200°, as Staudinger and Ruzicka reported, but analysis showed that it contained two hydrogen atoms less than Staudinger and Ruzicka found. This was confirmed by hydrogenating the pyrethrolone semicarbazone, yielding tetrahydropyrethrolone semicarbazone, which also contained two hydrogen atoms less than found by Staudinger and Ruzicka. Paraphenylphenacyl esters of chrysanthemum monocarboxylic acid and chrysanthemum dicarboxylic acid were pre-

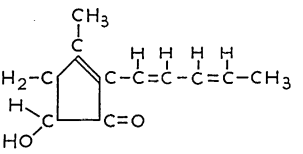
pared and these compounds served to identify the two chrysanthemum acids.

LaForge and Haller *(1769)* suggested revised formulas for pyrethrolone and tetrahydropyrethrolone, based on analyses of the free ketones and their derivatives. When the hydroxyl group of tetrahydropyrethrolone was replaced by chlorine and the resulting compound was reduced, an optically inactive ketone, tetrahydropyrethrone, was obtained. The semicarbazone of this ketone melted at 176° and was thought to be identical with the semicarbazone of dihydrojasmone.

Subsequently, LaForge and Haller *(1770)* confirmed the identity of tetrahydropyrethrone with dihydrojasmone by the mixed melting point of the semicarbazones. On the basis of the established structural formula assigned to dihydrojasmone and tentatively adopting the suggestion of Ruzicka and Pfeiffer *(2090)* regarding the side chain, LaForge and Haller suggested a revised formula for pyrethrolone, which is compared below with that of Staudinger and Ruzicka.



Pyrethrolone ($C_{11}H_{16}O_2$)
Staudinger and Ruzicka



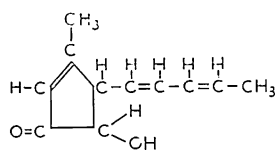
Pyrethrolone ($C_{11}H_{14}O_2$)
LaForge and Haller

The revision by LaForge and Haller, therefore, consisted of substituting a cyclopentenolone for the cyclopentanolone nucleus of Staudinger and Ruzicka and adopting the conjugated side chain of Ruzicka and Pfeiffer in preference to the allene grouping of Staudinger and Ruzicka.

Further work by LaForge and Haller *(1773)* failed to prove conclusively the structure of the side chain. The possibility of a second ring system in the side chain, easily cleaved by hydrogenation to form a saturated five-membered straight chain, was suggested. Acree and LaForge *(995, 996)* synthesized a number of allenes in order to compare them with pyrethrolone, but did not reach a definite conclusion as to the presence of a cumulated or conjugated system in the side chain of pyrethrolone.

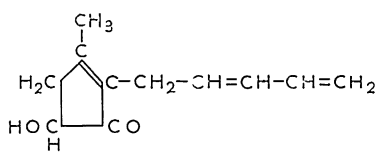
Gillam and West *(1493)*, in a most interesting investigation, compared the absorption-spectra data on the pyrethrins and related compounds with absorption spectra of compounds of

known structure and, using stepwise hydrogenation with absorption-spectra control, reached the following conclusions: "In the pyrethrolone fragment of the pyrethrin molecules, two *separate* chromophoric systems are present each containing more than one double linkage; it follows, therefore, that the trienone chromophoric system which has been postulated [by LaForge and Haller] cannot be present. An α, β-unsaturated ketone grouping is present in a five-atom ring. The unsaturation in the side chain is present as a conjugated diene, the presence of a cumulated diene system being precluded by the absorption spectra evidence." The possibility of a second ring in the side chain, as suggested by LaForge and Haller was ruled out by the data. Gillam and West suggested tentatively the following formula for pyrethrolone as being in accordance with the absorption data.



Pyrethrolone ($C_{11}H_{14}O_2$)
Gillam and West

LaForge and Barthel *(1764)*, unable to reconcile the absorption results of Gillam and West *(1492, 1493)* with the known chemical reactions of pyrethrolone, extended their investigation of the structure of pyrethrolone. They showed that pyrethrolone, when isolated in the usual manner, is not a homogeneous compound, as previously assumed, but a mixture of components differing with respect to the nature of the side chain. These components were partially separated by distillation and showed marked differences in refractive index. By determining the carbon-linked methyl groups in successive fractions, it was shown that one component has the conjugated system of double bonds while the other contains a side chain terminating with the group $C = CH - CH_3$. The mixture of compounds previously known as "pyrethrolone" consisted predominantly of the compound of the following structure, suggested by R. B. Woodward:



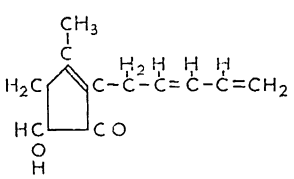
Continuing their investigation *(1765)*, LaForge and Barthel succeeded in isolating five pyrethrolone semicarbazones by fractional distillation of acetyl pyrethrolone prepared from pyrethrolone obtained by acid hydrolysis of pyrethrolone semicarbazone. The pyrethrolone present in largest proportion had the structure suggested by Woodward and was designated "pyrethrolone C."

Two isomers of "pyrethrolone C" were also isolated as their semicarbazones. These were designated "pyrethrolone B-1" and "pyrethrolone B-2." These three compounds behaved alike on hydrogenation and spectrographic data indicated that they all contain a conjugated side chain.

Two additional compounds, "pyrethrolone A-1" and "pyrethrolone A-2" were also isolated. They differed from the other three pyrethrolones by having one less carbon atom, one less unsaturated linkage and one more terminal-methyl group.

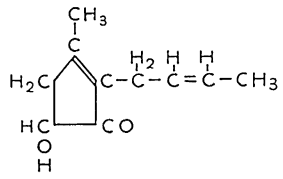
LaForge and Barthel state: "In what proportions the different pyrethrolones are combined with chrysanthemum acids is unknown. The terms "pyrethrin I" and "pyrethrin II," however, must henceforth be regarded as defining not compounds, but groups characterized only according to the acid component, which is each case is esterified with more than one and probably with several pyrethrolones."

Later, LaForge and Barthel *(1766)* showed that "pyrethrolone B-1" is the optically active isomer, "pyrethrolone B-2" is the racemic mixture and "pyrethrolone C" is a mixture of the optically active and the partly racemized compound. Hence, "pyrethrolone, as prepared by the acid hydrolysis of the semicarbazone, is a mixture the greater part of which consists of the dextro and racemic forms of the compound of the empirical formula $C_{11}H_{14}O_2$ and the following structure:"



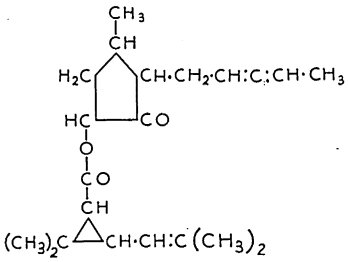
Pyrethrolone ($C_{11}H_{14}O_2$)
LaForge and Barthel

The two compounds, "pyrethrolone A-1" and "pyrethrolone A-2" were present in lesser amount, also in the dextro and racemic forms. This constituent was named "cinerolone." It has the empirical formula $C_{10}H_{14}O_2$ and the following structure:

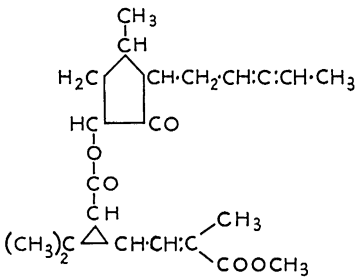


Cinerolone ($C_{10}H_{14}O_2$)
LaForge and Barthel

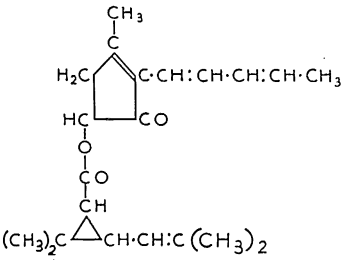
The formulas of the two pyrethrins, based on the structures suggested by Staudinger and Ruzicka, by LaForge and Haller, by Gillam and West and by LaForge and Barthel are given herewith:



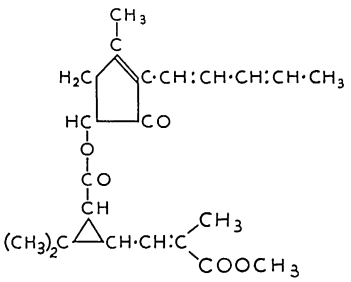
Pyrethrin I ($C_{21}H_{30}O_3$ = 330.45)
Staudinger and Ruzicka



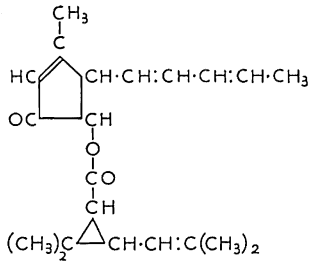
Pyrethrin II ($C_{22}H_{30}O_5$ = 374.46)
Staudinger and Ruzicka



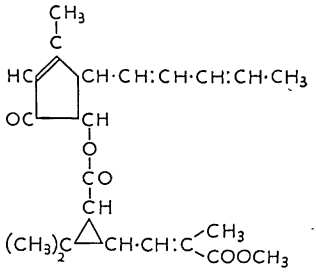
Pyrethrin I ($C_{21}H_{28}O_3$ = 328.43)
LaForge and Haller



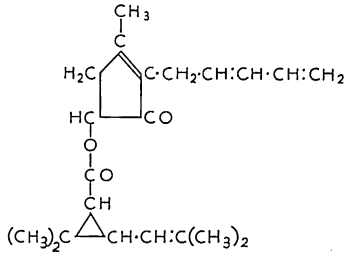
Pyrethrin II ($C_{22}H_{28}O_5$ = 372.44)
LaForge and Haller



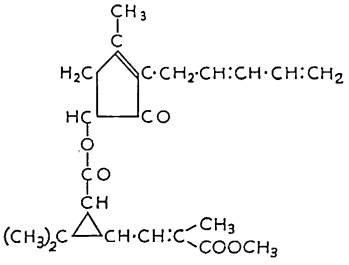
Pyrethrin I ($C_{21}H_{28}O_3$ = 328.43)
Gillam and West



Pyrethrin II ($C_{22}H_{28}O_5$ = 372.44)
Gillam and West



Pyrethrin I ($C_{21}H_{28}O_3$ = 328.43)
LaForge and Barthel



Pyrethrin II ($C_{22}H_{28}O_5$ = 372.44)
LaForge and Barthel

Haller and LaForge *(1576)* have found that pyrethrolone from pyrethrin I semicarbazone is identical with pyrethrolone from pyrethrin II semicarbazone. The melting points of the pyrethrolone semicarbazones as well as the specific rotations of pyrethrolones from the two pyrethrins were substantially the same. Synthesis of pyrethrin II by the method of Staudinger and Ruzicka gave a product which did not yield a crystalline semicarbazone. Tetrahydropyrethrolone and monomethyl ester chrysanthemum dicarboxylic acid chloride reacted to form tetrahydropyrethrin II, which yielded a semicarbazone identical with the product obtained on catalytic hydrogenation of pyrethrin II semicarbazone. Condensation of tetrahydropyrethrolone and chrysanthemum monocarboxylic acid chloride gave a tetrahydropyrethrin I which yielded a semicarbazone having a melting point slightly lower than the products obtained on hydrogenation of purified pyrethrin I semicarbazone.

Haller and LaForge *(1578)* concluded that the two enols obtained by treating pyrethrolone with sodium methylate solution are isomeric with pyrethrolone and not oxidation products as claimed by Staudinger and Ruzicka. In the lower boiling enol,

the ketone and hydroxyl groups are in the same positions as in pyrethrolone, but in the higher boiling enol the positions are reversed.

LaForge and Haller *(1772)* suggest the use of dilute hydrochloric acid for separating the semicarbazones of certain pyrethrin derivatives. The semicarbazones of pyrethrone, dihydropyrethrone, tetrahydropyrethrone and tetrahydropyrethrolone are soluble in dilute hydrochloric acid; the semicarbazones of pyrethrolone, pyrethrin I, pyrethrin II and hexahydropyrethrone are insoluble in dilute hydrochloric acid.

In their attempts to isolate pyrethrin I, LaForge and Haller *(1771)* were not able to obtain a pure product. Even after repeated purification, the semicarbazone of pyrethrin I was always found to be a mixture consisting chiefly of pyrethrin I semicarbazone, a little pyrethrin II semicarbazone and a third semicarbazone. Saponification of the mixed semicarbazones with sodium methylate in methanol yielded as the alcoholic component only pyrethrolone semicarbazone. The combined acid components, however, were found to be chrysanthemum monocarboxylic acid, from pyrethrin I, chrysanthemum dicarboxylic acid, from pyrethrin II, and a third acid, possibly a mixture, having the formula $C_{16}H_{30}O_2$.

This third acid melted at 41° and gave a color with Denigès' reagent similar to that obtained with chrysanthemum monocarboxylic acid (page 33). It was unsaturated, and a solution of its alkali salt was precipitated by barium chloride. LaForge and Haller concluded from this work that esters of pyrethrolone with acids other than the chrysanthemum acids are present in pyrethrum flowers. The toxicity of this new ester of pyrethrolone to insects was not determined.

Later, Acree and LaForge *(994)* identified this new ester as a mixture of the esters of pyrethrolone with palmitic and linoleic acids. Free palmitic and linoleic acids were also found in oleoresin of pyrethrum.

Haller and LaForge *(1575)* investigated the action of the pyrethrins on hydrogenation and found that they are cleaved, yielding a mixture of tetrahydro- and hexahydropyrethrone and chrysanthemum dicarboxylic acid monomethyl ester, in the case of pyrethrin II and the same pyrethrone derivatives and dihydrochrysanthemum monocarboxylic acid from pyrethrin I. This reaction was suggested as the basis of a quantitive method (page 451).

Haller and Sullivan *(1582)* determined the toxicity of hydrogenated pyrethrins to flies. Hydrogenation decreased the knockdown and mortality of pyrethrin I but only the knockdown of pyrethrin II. The mortality caused by pyrethrin I was considerably higher than that caused by pyrethrin II (page 506). Staudinger and Ruzicka found that hydrogenation of the pyrethrins rendered them inert (page 25).

On the basis of comparisons between chemical assays and biological tests, Roy and Ghosh *(2082)* concluded that pyrethrum flowers contain some active principle other than the pyrethrins. This claim has been characterized by Haller and LaForge *(1100)* as hardly warranted. West *(2304)* finds no evidence to support the suggestion that pyrethrum flowers contain a third active principle.

Rose and Haller *(2074)* have described a compound isolated from pyrethrum, to which they gave the name "chrysanthin" because it appeared to be identical with the compound of that name described by Chou and Chu *(180)*. This compound melted at 201° when crystallized from ethyl acetate and at 177-178° when crystallized from ethanol. It has the formula $C_{17}H_{22}O_5$.

Schechter and Haller *(2094)* have shown that the chrysanthin of Chou and Chu and of Rose and Haller is identical with the "pyrethrosin" of Thoms *(898)* and suggest the name pyrethrosin be retained for the compound. They also show that pyrethrosin is different from geigerin, a material isolated from the plant *Geigeria aspera* by Rimington and Roets *(2045)* and believed by the latter to be the same as pyrethrosin.

Harvill *(1615)* prepared a number of esters of chrysanthemum monocarboxylic acid and tested their toxicity against aphids and cockroaches. The lauryl, myristyl, cetyl and diethanolamine esters were about as toxic to *Aphis rumicis* at a concentration of 0.03 per cent as pyrethrins at the same concentration, but they did not produce the same effect on cockroaches as the pyrethrins. The furfuryl and vanillin esters were unstable.

Action of Pyrethrins on Cold-Blooded Animals

An extensive investigation of the action of isolated pyrethrins has been made by Gaudin *(1478)* on many different cold-blooded animals, including the following:

Infusoria:	Vorticella, paramecium
Actinozoa:	Sea anemone
Echinodermata:	Sea urchin, starfish

Arthropoda:	Crabs, scorpion, flies, mealworm
Annelida:	Sandworm, earthworm, leech
Mollusca:	Oyster, snail, octopus, cuttlefish
Tunicata:	Sea-squirt
Vertebrata:	Goldfish, frog, lizard, turtle, snake

The pyrethrins, prepared by the method of Ripert *(748)* were injected as an emulsion intraperitoneally or administered by mouth except in the case of the Infusoria, Tunicata and flies, where dosage was by contact. The threshold of toxic action and lethal dose were determined for each animal. The toxicity of the pyrethrins appears to be related to the development and functional importance of the nervous system. The Infusoria are only slightly affected by solutions of pyrethrins of much greater concentration than necessary to kill fish. The sea-squirt, having a poorly developed nervous system, reacts like the Infusoria. The lethal dose for sea anemone and echinoderms is between 200 mg. and 1000 mg. per kilo; for worms and leeches, 100 mg. per kilo. For mollusks the lethal dose is 250 mg. per kilo, excepting the cephalopods which are killed by 1 mg. per kilo, as would be expected from the high development of their nervous systems.

Among the arthropods, the scorpions, flies and mealworms are killed by doses of 17 to 75 mg. per kilo but the crustaceans are exceptionally susceptible to the action of pyrethrins and are killed by doses of 0.01 mg. per kilo. The cold-blooded vertebrates react variably, turtles requiring a lethal dose of 175 mg. per kilo, while the more lively snakes and lizards are killed by 12 mg. to 15 mg. per kilo. The effect of pyrethrins on snakes and frogs was the same when injected intraperitoneally as when given by mouth.

Gaudin *(1477)*, using frogs as test animals, also studied the effect of the pyrethrins on isolated nerve and muscle and on centers controlling peripheral chronaxia. Similar experiments were conducted by Monnier and Gaudin *(1901)*.

Rosen and Thompson *(2076)* also investigated the effects of pyrethrins on nerves and muscles of frogs. Using a hydro-alcoholic extract of pyrethrum, injected into the ventral lymph sac of the frog, they found that skeletal muscle and motor nerves supplying this type of muscle are not affected by pyrethrum. The principal site of action is the spinal cord. Toxic doses produce a transitory stimulation of both the anterior and posterior horns of the spinal cord, followed by an intense depression and paralysis of distinctly ascending type, ultimately

reaching the medullary centers. The autonomic nervous system appears not to be directly affected by pyrethrum.

Klinger *(1734)* concluded that the pyrethrins are nerve poisons and that the susceptibility of different insects to pyrethrum depends largely on the permeability of the cuticle and nature of the nerve system and perhaps on the pH of the body fluids. He demonstrated a measurable reduction in flow of nerve impulse by means of a galvanometer and furnished direct proof that pyrethrum acts on the nervous system in insects. The flow in a pyrethrum-injured nerve was about one-fifth that of a normal nerve.

Woke *(2342)* fed turnip-leaf sandwiches containing pyrethrum powder or pyrethrum extract to southern armyworms, *Prodenia eridania.* After a few hours neither the feces nor any part of the body was toxic to mosquito larvae. That this inactivation was not due merely to passage through the digestive tract was proved by incubating pyrethrum with the ground tissues of the armyworm. The toxicity of the pyrethrum was rapidly destroyed, the inactivating effect of the tissues decreasing in the following order: fat body, skin and muscles, digestive tract, blood.

Yeager and Munson *(2352)* found no marked hematological change following the administration of pyrethrum to southern armyworms.

Hoskins *(1656)* has made a critical study on the relation of insect physiology to insect toxicology and control. This valuable paper should be read by everyone interested in the mode of action of pyrethrum on insects. Hoskins has mentioned the importance of the fatty epicuticle in determining the ease of penetration of contact insecticides and has reviewed the work of Buchmann, Klinger, Wigglesworth and others, in which emphasis was laid on the lipoid layer of insect integument as the chief cause of impermeability. He also points out that the solubility of the pyrethrins in oils should allow them to penetrate insect integument readily.

Bredenkamp *(1246)* concluded that the penetration of insect cuticle by pyrethrum is a purely mechanical phenomenon. Neither the outer layer of the cuticle nor the insecticide was altered during penetration.

The larva of the tick, *Ornithodorus moubata,* remains motionless for about four days after hatching. If, however, it is sprayed with oil extract of pyrethrum, it soon begins to move its legs. Robinson *(2065)* has used the rapidity of this response to

measure the rate of penetration of pyrethrum through the cuticle. A logarithmic relation was found between pyrethrin concentration and speed of entry. Penetration was slower as the larva aged. Mineral oils caused more rapid penetration than vegetable oils.

In a study of the integument of insects in relation to the entry of contact insecticides, Wigglesworth *(2318)* observed that when insects are immersed in petroleum oils, minute drops of water appear on the surface of the cuticle. Light oils cause the droplets to appear more rapidly than heavy oils, and the appearance is more rapid in young insects than in old insects. Delay in the penetration of heavy oils can be eliminated by first extracting the lipoid layer with petroleum ether. Penetration of oil extracts of pyrethrum into the blood-sucking insect, *Rhodnium prolixus*, is more rapid with light petroleum oils than with heavy oils and rapidity of penetration is greatly increased if the cuticle is first extracted with petroleum ether. Wigglesworth found that penetration of pyrethrins in vegetable oils is very slow, confirming the observations of Robinson *(2065)*. Penetration of pyrethrum in oils is accelerated by the addition of 5 per cent of oleic or other fatty acid.

Hartzell and Scudder *(1610)* found the action of pyrethrins on the housefly quite different from that of the "activator" isobutylundecylenamide. Pyrethrum caused clumping of the chromatin of the nuclei in all of the body tissues, while the amide caused dissolution of the chromatin. Pyrethrum also caused drastic destruction of the fiber tracts of the brain (see cut). Hartzell and Scudder suggest that the interaction of the two types of nuclear destruction caused by pyrethrins and the amide may be the true basis of "activation." The effects of the pyrethrins are not confined to the nervous system.

The action of piperine on houseflies was investigated by Hartzell and Strong *(1611)* and compared with the effect of pyrethrum. The effect of piperine was much less general than that of pyrethrum, and piperine did not show the clumping of the chromatin in the nuclei, which is characteristic of pyrethrum.

Richards *(2034)* concluded that insect nerve cells and their processes are surrounded and insulated by bound lipid sheaths of submicroscopic thickness, probably phospholipids, perhaps with the addition of cholesterol. These lipid sheaths are correlated with and so presumably condition the penetration of oil solvents and oil-borne toxins, such as pyrethrins, into the nervous

VERTICAL SECTION THROUGH BRAIN OF HOUSEFLY, SHOWING CORPUS CENTRALE (UPPER RIGHT) AND FIBER TRACTS LATERAD OF IT. SHOWS THE EFFECT OF PYRETHRUM ON FIBERS.

(Courtesy Hartzell and Scudder, Boyce Thompson Institute)

VERTICAL SECTION THROUGH BRAIN OF HOUSEFLY IN REGION OF CORPUS CENTRALE. NORMAL TISSUE.

(Courtesy Hartzell and Scudder, Boyce Thompson Institute)

system from tracheae. Some data suggest that the pyrethrins destroy nerve sheaths.

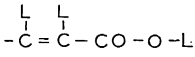
Sweetman and Gyrisko *(2197)* in a series of tests on firebrats, *Thermobia domestica,* observed that survivors of the acute toxic effects of pyrethrum frequently suffered from a latent injury, characterized by discoloration and sloughing of afflicted appendages. When discoloration occurred, the injury was progressive and death followed. Some samples of pyrethrum did not cause the typical latent symptoms. Rotenone dusts caused similar injury.

Coon *(1308)* studied the blood circulation of the American cockroach by injecting a fluorescent indicator solution into one of the cerci and observing its course under ultra violet light. Similar studies were made on cockroaches that had been paralyzed with liquid pyrethrum insecticide or powdered pyrethrum. Roaches that had received a lethal dose showed a continuous decline in heart beat, and blood circulation ceased before the heart stopped beating. In roaches receiving a sublethal dose the heart beat increased and then returned to normal; there was little effect on the blood circulation.

Pyrethrum has a violent activating effect on roaches, *Blatella germanica,* causing them to desert their hiding places. This action is believed by Hutzel *(1681)* to depend on the rate of diffusion of the pyrethrins through the oily film which covers the ventral surface. The initial excitation results from stimulation of the sensory endings in the integument. Final paralysis depends on the speed of diffusion of the pyrethrins to the thoracic ganglion, which is inactivated.

McGovran and others *(1880)* injected cockroach nymphs with Chinese ink and trypan blue; the former loads the hemocytes with carbon particles and the latter stains the nephrocytes. An acetone extract of pyrethrum containing 0.4 mg. pyrethrins per cc. was applied externally to the injected insects at the rate of 0.005 cc. per g. of body weight. The resistance of the insects to pyrethrum was not appreciably changed by either treatment.

Anderson and Hook *(1011)* found newly emerged blowflies, *Phormia regina,* more resistant to pyrethrins than older blowflies, either because the pyrethrins do not penetrate the new cuticle so readily, or because the nervous system of the new flies has not reached its maximum susceptibility to pyrethrins. Flies that recovered from the effects of pyrethrins mated and laid fertile eggs.

Läuger, Martin and Müller *(1791)* propose the theory that compounds which are highly effective contact insecticides contain in the molecule a toxic component and a solubilizing component. The latter gives the compound high lipoid solubility, which is essential for penetration of the integument of insects. They support their theory by comparisons of the constitution of synthetic insecticides and natural insecticides. Their suggested generalized formula for most insecticides derived from plants, including pyrethrins, is  in which L is the lipoid-solubilizing component.

The physiological action of the pyrethrins on the larvae of the wax moth, *Galleria mellonella, Daphnia* and crawfish has been studied by Belleuvre *(1203)*. Functioning of the crawfish heart was arrested by minute doses of pyrethrins, but the *Daphnia* heart and dorsal vessel of the *Galleria* larva tolerated doses 20,000 times as large as those which act on the crawfish heart.

The mechanism of action of pyrethrum insecticides has also been investigated by Roy and Ghosh *(2083)*.

Pepper and Hastings *(1987)* noted the effectiveness of pyrethrum dusts and sprays on first and second larval instars of the sugar beet webworm, *Loxostege sticticalis*. They also observed that these insecticides were less effective against the third instar, of little effect against the fourth and practically ineffective against the fifth larval instar. They considered it doubtful that this decreasing effectiveness is due to physiological or morphological changes within the insect as its development proceeds. "More probably the reason lies in functional differences in the exoskeleton, through which structure the insecticide must pass before exerting its toxic effect." The exoskeletons of third, fourth and fifth instar larvae were separated from all cellular structures and subjected to chemical analysis. As the larvae develop, there is a marked decrease in fat content of the exoskeleton and an increase in protein, chitin and ash. Membranes high in fat content, such as third instar exoskeletons would probably be more permeable to fat soluble insecticides like the pyrethrins than the exoskeletons of fourth and fifth instars, which are low in fat.

Pyrethrum is toxic to frogs and snakes when applied to the skin. The author has seen frogs dying from the effects of pyrethrum dust applied to an orchard for insect control. A large bull snake 30 inches long and more than an inch in diameter was

placed in contact for 5 minutes with 30 cc. of oil-pyrethrum insecticide containing 2.5 per cent pyrethrins. After 20 minutes the snake was almost completely paralyzed, having shown all the symptoms of pyrethrum poisoning usually exhibited by cabbage worms and similar insects. Death occurred in about 18 hours.

Effect of Pyrethrins on Warm-Blooded Animals

Gaudin *(1478)*, in experiments on guinea pigs and mice, showed that intraperitoneal dosage of pyrethrins caused death, while the effect of oral administration of 5 g. of pyrethrins per kilo of body weight was practically nil. In general, Gaudin found that the action of pyrethrins is the same on cold-blooded animals whether administered by mouth or intraperitoneally. To warm-blooded animals, however, the pyrethrins are toxic when given intraperitoneally but are non-toxic by mouth. The relative toxicity of pyrethrins I and II varied according to the species of animal studied.

Rosen and Thompson *(2076)* injected olive oil solutions of pyrethrins subcutaneously into rats and intraperitoneally or intravenously into cats. Death resulted in all cases, but most rapidly following intravenous injections. The symptoms produced in these experiments indicated that pyrethrum causes an ascending paralysis of the spinal cord. Large intravenous doses produced no change in the blood pressure of the cat. Pyrethrum powder was mixed with food to the extent of 10 per cent and fed to cats over long periods of time. No toxic symptoms developed and the cats appeared normal in all respects. Pyrethrum produced a decrease in amplitude of contraction and a decrease in tonus of isolated rabbit intestine.

Leonard *(1796)*, using pyrethrum concentrates prepared by LaForge, administered solutions of pyrethrins in sesame oil to white mice, white rats, guinea pigs and rabbits, by intraperitoneal injection. The injections in white mice caused diarrhea, clonic convulsions, prostration and death from respiratory paralysis. The lethal doses for mice were much higher than reported by Gaudin *(1478)*. Pyrethrin II was much more toxic to mice than pyrethrin I. Pyrethrins caused a gradual decrease of amplitude of contraction and loss of tonus in isolated rat intestine. The dose of 240 mg. of pyrethrin II per kilo, which killed 66 per cent of the white mice, had little effect on white

rats and guinea pigs but caused incoordination of the hind legs and tremor in a rabbit.

De Ong *(1361)* conducted extensive experiments to determine the effect of pyrethrum extracts on sheep. The extracts were made with light mineral oils and were of the type used to control hibernating sugar beet leafhoppers. The extracts were applied to hay, alfalfa and other feeds which the sheep were allowed to eat. The sheep were not repelled by the pyrethrum sprays, which were fed in excessive quantities. There was no injury to feeding lambs or ewes.

Dermatitis among workmen in the pyrethrum industry of Kenya has been discussed by Tonking *(2239)* and by Sequeira *(2112)*. The condition is aggravated by hot weather and perspiration. Some individuals may work with pyrethrum two or three years before dermatitis develops. Others may bring on a rash by merely going into areas where the plants are growing. The dermatitis is characteristic of eruptions caused by contact with an irritant and in some cases distinct allergic phenomena are present. All cases are cured by removal from the irritant.

Sweitzer *(2198)* has reported a series of 1213 cases of scabies treated with pyrethrum ointment. Only 5 of these cases showed sensitivity to pyrethrum.

Dr. J. T. Martin of Rothamsted Experimental Station, Harpenden, England, developed a susceptibility to pyrethrum dermatitis after working with pyrethrum for several years. So acute was his sensitization that he could not go near a field of pyrethrum without experiencing intense itching of the hands and face and swelling of the eyelids. In conjunction with K. H. C. Hester, Martin investigated the cause of pyrethrum dermatitis, using himself as the test subject *(1851)*.

Patch tests made with different parts of the flower indicated that the cause of dermatitis was located in the achenes, involucre and receptacle, and not in the pollen, petals and disk florets. Ether almost completely extracted the active agent from the flowers. When the ether extract was evaporated and the resulting oleoresin was extracted with petroleum ether, the material insoluble in petroleum ether was much more irritating than that soluble in petroleum ether. Since the pyrethrins are readily soluble in petroleum ether, this indicated that the pyrethrins are not the cause of the dermatitis.

A colorless extract was prepared by extracting a mixture of pyrethrum and activated charcoal with petroleum ether and evaporating the solvent. This gave an intense reaction when

applied to the forearm. The fatty material present in the colorless extract caused little irritation and was apparently not the material causing dermatitis.

A highly concentrated purified extract containing 93 per cent pyrethrins caused only a very slight reaction when applied to the skin, again indicating that the pyrethrins are not the cause of dermatitis.

The volatile oil of pyrethrum was prepared by distilling ground flowers with steam, extracting the distillate with ether, drying the ether solution over sodium sulfate and evaporating the ether. The yield was about 0.1 per cent. This volatile oil gave a very strong positive reaction when applied to the skin, but the ground flowers from which it was distilled also gave a strong positive reaction. However, this may have been due to incomplete removal of the volatile oil. The volatile oil, freed of acids, gave a slightly less positive reaction than the original oil.

At one stage of the work Martin experienced such an acute reaction that he was obliged to remain in bed for three days. He experienced faintness and shivering; temperature 99.4; pulse 100; smarting of skin and swelling of eyes. There were no abnormal signs in the respiratory, abdominal and nervous systems. The lack of respiratory disturbance supports the views of Feinberg *(265)* and Sequeira *(2112)*, that the agent responsible for dermatitis differs from that causing respiratory troubles.

Although Martin and Hester did not succeed in isolating the cause of pyrethrum dermatitis, they made an interesting contribution to our knowledge of the subject. The problem of pyrethrum dermatitis and pyrethrum allergy has been an important one in connection with the use of pyrethrum for malaria control by the armed forces.

CHAPTER XIX

EVALUATION OF PYRETHRUM BY CHEMICAL METHODS

The chemical assay methods that have been most widely used during the last ten years are the Seil acid method for pyrethrin I and pyrethrin II, the Wilcoxon mercury reduction method for pyrethrin I and the Gnadinger-Corl copper reduction method for total pyrethrin content. The Seil method and the Wilcoxon method are applicable both to pyrethrum flowers and oil solutions of pyrethrins. The Gnadinger-Corl method is applicable to flowers, but cannot be used on oil-pyrethrum extracts unless the pyrethrin content exceeds about ten per cent (page 228).

The Seil method (page 67) and the Gnadinger-Corl method (page 52) have previously been described. The Gnadinger-Corl method was officially used for the assay of pyrethrum flowers in Yugoslavia and Japan until 1941 and in Kenya until 1943, when the U. S. Commodity Credit Corporation specified the Seil method in its sales contracts with American processors.

Mercury Reduction Method of Wilcoxon

Wilcoxon *(2324)*, in a study of the determination of pyrethrin I by the Seil method, prepared chrysanthemum monocarboxylic acid by saponifying a petroleum ether oleoresin of pyrethrum with alcoholic sodium hydroxide solution, diluting with water, boiling off the alcohol and adding barium chloride as in the Seil method. The filtrate, after the barium chloride treatment, was acidified with sulfuric acid and extracted with petroleum ether. The petroleum ether extract was washed with aqueous sodium hydroxide and discarded; the sodium hydroxide solution was acidified with sulfuric acid and extracted with petroleum ether which was washed free from sulfuric acid, dried over anhydrous sodium sulfate and evaporated in vacuo. The yield was 11 g. of crude acids from 188 g. of oleoresin.

To obtain pure monocarboxylic acid, the 11 g. of crude acids were twice fractionated by vacuum distillation (2.5 mm. pressure). The purest fraction was recrystallized from 80 per cent methanol. The identity and purity of the monocarboxylic acid were established by microchemical combustion, preparation and analysis of the silver salt, physical constants, titration and the

parachor of Sugden *(2191)*. The acid was 99.8 per cent pure, by titration.

Weighed portions of the pure monocarboxylic acid were distilled with steam in the presence of sulfuric acid and the distillate was extracted with petroleum ether as in the Seil method. Titration of the petroleum ether extract showed only 69 to 71 per cent recovery of the weight taken. Wilcoxon concluded that the monocarboxylic acid is "either not entirely volatile with steam, or it is partly decomposed by heating in the presence of sulfuric acid."

Wilcoxon next investigated the reaction of Denigès' reagent with chrysanthemum monocarboxylic acid. This reaction has been described by Audiffren *(85)* and Seil *(794)*. Wilcoxon developed the following method for determining the monocarboxylic acid and, indirectly, pyrethrin I, based on the reaction between the monocarboxylic acid and Denigès' reagent.

> "The sample should be of such a size that it will contain 50 to 70 mg. of pyrethrin I. The preliminary treatment is the same as that described by Seil. The 200 cc. aliquot obtained after the barium chloride treatment is treated in a separatory funnel with 1 cc. conc. H_2SO_4, and extracted with two 50 cc. portions of low boiling petroleum ether. The barium sulfate precipitate does not seem to cause any difficulty. The petroleum ether extracts are washed successively with several small portions of water, and filtered through a small plug of cotton into a fresh separatory funnel. The combined petroleum ether extract is shaken vigorously with a slight excess of 0.1*N* NaOH and the aqueous layer is run off into a 100 cc. beaker. The ether layer is washed once with a few cc. of water and the washings added to the beaker. The beaker containing about 10 to 12 cc. is treated with 10 cc. of Denigès' reagent *(1021)* and allowed to stand one hour. Three cc. of saturated NaCl solution are added after which the precipitated calomel (and possibly other substances) are filtered and washed several times. The precipitate and filter paper are introduced into a titrating bottle containing 20 cc. H_2O, 30 cc. conc. HCl, cooled, and 6 cc. chloroform added. The titration with 0.01*M* KIO_3 is performed as described by Jamieson *(1693)*, until the iodine color disappears from the chloroform. In the case of a very small sample, a little ICl solution may be added, as suggested by Jamieson. The end point is not entirely permanent, so the titration should be carried out promptly with vigorous shaking.

"According to Jamieson 1 mol. of iodate is equivalent to 4 atoms of mercurous mercury, while we have found 3 atoms of mercurous mercury equivalent to 1 mol. of pyrethrin I. Hence the factor for converting cc. 0.01*M* KIO_3 to pyrethrin I is:

$$1 \text{ cc. } 0.01M \text{ } KIO_3 = \frac{0.04}{3} \times 330 = 4.4 \text{ mg. pyrethrin I}$$

"Since a 200 cc. aliquot was taken out of 250 cc., this factor (4.4) must be multiplied by 1.25 to give mg. pyrethrin I in the sample."

Wilcoxon pointed out two sources of error in the Seil method:

1. About 25 per cent of the monocarboxylic acid was lost during steam distillation.
2. The steam distillate is contaminated with acid material soluble in petroleum ether, which is not chrysanthemum monocarboxylic acid.

Wilcoxon's comparison of his method for pyrethrin I with Seil's method showed that Seil's method gives low results for pyrethrin I. For example, a sample of "pyrethrin resins" giving 10.38 per cent pyrethrin I by the Seil method gave 13.16 per cent by the Wilcoxon method.

Seil's method for determining the pyrethrins in perfumed oil-pyrethrum extracts specifies a preliminary distillation with steam to remove the perfumes that might interfere with the assay method. Graham *(1540)* applied this modified Seil method to pyrethrum extracts that did not contain perfume. When he subjected these extracts to the preliminary steam distillation, Graham got much lower pyrethrin I and pyrethrin II contents than when the same extracts were assayed without the preliminary steam distillation. Furthermore, the distillate from the preliminary steam distillation contained appreciable amounts of pyrethrin I and pyrethrin II. Graham concluded: "A study of the Seil method for the determination of pyrethrins in mineral oil extracts of pyrethrum shows that there is a loss of pyrethrins during the steam distillation for the removal of perfume. The loss is due to volatilization with steam and to decomposition or other chemical change. In the case of the materials here reported the loss approximated 25 per cent of the pyrethrins."

Ripert *(2048)* was unable to confirm Graham's conclusions, but Pantsios *(1965)* found that "because of the destructive effect of steam distillation on the chrysanthemum monocarboxylic acid, the acid methods of pyrethrin I analysis are inaccurate and un-

reliable. It is possible to separate the two chrysanthemum acids by the selective extraction of the monocarboxylic acid with low-boiling petroleum ether, but this method requires further study before it can be applied to the estimation of the pyrethrin content of pyrethrum flowers and their commercial extracts."

Graham *(1541)*, as referee on insecticides for the Association of Official Agricultural Chemists, submitted two samples of pyrethrum powder to five collaborators, to be assayed by the Seil method and the Haller-Acree method for pyrethrin II (page 71). The analysts had no difficulty in duplicating their own results by the Seil method, but agreement between different analysts was poor. Only two analysts used the Haller-Acree method, reporting good agreement on one sample and a wide difference on the other.

Acree and LaForge *(993)* failed to find any toxicity to flies in an extract of daisy flowers and concluded that these flowers contain no appreciable quantity of pyrethrins. They then applied the Seil method to petroleum ether extract of daisy flowers and found 0.09 per cent pyrethrin I and 0.09 per cent pyrethrin II, although no pyrethrins were present. Determination of pyrethrin II by the Haller-Acree method showed 0.06 per cent. Acree and LaForge state: "It must be concluded that no appreciable quantity of the pyrethrins is present in the daisy flower and that what is indicated as such by the usual methods actually consists of other substances containing methoxyl and acids with properties similar to those of the pyrethrins."

LaForge and Haller *(1771)* announced the presence of a new ester of pyrethrolone in pyrethrum flowers. The acid portion of the new ester formed an insoluble barium salt and hence would not be determined as a pyrethrin by the Seil acid method. Later, Acree and LaForge *(994)* identified this new ester as a mixture of esters of pyrethrolone with palmitic and linoleic acids.

Fischer *(1444)* described a colorimetric method for determining pyrethrin I based on the reaction between Denigès' reagent and chrysanthemum monocarboxylic acid.

Holaday *(1649)* obtained satisfactory results with Wilcoxon's mercury reduction method when applied to pyrethrum flowers, but the products of saponification in perfumed oil-pyrethrum insecticides interfered with the iodate titration by absorbing iodine, thus causing high results. Holaday removed these interfering substances by washing the precipitate of mercurous chloride with acetone and chloroform, thereby making Wilcoxon's method applicable to oil-pyrethrum extracts containing other

insecticidal materials and perfumes. Holaday assumed that one cubic centimeter of 0.01*M* iodate solution is equivalent to 4.4 mg. pyrethrin I. He confirmed Wilcoxon's conclusions on the sources of error in the Seil method. Holaday's modification of Wilcoxon's method was adopted by the Association of Official Agricultural Chemists as a tentative method in 1937 *(1047)*.

Graham *(1543)* reported that the modified Wilcoxon mercury reduction method for pyrethrin I is satisfactory when applied to pyrethrum powder, to pyrethrum powder mixtures and to mineral oil pyrethrum extracts. Pine oil, oleic acid and derris resins do not interfere. The method seems to be specific for pyrethrin I and is not affected by the usual ingredients of pyrethrum spray materials.

TABLE CVII. COMPARISON OF ANALYSES OF PYRETHRUM FLOWERS BY DIFFERENT METHODS (MARTIN).

Sample	Tattersfield Method	Seil Method	Ripert Method	Haller-Acree Method
		Pyrethrin I, %		
W1	0.47	0.47	0.52	...
W2	0.44	0.42	0.49	...
W3	0.34	0.36	0.37	...
W4	0.41	0.40	0.41	...
K1	0.58	0.59	0.64	...
K2	0.64	0.65	0.68	...
K3	0.67	0.67	0.70	...
K4	0.66	0.63	0.66	...
K5	0.56	0.65	0.54	...
		Pyrethrin II, %		
W1	0.49	0.54	0.70	0.56
W2	0.49	0.57	0.69	0.54
W3	0.37	0.39	0.47	0.40
W4	0.37	0.41	0.44	0.45
K1	0.58	0.57	0.64	0.60
K2	0.46	0.50	0.65	0.64
K3	0.52	0.52	0.68	0.59
K4	0.52	0.50	0.57	0.50
K5	0.52	0.53	0.52	0.46
		Total Pyrethrins, %		
W1	0.96	1.01	1.22	...
W2	0.93	0.99	1.18	...
W3	0.71	0.75	0.84	...
W4	0.78	0.81	0.85	...
K1	1.16	1.16	1.28	...
K2	1.10	1.15	1.33	...
K3	1.19	1.19	1.38	...
K4	1.18	1.13	1.23	...
K5	1.08	1.18	1.06	...

Martin *(1848)* made a careful comparison of the results obtained by applying the Tattersfield, Seil, Ripert (page 69) and Haller-Acree methods to pyrethrum flowers. His analyses are given in Table CVII.

The Seil and Tattersfield methods gave values that agreed closely, but Ripert's method yielded high results. Results by the Haller-Acree method were sometimes higher and sometimes lower than those obtained by the Seil method.

Martin agreed with Wilcoxon that pyrethrin I is partly lost in the determination by the Seil and Tattersfield methods, but this was overcome by reducing the excess of sulfuric acid present during the steam distillation to 1 cc. of 1*N* acid.

Martin also compared the Seil and Wilcoxon methods and found that the latter gave higher values for pyrethrin I, especially in flowers rich in pyrethrins. Martin found that the relationship between the amount of chrysanthemum monocarboxylic acid present and the volume of iodate solution required, in the Wilcoxon method, is not a linear one. This was proved by the fact that different aliquots of the same solution analyzed by Wilcoxon's method gave different percentages of pyrethrin I. If this were true the constant ratio of 4.4 mg. of pyrethrin I to 1 cc. 0.01*M* iodate solution, suggested by Wilcoxon, would be incorrect.

Holaday and Graham *(1650)* applied Holaday's modification of Wilcoxon's method of aliquots of an alkaline solution of chrysanthemum monocarboxylic acid. The aliquots varied from 5 cc. to 200 cc., equivalent to 2.9 mg. to 114 mg. of pyrethrin I. The reduction from mercuric to mercurous sulfate was found to be linear. They concluded: "The linearity of results obtained [by Holaday's modification] shows that the erroneous values obtained using the original Wilcoxon procedure are not due to compounds other than chrysanthemum monocarboxylic acid reacting to give mercurous sulfate. The interferences are no doubt due to unsaturated organic compounds which absorb iodine during the titration with potassium iodate. These compounds which are precipitated along with the calomel are removed from the precipitate by washing with alcohol or acetone, followed by chloroform.

"The linearity of the results obtained indicates that the reduction of the Denigès' reagent is a clear-cut reaction which is not affected by the quantity of chrysanthemum monocarboxylic acid within the limits of the experiment."

De Ong *(1362)* compiled a report on a study of methods of analysis of fly sprays, conducted by the Pacific Coast Insecticide Association. His conclusions were: "The results of this cooperative work demonstrate that the Modified Seil procedure is capable of yielding reasonably uniform figures for pyrethrins I and II on a variety of household insecticides, the determinations being made independently by different laboratories. The original Seil method gives fairly consistent results; the Ripert method *(745)* proved to be unreliable."

The Modified Seil procedure referred to included minor changes introduced by the California State Division of Chemistry.

Graham *(1545)* reported on collaborative work on pyrethrum analysis conducted by the Association of Official Agricultural Chemists. Both the Seil method and the Holaday modification of Wilcoxon's method gave values for pyrethrin I in powdered pyrethrum that agreed fairly well. On mineral oil extracts the Wilcoxon-Holaday method yielded more consistent values for pyrethrin I than the Seil method. Results for pyrethrin II showed wide variation.

On the basis of further collaborative work the Wilcoxon-Holaday mercury reduction method was adopted in 1939 by the Association of Official Agricultural Chemists as an "Official Method" for the determination of pyrethrin I in pyrethrum flowers and as a "Tentative Method" for pyrethrin I in mineral oil extracts. A slight modification of Seil's method was adopted as a "Tentative Method" for pyrethrin II in pyrethrum flowers but no method was adopted for pyrethrin II in mineral oil extracts *(1547)*.

Graham *(1546)* considers the Wilcoxon-Holaday method the most accurate yet proposed for pyrethrin I, but found none of the methods for pyrethrin II entirely reliable. The Seil method gave high results in the presence of isobutylundecylenamide.

Yip *(2354)* has suggested an improved apparatus for the extraction of pyrethrum flowers with petroleum ether.

The nature of the color change that occurs in the Wilcoxon method has been investigated by Sherman and Herzog *(2122)*. The solution becomes successively, faintly cloudy, pink, red, light red-violet, blue violet, dull blue, blue violet at the top, greenish blue at the bottom. All of these changes take place within 50 minutes. Sherman and Herzog confirmed the conclusions of Martin *(1848)* and of Holaday and Graham *(1650)* on the linearity of results by the mercury reduction method.

Hartz, Hendrickson and Hoyer *(1605)* assayed thirty samples of pyrethrum flowers and thirty samples of concentrated mineral oil-pyrethrum extracts by the Seil method and the A. O. A. C. official method. In the latter method, pyrethrin I is determined by the Wilcoxon-Holaday mercury reduction method and pyrethrin II by a slight modification of the Seil method. Assays of the ground flowers by the official method showed only 75.8 per cent as much pyrethrin I and only 87.7 per cent as much total pyrethrins as the Seil method. Analyses of the mineral oil-pyrethrum extracts by the official method showed only 73.5 per cent of the pyrethrin I and 86.9 per cent of the total pyrethrins found by the Seil method.

The presence of certain aliphatic thiocyanates (Lethane) in oil-pyrethrum extracts cause low results for pyrethrin I determined by the Wilcoxon-Holaday mercury reduction method. Graham *(1549)* was able to overcome this difficulty by increasing the amount of alcoholic sodium hydroxide solution used for saponification and by washing the precipitate of mercurous chloride with hot water.

Ripert *(2048)* prefers to use ethyl ether for the extraction of the pyrethrins in assaying pyrethrum flowers. He objects to the use of petroleum ether because of the possibility of incomplete extraction, due to a film of insoluble oxyacids which surrounds the pyrethrins. Gnadinger (pages 69 and 85) has emphasized the danger of using ethyl ether because oxidized or altered pyrethrins are more soluble in it than in petroleum ether.

Martin *(1848)* compared analyses, by the Seil and Tattersfield methods, using petroleum ether and ether for extracting the flowers. A sample of flowers that had been stored for six months was assayed with these results.

	Pyrethrin I		Pyrethrin II	
	Petroleum Ether Extraction %	Ether Extraction %	Petroleum Ether Extraction %	Ether Extraction %
Tattersfield Method.....	0.66	0.82	0.56	0.83
Seil Method.....	0.66	0.72	0.64	0.86

Martin also extracted ground flowers in a Soxhlet extractor with petroleum ether for 20 hours. The extracted flowers were then air dried at low temperature and extracted for 20 hours with ethyl ether. The ethyl ether extracts were assayed by the Seil method and the quantities of pyrethrins found were:

Sample	Pyrethrin I	Pyrethrin II
W10	0.02	0.07
W11	0.03	0.07
W12	0.03	0.08
H	0.03	0.10

Since the flowers contained about 1 per cent total pyrethrins, the amounts recovered by extracting with ether after petroleum ether extraction were about 10 per cent of the total pyrethrin content.

Martin *(1849)* later reported the results of biological tests carried out on ether extracts of the flowers made after preliminary extraction with petroleum ether. Flowers containing about 1 per cent total pyrethrins were extracted for 5½ hours with petroleum ether. The extracted flowers were dried at 30°-35° for 1 hour and then extracted with ethyl ether for 3 hours. The ethyl ether extract was dissolved in alcohol and tested against the flour beetle, *Tribolium castaneum*, and bean aphid, *Aphis rumicis*. There was no toxicity to *Tribolium castaneum* and the toxicity to *Aphis rumicis* was much less than the apparent pyrethrin content indicated. Martin concluded: 95 per cent of the pyrethrins was extracted from flowers one year old after only 3 hours extraction with petroleum ether. "In view of its selective action, petroleum ether should therefore be retained for the extraction of the flowers for analysis, a minimum period of 8 hours being employed."

Graham *(1550)* was unable to get complete extraction of the pyrethrins in a mixture of 30 per cent pyrethrum powder and 70 per cent clay, using petroleum ether as the solvent. This was true whether the extraction was made in a Soxhlet extractor or in a shaking machine. Other solvents tried were acetone, carbon tetrachloride and chloroform. None of these gave complete extraction in a Soxhlet extractor. Complete extraction was obtained with acetone and chloroform in a shaking machine or by boiling under a reflux condenser for one hour; carbon tetrachloride gave incomplete extraction by these procedures. The acetone extraction gave satisfactory results for pyrethrin I, but the value for pyrethrin II was extremely high. Chloroform also gave satisfactory results for pyrethrin I, but the pyrethrin II values, while better than with acetone, were still too high.

Analyses of mixtures of pyrethrum powder with cubé, derris, tobacco, lime, sodium fluoride, borax, sugar, flour, soap bark and sulfur by the Wilcoxon-Holaday method gave satisfactory results for pyrethrin I, when chloroform was used for the extraction,

using a shaking machine or boiling under a reflux condenser. No satisfactory method for pyrethrin II was found.

Graham did not investigate the effect of chloroform extraction on the pyrethrin I content of old flowers containing oxidized or altered pyrethrins. It is possible that oxidized pyrethrin I does not reduce the mercuric sulfate solution used in the Wilcoxon-Holaday method.

Graham *(1548)*, reporting on additional collaborative work of the A. O. A. C., noted good agreement between the results for pyrethrin I by the Seil method and the Wilcoxon-Holaday method. Collaborators' results for pyrethrin II by the Seil method were in good agreement, but those by the modified Seil method, following determination of pyrethrin I by the Wilcoxon-Holaday method, were not so good.

Green and his associates *(1560)* suggested the use of 0.5*N* solution of potassium hydroxide in ethylene glycol monoethyl ether for the saponification in the Seil method and in the Wilcoxon-Holaday method. Other minor modifications were also suggested. The agreement between the Seil method and Green's Modified Seil method was good and so was that between the Wilcoxon-Holaday official method and Green's modification of it. However, the Wilcoxon-Holaday methods gave lower results for pyrethrin I and total pyrethrins than the Seil methods. Green used a molecular weight of 328 for pyrethrin I and 372 for pyrethrin II instead of the old values of Staudinger and Ruzicka, 330 and 374.

In his original paper *(2324)* Wilcoxon stated that 1 cc. of 0.01*M* potassium iodate solution is equivalent to 4.4 mg. of pyrethrin I. This relationship was determined on purified chrysanthemum monocarboxylic acid, 99.8 per cent pure. It had been approved by the Association of Official Agricultural Chemists, apparently without verification, and all of the comparisons of the Wilcoxon method and the Wilcoxon-Holaday method with other methods, made by various investigators from July, 1936, to November, 1943, had been based on this relationship.

Graham and LaForge *(1552)* redetermined the relation between the reduced mercury and pyrethrin I in the Wilcoxon method. They prepared four samples of chrysanthemum monocarboxylic acid, three from pyrethrin I semicarbazone and one according to Wilcoxon's procedure. Nineteen determinations of the ratio were made on the four samples of acid. It was found that 1 mol. of the chrysanthemum acid reduces 2.38 atoms of mercury instead of 3 reported by Wilcoxon. By dividing the

quantity of pure acid taken for reduction, expressed as pyrethrin I, by the number of cc. of 0.01*M* potassium iodate used in the titration, Graham and LaForge determined the factor to be: 1 cc. 0.01*M* potassium iodate is equivalent to 5.70 mg. of pyrethrin I. This would mean that all calculations of results for pyrethrin I based on Wilcoxon's factor (1 cc. = 4.4 mg.) were 22.8 per cent too low.

In England, cooperative research between the Imperial Institute, the Rothamsted Experimental Station, S. H. Harper and T. F. West indicates a factor in close agreement with that determined by Graham and LaForge. There was strong evidence that the factor depends on temperature and the time allowed for the reaction.

The methods of the Association of Official Agricultural Chemists for determining pyrethrin I were slightly revised in 1944 *(1130)*. The revised methods provide for a reduction temperature of 25°, ± 2° for a period of 1 hour, and the factor for 1 cc. of 0.01*M* KIO_3 was changed to 5.7 mg. of pyrethrin I, as determined by Graham and LaForge *(1552)*.

If the analyses reported by Hartz and his co-workers *(1605)* are re-calculated on the basis of the Graham-LaForge factor, the agreement between the Seil method and the Wilcoxon-Holaday official method becomes much closer. In the re-calculated analyses of the 30 samples of flowers, the official method yields 98.2 per cent as much pyrethrin I and 99.2 per cent as much pyrethrin II as the Seil method. On the 30 samples of oil-pyrethrum concentrate the official method shows 95.3 per cent of the pyrethrin I content and 98.6 per cent of the pyrethrin II content found by the Seil method.

The following analyses of commercial lots of pyrethrum flowers were made by the Gnadinger-Corl method, Seil method and Wilcoxon-Holaday official method under the author's supervision. The factor 5.70 was used in the official method for pyrethrin I instead of 4.4.

Source	Crop Year	Total Pyrethrin Content		
		Gnadinger-Corl	Seil	Wilcoxon-Holaday
		%	%	%
Kenya	1941	1.26	...	1.44
Kenya	1941	1.30	1.44	1.41
Kenya	1942	1.23	1.39	1.45
Kenya	1942	1.26	1.38	1.45
Kenya	1942	1.20	1.23	1.30
Brazil	1943	0.73	0.79	0.74
Brazil	1943	0.87	0.97	0.91
Brazil	1943	0.81	0.91	0.81
Tanganyika	1943	1.20	1.27	1.17

A. O. A. C. Methods

The methods of the Association of Official Agricultural Chemists for determining the pyrethrins are given in "Official and Tentative Methods of Analysis," 5th edition 1940 *(1069)*, and are reprinted herewith, as revised in 1944 *(1130)*, by permission of the Association.

Pyrethrum Powder

Pyrethrin I. Mercury Reduction Method—Official, First Action

Reagents

(a) Denigès' reagent.—Mix 5 g of yellow HgO with 40 ml of H_2O and, while stirring, slowly add 20 ml of H_2SO_4; then add another 40 ml portion of H_2O and stir until completely dissolved. Test for absence of mercurous Hg by adding a few drops of (b) to 10 ml and titrating with (c) as directed under "Determination," beginning "Add 30 ml of HCl."

(b) Iodine monochloride soln.—Dissolve 10 g of KI and 6.44 g of KIO_3 in 75 ml of H_2O; add 75 ml of HCl and 5 ml of $CHCl_3$ in glass-stoppered bottle and adjust to faint I color (in $CHCl_3$) by adding dilute KI or KIO_3 soln. If there is much I set free, use a stronger soln of KIO_3 than 0.01*M* at first, making final adjustment with 0.01*M* soln. Keep in dark cupboard and readjust when necessary.

(c) Standard potassium iodate soln.—0.01*M*. Dissolve 2.14 g of pure KIO_3, previously dried at 105°, in H_2O and dilute to 1 liter, 1 ml of this soln = 0.0057 g of Pyrethrin I, and needs no further standardization.

Determination

Extract quantity of sample that will contain 20-75 mg of Pyrethrin I (12.5-20 g) in Soxhlet or other efficient extraction apparatus 7 hours with petroleum benzin, and evaporate petroleum benzin on water bath, heating no longer than necessary to remove solvent. Do not pass current of air through flask during evaporation.

Add 15-20 ml of 0.5*N* alcoholic NaOH soln to flask containing pyrethrum extract, connect to reflux condenser and boil gently 1-1.5 hours. Transfer to 600 ml beaker and add sufficient H_2O to bring volume to 200 ml. Add a few glass beads or preferably use boiling tube, and boil down to 150 ml. Transfer to 250 ml volumetric flask, add 1 g of filter-cel and 10 ml of 10% $BaCl_2$ soln. Do not shake before making to volume.

Make to volume, mix thoroughly, filter off 200 ml, neutralize with H_2SO_4 (1 + 4), and add 1 ml in excess, using 1 drop of phenolphthalein as indicator. (If necessary to have soln stand overnight at this point, it should be left in alkaline condition.) Filter through 7 cm filter paper that has been coated lightly with suspension of filter-cel in H_2O, on Büchner funnel and wash several times with H_2O. Transfer into 500 ml separatory funnel and extract with two 50 ml portions of petroleum benzin. Wash extracts with 2 or 3 10 ml portions of H_2O, and filter petroleum benzin extract through plug of cotton into clean 250 ml separatory funnel. Wash cotton with 5 ml of petroleum benzin. Extract petroleum benzin with 5 ml of 0.1*N* NaOH, shaking vigorously. Draw off aqueous layer into 100 ml beaker, wash petroleum benzin with 5 ml of H_2O or with additional 5 ml of 0.1*N* NaOH, and add this to the beaker. Add 10 ml of Denigès' reagent to beaker and let stand 1 hour at 25° ± 2°. Add 20 ml of alcohol to beaker and precipitate HgCl with 3 ml of saturated NaCl soln. Warm to ca 60°, and filter through small filter paper, transferring all precipitate to filter paper, and wash with 10 ml or more of hot alcohol. Wash with two or more 10 ml portions of hot $CHCl_3$, and place filter paper and contents in 250 ml glass-stoppered Erlenmeyer flask. Add 30 ml of HCl and 20 ml of H_2O to flask and cool; add 6 ml of $CHCl_3$ or CCl_4 and 1 ml of ICl soln and titrate with the iodate soln, shaking vigorously after each addition, until there is no iodine color in $CHCl_3$ layer. From number of ml of the standard iodate soln used in titration calculate percentage of Pyrethrin I in sample.

KIO_3 reacts with mercurous Hg to form mercuric Hg and I. Further addition of iodate in presence of HCl oxidizes I to ICl.

$$2Hg_2Cl_2+KIO_3+6HCl = 4HgCl_2+ICl+KCl+3H_2O$$

Addition of ICl does not change volume relationship between mercurous Hg and iodate soln and aids in determining end point in titration of small quantities of Hg. The end point is taken when red color disappears from $CHCl_3$ layer. The end point is not permanent, therefore titration should be completed rapidly with vigorous shaking after each addition of iodate.

Pyrethrin II

Filter, if necessary, aqueous residue from petroleum benzin extraction through Gooch crucible. Concentrate filtrate to ca 50 ml, transfer to separatory funnel, and neutralize with

$NaHCO_3$. Extract twice with $CHCl_3$ and wash $CHCl_3$ extract through ca 15 ml of H_2O in each of two separatory funnels. Combine aqueous soln and washings, acidify strongly with HCl (ca 8 ml), saturate with NaCl, adding cautiously at first to prevent excessive ebullition of CO_2, and extract with 50 ml of ethyl ether. Draw off aqueous layer into a second separatory funnel and extract again with 50 ml of ether. Continue this extraction and drawing off of aqueous layer, using 35 ml for third and fourth extractions. Wash the four ether extracts successively with 10 ml of H_2O, and repeat with second successive washing with another 10 ml of H_2O. Combine ether solns, draw off any H_2O that separates, and filter through plug of cotton into 500 ml Erlenmeyer flask. Evaporate ether on water bath and dry residue at 100° for 10 minutes. Add 2 ml of neutral alcohol and 20 ml of H_2O and heat to dissolve acid. Cool, filter through Gooch crucible, add drop or two of phenolphthalein indicator soln, and titrate with 0.02*N* NaOH soln, of which 1 ml = 0.00374 g of Pyrethrin II.

Pyrethrum Extracts in Mineral Oil

Pyrethrin I. Mercury Reduction Method—Tentative

Determination

Weigh or measure a quantity of sample that will contain 20-75 mg of Pyrethrin I, and transfer into 300 ml Erlenmeyer flask.

Add 20 ml or more if necessary of normal alcoholic NaOH soln to flask containing pyrethrum extract, connect to reflux condenser, and boil gently 1-1.5 hours. Transfer to 600 ml beaker and add sufficient H_2O to make aqueous layer to 200 ml. If more than 20 ml of alcoholic soda has been used, add sufficient H_2O so that all alcohol will be removed when volume has been reduced to 150 ml. Add a few glass beads, or preferably use boiling tube, and boil aqueous layer down to 150 ml. Transfer contents of beaker to 500 ml separatory funnel and draw off aqueous layer into 250 ml volumetric flask. Wash oil layer once with H_2O and add wash H_2O to aqueous portion. After drawing off aqueous layer and washings, if slight emulsion still persists, it may be broken by addition of 2-3 ml of 10% $BaCl_2$ soln. Do not shake vigorously after adding the $BaCl_2$, otherwise reversed emulsion that is difficult to separate may be formed. To aqueous soln in the 250 ml flask, add 1 g of filter-cel and 10 ml or more of the $BaCl_2$ soln. Do

not shake before making to volume. Make to volume, mix thoroughly and filter off 200 ml. Test filtrate with $BaCl_2$ to see if sufficient has been added to obtain clear soln. Neutralize with H_2SO_4 (1 + 4) and add 1 ml in excess, using 1 drop of phenolphthalein as indicator. From this point, proceed as directed above, beginning "Filter through 7 cm filter paper."

NOTE: Chrysanthemum monocarboxylic acid reacts with Denigès' reagent to form a series of colors beginning with phenolphthalein red, which gradually changes to purple, then blue, and finally to bluish green. The color reaction is very distinct with 5 mg of monocarboxylic acid and quantities as low as 1 mg can usually be detected. Therefore no Pyrethrin I should be reported if color reaction is negative.

When analyzing samples containing much perfume or other saponifiable ingredients such as thiocyanates, it may be necessary to use as much as 50 ml of normal alcoholic NaOH.

Other Methods

Haller and LaForge *(1573)* established that the pyrethrins contain two hydrogen atoms less than found by Staudinger and Ruzicka. The correct molecular weights are, therefore, 328.4 for pyrethrin I and 372.4 for pyrethrin II. These values should be used in the Seil and other acid methods. This would make 1 cc. 0.02*N* alkali equivalent to 0.00657 g. pyrethrin I, or 0.00372 g. pyrethrin II.

Gillam and West *(1493)* in their study of the molecular structure of the pyrethrins suggested the use of absorption spectra for determining the pyrethrins quantitatively. Later *(1494)* they studied such a method but concluded that it had little advantage to offer.

Haller and LaForge *(1575)* observed that on hydrogenation the pyrethrins undergo cleavage; pyrethrin I yields hexahydropyrethrone and the dihydro derivative of chrysanthemum monocarboxylic acid; pyrethrin II yields hexahydropyrethrone and chrysanthemum dicarboxylic acid monomethyl ester. This reaction has been used by LaForge and Acree *(1763)* as the basis of a quantitative method for determining the pyrethrins.

The hydrogenation is carried out in a glass tube about 18 cm. long by 4 cm. in diameter and 2 to 3 mm. thick, drawn down at the top to a 2 cm. neck. The tube is closed with a rubber stopper, through which the tube from the hydrogen tank passes. It is provided with a side arm which terminates in a small upright

funnel and which may be closed with a stopcock. The tube is agitated with the standard Burgess-Parr apparatus or any other suitable device. About 30 minutes at a pressure of 20 to 25 pounds per square inch is sufficient for the reaction.

Oleoresins and extracts of flowers and some concentrates contain free higher fatty acids and, to a small extent, fatty acid esters of pyrethrolone, which would probably yield such acids on hydrogenation. These acids must therefore be removed, and this is accomplished by precipitation with dilute aqueous barium hydroxide.

The precipitated material, together with inert water-insoluble constituents, is filtered off with suction, the alkaline solution of the chrysanthemum acid barium salts being received directly in the distillation apparatus. This is an ordinary 250 cc. distilling flask with a neck 18 cm. long having a side arm about 1.5 cm. in diameter located about midway. The side arm is bent upwards and terminates in a bulb about 3 cm. in diameter, from the top of which a tube leads to the condenser. The steam enters through a tube that passes through a rubber stopper to the bottom of the flask. The following is the analytical procedure.

> "About 1-1.2 g. of palladium-calcium carbonate catalyst is reduced in about 5 cc. of absolute ethanol for 3 or 4 minutes at 20-25 pounds pressure in the hydrogenating tube. The pressure is then released, and the sample of concentrate, oleoresin, or extract of flowers, containing 0.1-0.3 g. of total pyrethrins dissolved in 5 cc. of acetone, is introduced through the funnel and washed in with an additional 5 cc. of the same solvent followed by 5 cc. of the same solvent followed by 5 cc. of ethanol. If a number of determinations are to be made on an oleoresin, for instance, a larger sample is weighed and an absolute ethanol solution is made up to standard volume, from which convenient aliquots are taken. The volume of organic solvents present during the hydrogenation should be about 20 cc. After introduction of the sample, the tube is evacuated and filled with hydrogen three times and then shaken under 20-25 pounds pressure for at least 30 minutes. The pressure is then released, 20 cc. of about 0.05*N* aqueous barium hydroxide is added through the funnel, and the tube is shaken for 5 minutes to ensure thorough mixing and neutralization of the acids present.
>
> "About 0.3 g. of Filter-cel and 15 cc. of water are added, and the solution is filtered with suction directly into the distillation flask, by means of a small glass funnel with flat perfo-

rated bottom covered with a disk of filter paper. The insoluble material is washed with water to bring the volume in the flask to 75 cc. Owing to the presence of hexahydropyrethrone the filtered solution may be slightly cloudy and it is sometimes colored light yellow. About 1 cc. of 6 per cent hydrochloric acid is added, and steam from a suitable generator is passed through the solution, the volume of which is maintained constant by a small flame, and 75 cc. of distillate is collected.

"The distillate is titrated with 0.05*N* sodium hydroxide solution after addition of phenolphthalein, and the pyrethrin I is calculated from the alkali required for neutralization.

$$\frac{\text{cc. } 0.05\ N \text{ alkali} \times 1.64}{\text{Wt. of sample}} = \%\ \text{pyrethrin I}$$

"The contents of the distillation flask, after being cooled, are poured into a separatory funnel, and the flask is washed out with 30 cc. of ether, which is added to the aqueous solution. The flask is then washed with another portion of 30 cc. of ether, which is poured into another separatory funnel. After being shaken the aqueous layer is transferred from the first funnel into the second, and after extraction with the second ether washing it is discarded. The second ethereal solution is added to the first and the combined solution is washed twice with 7 cc. of water. The combined washings are in turn extracted with 15 cc. of ether, which is added to the main solution, and about 10 cc. of water and a few drops of phenolphthalein indicator are added.

"A measured quantity of 0.05*N* sodium hydroxide solution in slight excess is introduced, and the acids are extracted from the ether by shaking. The alkaline layer is then removed and the ether solution washed with two 5 cc. portions of water, which are combined with the first extract. The aqueous solution is then back-titrated with 0.05*N* acid. The content of pyrethrin II is calculated on the basis of a monobasic acid, chrysanthemum dicarboxylic acid monomethyl ester.

$$\frac{\text{cc. } 0.05\ N \text{ alkali} \times 1.86}{\text{Wt. of sample}} = \%\ \text{pyrethrin II}$$

"It is most convenient to overneutralize with the standard acid and then to titrate back to a faint pink with the standard alkali. When oleoresins or flower extracts are analyzed, the color change is disturbed by the yellow tinge of the alkaline solution but the end point is easily observed with a little practice.

"All the operations described can be carried out in a little over 1 hour."

LaForge and Acree present a full discussion of the sources of error in the method. LaForge and Bendigo *(1767)* suggested the following modification of the LaForge-Acree method:

"A somewhat lower and more correct value for pyrethrin I is found if the steam distillate obtained by following the original procedure is made slightly alkaline or it may be neutralized by titrating with 0.05*N* alkali solution as originally proposed and is then boiled down to about half its volume to expel the acetone or alcohol used as solvent, the solution acidified with about 0.5 cc. of 6 per cent hydrochloric acid, and the dihydrochrysanthemum acid extracted with ether, or, preferably, petroleum ether. The titration for the determination of pyrethrin I is then done exactly as originally described for pyrethrin II."

CHAPTER XX

BIOLOGICAL METHODS FOR EVALUATING PYRETHRUM

Importers of pyrethrum flowers buy on the basis of the total pyrethrin content determined by chemical analysis. Manufacturers of concentrated extracts ordinarily sell on the basis of total pyrethrin content chemically determined. This is because the chemical methods, while not entirely satisfactory, are more accurate than biological methods. Household insecticides containing only pyrethrins as active principles can be rather accurately assayed chemically, but mixtures of pyrethrum with other active principles, or with synergists, can usually be evaluated only by means of biological tests.

Peet-Grady Method

The Peet-Grady method was adopted as an official method by the National Association of Insecticide and Disinfectant Manufacturers (NAIDM) in 1932. Since then it has been the subject of study by many investigators. One of the most important steps in the development of the present Peet-Grady procedure was the adoption of the official control insecticide. As early as 1932 the development of a suitable standard for measuring the resistance of flies of various origins had been suggested (page 100). In 1935 Campbell *(1263)* undertook co-operative tests to find an insecticide that could be used as a standard of comparison in the Peet-Grady method. Tests were made by ten laboratories on three oil sprays containing respectively 0.05 per cent pyrethrins, 0.10 per cent pyrethrins and 5.0 per cent benzophenone. As a result of these tests the insecticide containing 0.10 per cent pyrethrins was adopted by the NAIDM as the Official Control Insecticide in 1936 *(2293)*. The Official Control Insecticide (O.C.I.) was made by diluting pyrethrum extract (2 grams pyrethrins per 100 cc.) with insecticide base oil in the proportion of 1 to 19. Both pyrethrum extract and base oil were mixtures prepared from various commercial products. The following grades were also adopted by NAIDM in 1936 *(2294)*, based on the difference in per cent kill between the unknown and the O.C.I.:

Grade AA, excellent, 21% or more above the O.C.I.

Grade A, good, 11% to 20% above the O.C.I.

Grade B, equal O.C.I., 10% above to 10% below the O.C.I.

Grade C, poor, 11% to 20% below the O.C.I.

Grade D, little value, 21% or more below the O.C.I.

The detailed instructions furnished by NAIDM with each bottle of O.C.I. were:

"1. The tests shall be conducted in accordance with the current Peet-Grady test procedure. After each test thoroughly wipe out the chamber using a cloth saturated with carbon tetrachloride. When paper is used on the floor, it should be changed after each group of two or three tests.

2. The tests must show an average kill for the O.C.I. to lie within the range between 30 and 70% kill. The dosage of the O.C.I. and Unknowns compared with it may be altered, if necessary, to bring the average kill of the O.C.I. within the required range. It is desirable to cause the O.C.I. to kill between 50 and 60% of the flies.

3. No more than two Unknowns may be tested in conjunction with the O.C.I. in any one series. Ten tests of the O.C.I. and of each of the Unknowns shall be made in parallel; i.e., test each sample of the series the same number of times with flies of the same batch, and test every sample of the series the same number of times during any one day. The three samples in a series should be randomized in order of testing. For example, 1, 2, 3; 2, 3, 1; 3, 1, 2; etc., until thirty tests have been made. When only one Unknown and the O.C.I. comprise the series, the order should likewise be randomized. For example, 1, 2; 2, 1; 2, 1; 1, 2; etc., until twenty tests have been completed.

4. The standard error of the mean difference between the average O.C.I. kill and the average Unknown kill must be less than 3. If it is 3 or greater, the differences between pairs were too variable and to make the results valid additional paired tests must be run to bring the standard error of the mean difference down to 3 or lower.

5. Calculate the mean difference between kill obtained with the Unknown and that obtained with the O.C.I. Denote the difference between kills by the appropriate grade according to letter.

Suggested Additional Method of Reporting Kills. If it is necessary to supply a numerical value for the Unknown in addition to the grade designation, add a positive difference between the Unknown and O.C.I. to 60 or subtract a negative difference from 60, and write $\pm$ 3 after the result. Thus, if an Unknown gives a kill 7 points higher

than the O.C.I., it is given a B rating and a numerical evaluation of 67 ± 3. The latter figure indicates that other laboratories reporting on the same sample would probably report a kill between 64 and 70."

Ford *(1451)* pointed out the inaccuracy of the two methods of reporting results. In reporting by grades, designated by letters, the differences in kills between the Unknown and the O.C.I. are absolute differences and are the same whether the O.C.I. kill is low or high. In the "suggested additional method," kills greater than 100 per cent may be reported when the O.C.I. is low and the Unknown is high. Ford suggested a different method of expressing kills on a percentage basis.

Simanton *(2130)* reported results obtained by the official method on an unknown sample tested by eight laboratories. The grade ratings ranged from B to AA, on the same sample.

Murray *(1925)* in tests of household sprays with the Peet-Grady method concluded that fly mortality varied too much to give an accurate evaluation. He also found increased susceptibility in unfed flies and much greater susceptibility in male flies than in female flies.

Simanton and Miller *(2134)* confirmed Murray's conclusion that male flies are much less resistant to oil-pyrethrum sprays than female flies. They also found very young flies more difficult to kill than older flies, although the former were more easily paralyzed.

Murray *(1926)* also considered the statistical procedure recommended by NAIDM very inaccurate and suggested a method described by Bliss, who later discussed the theory of evaluating liquid household insecticides by the Peet-Grady method *(1216)*.

Miller and Simanton *(1895)* obtained varying slopes to toxicity curves on different cultures of flies, indicating differences in resistance. When flies emerge from cultures, males predominate in the first half and females in the last half. When successive samples of flies are drawn from stock cages, the proportion of males is high in the first samples and low in the last samples. In order to avoid these sources of error and the necessity of sexing the flies as suggested by Murray *(1925)*, Simanton and Miller *(2135)* devised the "Large Group" modification of the Peet-Grady method. This method was also adopted as an official method by NAIDM in December, 1938. The following is the procedure which Simanton and Miller recommended:

"1. Three battery-jar cultures are prepared at the same time, each receiving sufficient eggs to produce between 1300-

1700 flies per jar. With jar populations not exceeding 1700, all larvae tend to pupate on the same day.

2. As soon as all larvae have transformed into dark-colored pupae, the top layer of rearing medium is removed, the exposed pupae then being brushed into a cafeteria tray. The material on the tray usually comprises about half pupae and half medium.
3. The tray is placed in a fan blast until the pupae-medium mixture is moderately dry. Then the tray is tipped toward the fan blast and the mixture stirred upward with a card. The pupae roll down the tray, while the medium is held at the top of the incline. Five thousand pupae may be separated by this method in about twenty minutes.
4. The separated pupae from the three jars are gently but thoroughly mixed, then carefully weighed into groups of 500 each. Each group is placed in a shallow dish, and the dish is then placed in a screen cage fitted with a sleeve opening. The cage has a capacity of about one cubic foot, has a floor area at least one square foot, and is equipped with a removable wooden floor.
5. The flies are held for five days after peak emergence, during which time they are supplied with fresh milk daily. On the fifth day, the tests are run in the Peet-Grady chamber according to the usual procedure with the exception that 500 flies are sprayed at one time. All flies in one cage are sprayed with the Official Test Insecticide. During the ten-minute exposure period, the cage is cleaned and made ready to receive the paralyzed flies. In a similar manner, one cage is used for each unknown sample in the series to be evaluated in comparison with the O.T.I.
6. The number of "up" flies is recorded at the time of testing. Paralyzed flies are returned to their original cage, supplied with cotton saturated with sugar solution, and then held in the rearing room. Care should be taken to distribute the paralyzed flies evenly over the cage floor and to space the cages to assure uniform aeration during the recovery period. After 24 hours, the dead flies are removed through the sleeve opening and counted; then the recovered flies are killed and counted. The percentage dead flies of total flies (including "up" flies) is calculated.
7. The series of samples, randomized as to order of testing, is again tested once or preferably twice, cages from a

different culture being used for each series. The evaluation reported for the sample is the mean difference between the O.T.I. and sample kills obtained from comparisons on two or more cultures."

"The above procedure employs the regular Peet-Grady equipment with the exception of the stock and observation cages which are replaced by the one type of cage previously described. Thirty to thirty-six cages are sufficient to keep one chamber in continual operation. The modified method permits one operator to evaluate twelve to eighteen samples a week, with equal or better accuracy than is obtained by the present method which does not allow more than five samples to be evaluated in the same period. Perhaps the greatest advantage of the method, in addition to providing improved testing capacity, lies in the fact that the tedious sexing of flies is avoided because each test unit contains approximately 50 per cent males at the time of spraying. Groups of 500 flies are handled almost as easily as groups of 100 flies. Test units of 500 or more flies, when derived from thoroughly mixed pupae, are very uniform with regard to sex ratio. In addition, the method of obtaining the large test units results in the use of a true sample of the reared population. In the large group modification, the number of replications has been greatly reduced, because several important sources of variation, formerly taken into account only by replicating tests, have been controlled by other means."

In December, 1937, the NAIDM dropped grades C and D, which were lower than the official test insecticides (O.T.I.) and changed the other grades, as follows:

Grade AA, sprays giving a kill at least 16 per cent higher than the Official Test Insecticide.

Grade A, sprays giving a kill of 6 per cent to 15 per cent higher than the Official Test Insecticide.

Grade B, sprays giving a kill between 5 per cent lower and 5 per cent higher than the Official Test Insecticide.

At the time the grading system was established, action was taken by the NAIDM to secure its use as a commercial standard. The Association requested the U. S. Bureau of Standards to establish the Peet-Grady method and the grading system as a commercial standard for household insecticides. The U. S. Bureau of Standards, with the approval of the U. S. Department of Agriculture, complied with this request *(1038)*. The Commercial Standard states: "The Peet-Grady method of determining efficiency of contact liquid insecticides is subject to variations that

necessitate close attention to details of equipment and procedure to obtain comparable results. Refinements that result in higher precision are being developed by constant research and are incorporated in the official method from time to time."

Murray *(1927)* attempted, during a period of about two years, to determine why the results obtained by the Peet-Grady method are not more accurate. Such factors as temperature, humidity, interval between feeding and spraying, the type of food available for recovering flies, whether recovery is the same in light or darkness, and fly rearing were thoroughly investigated. Attempts were made to evaluate the effects of using different pressures for spraying, spraying from different sides of the chamber and varying the amount of spray delivered. Much detailed information was gained by studying the effects of such factors as different types of bran and alfalfas with different protein contents.

The effect of the amount of water present, the pH and temperature of the medium and its decomposition were also investigated. His investigation was thorough and prolonged, and while he obtained valuable information on the production of flies of uniform size and vitality, some unknown factor continued to cause erratic results in the Peet-Grady tests. At the suggestion of the author, Murray began a study of the uniformity of the dose received by individual flies. An oil-soluble red dye was dissolved in an oil-pyrethrum spray containing 1 mg. of pyrethrins per cc. and this colored insecticide was applied to flies by the Peet-Grady method. After spraying, each fly knocked down was placed in a small calibrated vial, the dye was washed off with mineral oil and the solution was diluted to 5 cc. with oil. This oil solution was then compared colorimetrically with a standard solution of the red dye in oil, from which the dose of pyrethrins received by each fly was calculated. Six tests were made on male flies and six on female flies, using 100 flies for each test. The ratios of the greatest dose to the least dose in the six tests on male flies were 2.60, 3.16, 3.60, 3.94, 4.81, and 6.44. For the female flies, the ratios were 2.58, 3.95, 4.30, 4.63, 5.13, and 5.15. These differences indicated little uniformity in dosage. Even in pairs of successive Peet-Grady tests the flies do not receive the same amount of spray.

Three more tests were then made in each of which 500 flies were sprayed with 12, 24 or 36 cc. of dyed insecticide. The individual doses were not determined, but the total dose was estimated by dissolving the color from all the flies of each 500-fly test, diluting to 200 cc., and comparing with the color standard. The average individual dose was then calculated.

Murray concluded: "A fundamental error in the Peet-Grady method has been exposed. The variation in average fly dose and per cent kill is of the same magnitude as if the operator did not measure the amount of insecticide sprayed into the chamber, but took at random anywhere from 12 to 36 cc. of spray for each test."

A characteristic of pyrethrum household insecticides is the quick knockdown when tested by the Peet-Grady method. A good pyrethrum spray will usually knock down 95 to 100 per cent of the flies within 10 minutes. In the official method, counts are made 24 hours after spraying to determine the percentage killed. At that time the flies are generally dead or able to fly, with only a few moribund. With rotenone and its derivatives and certain other compounds, there is a considerable percentage of moribund flies at the end of 24 hours, but nearly all of these die within 72 hours. This has led to the suggestion, on the part of those interested in the slower acting materials, that some allowance for moribund flies be made in rating insecticides. A moribund fly has been defined as one that cannot walk or fly.

The question of counting moribund flies as dead in the Peet-Grady method has been discussed by Stoddard *(2185)*, Whitmire *(2316)*, Weed *(2296)*, Benedict *(1204)*, and Simanton *(2133)*. The problem is not a simple one.

By determining the knockdown in the Peet-Grady test at intervals of 1, 3, 5, 7 and 10 minutes, Lederer *(1793)* has shown that household insecticides may have the same percentage kill or rating but may differ widely in speed of knockdown. Fractional knockdown is determined by spreading sheets of paper the size of the chamber floor over the flies knocked down at intervals of 1, 3, 5, 7 and 10 minutes, afterward counting the flies on each sheet and on the floor. Kills must be determined separately.

Badertscher *(1150)* concluded that to evaluate concentrated oil-pyrethrum sprays containing 300 to 400 mg. of pyrethrins per 100 cc. by the Peet-Grady method, they should first be so diluted that the killing power is 50 per cent and then compared with the O. T. I. Sullivan *(2192)*, using the Campbell turntable method (page 112) agreed with Badertscher that the best method for the biological testing of semi-concentrate fly sprays is to dilute them and compare them with the O. T. I.

Murray and Caler *(1929)* collected wild flies and compared their susceptibility to oil-pyrethrum sprays with that of flies reared as in the Peet-Grady method. Unlike reared flies, wild male and female flies were about equally susceptible and were

about as resistant as male reared flies. Wild female flies were much more susceptible than reared female flies. Samples of wild flies contained many more females than males, but the sexes were about evenly divided among reared flies.

MacCreary and Pearson *(1819)* subjected horseflies to tests by the Peet-Grady method. The flies used were *Tabanus nigrovittatus, T. lineola, T. atratus* and *T. daeckei.* All were considerably less resistant to pyrethrum sprays than houseflies.

NAIDM Methods

The Peet-Grady method, as officially adopted by the NAIDM, for evaluating liquid household insecticides, is reprinted herewith, from Soap Blue Book 1945, by permission of MacNair-Dorland Company.

"Rearing Room.—This room may be of any convenient size constructed so as to be free from strong drafts, and maintained at a temperature between 80 and 85 degrees Fahrenheit with a range in relative humidity between 40 and 70 per cent. It should be separate from the testing room in order to eliminate the possibility of traces of insecticide coming in contact with the test insects prior to the test.

"Testing Room.—This room may be of any convenient size capable of holding the standard Peet-Grady Test Chamber and permitting adequate additional space for the operator to handle the test efficiently. While conducting tests this room shall be maintained at a temperature of 75 to 85 degrees Fahrenheit. It is not necessary to control the humidity, although it is suggested that it be held between 40 to 70 per cent relative humidity. Since the exhaust fan of the Chamber will remove relatively large quantities of air, the air inlet to this room should be constructed to permit air to approximately the specified temperature to enter the room.

"Peet-Grady Test Chamber.—The Test Chamber shall be constructed of wood or metal, or other suitable material. The inner surface shall be smooth and impervious to the usual household type of insecticide. The chamber must be rigidly constructed and the inside must be free from cracks, projections, ledges, etc. The Chamber shall be a 6-ft. cube by internal measurements, with a tolerance of plus or minus 1 in. for any dimension. One wall shall contain a tight-fitting door large enough for a man to enter conveniently, with the interior side flush with the wall when closed. One or more of the walls, or the ceiling, shall contain an

observation window. (It is suggested that at least two opposite walls have observation windows.) Illumination is provided by means of a glass window in the ceiling above which is placed an electric lamp in a reflector, or by any other satisfactory means such as fluorescent lights. A wire screen-covered air duct (10-mesh screening) shall be provided to permit the ventilation of the chamber after each test. The location of this exhaust duct in relation to ventilation openings in the walls must be such that thorough ventilation of the chamber is obtained. Preferably, this is accomplished by ports approximately 6 x 6 in. in size, covered with screen on the inside and provided with tight-fitting hinged covers on the outside. Four ports located in the 4 lower corners, or 8 ports located in both the 4 upper and 4 lower corners are satisfactory. The ventilation ports should not be on the same level as the exhaust port. The entrance door may be used alone or in conjunction with the ventilation ports if a screen door is provided and thorough ventilation of the chamber is obtained. Satisfactory openings shall be provided for the introduction of the insecticide; these shall be so constructed and so located that uniform distribution of the spray is effected. These openings may be round 1 in. holes located not less than 6 in. or more than 18 in. from the ceiling and 1 in. from the nearest corner on each wall or a single hole may be provided in the center of the wall 6 to 18 in. from ceiling. Any spraying arrangement that may be used should not furnish hiding places for the insects nor allow undue ventilation. The nozzle of the atomizer shall be oscillated slowly in a horizontal plane to avoid spraying walls and ceilings and to effect uniform distribution of the spray.

"Fly Cages.—Any satisfactory cages of any convenient type may be used. It is suggested that the base be square in shape to provide sufficient floor space, and that they provide at least 1 cubic inch of space per fly. The floor of the cage is preferably detachable, to facilitate cleaning the cages and inserting a paper floor covering. The cages are constructed of wood or other suitable material and 16 mesh wire screening, and are fitted with a sleeve opening or rubber membrane. At least 2 sides and the top shall be screened.

"Atomizer.—The atomizer shall be the special one constructed by the DeVilbiss Company for the N.A.I.D.M. Standardization Committee and obtainable from that company, by referring to 'special atomizer for Peet-Grady Insecticide Test—DeVilbiss Special No. 5004.' The sprayer shall be operated with air maintained at a constant pressure of 12.5 plus or minus 0.5 lb. per sq.

in. and which must be free of oil, dust particles, or condensed moisture. The atomizer should deliver 12 cc. of base oil in 24 seconds (tolerance ± 1 second) and this should be checked frequently.

"Apparatus for Picking Up Flies.—Any convenient means of picking up the paralyzed flies without injuring or appreciably disturbing them may be used. A vacuum device providing gentle suction and a sufficiently large receptacle to prevent crowding of the flies has been found satisfactory. The pick-up device shall be cleaned after each test with the same solvent used in cleaning the chamber. However, careful picking up by hand or other satisfactory methods is permissible.

"Exhaust Fan.—An exhaust fan moving not less than 1,000 cu. ft. of air per min. is used to ventilate the chamber after each test. It shall be arranged with adequate piping to exhaust the chamber vapors outside of the building.

"Test Insect.—For evaluation purposes, the house fly (*Musca domestica,* L.) is used. Healthy test groups having an average age of not less than 4 nor more than 6 days are to be used. Individual flies in the test groups shall not be less than 3 nor more than 7 days old at the time of the testing. Under the usual rearing conditions (temp. 80-85 F.) the flies are ready for use 15 days after the culture was prepared (5 days after peak emergence) or about the second day of oviposition. The strain shall be of such susceptibility that the O. T. I. will cause a mortality of from 30 to 55 per cent.

"Reference Insecticide.—The primary reference insecticide used in this test for evaluating the unknown shall be the current Official Test Insecticide (O. T. I.) and shall be prepared by the National Association of Insecticide and Disinfectant Manufacturers, Inc., each year.

"Insecticide Paper.—Any unsized, non-glazed absorbent paper such as brown kraft wrapping paper or gray bogus paper of proper width may be used on the chamber floor. The paper surface should be renewed for each test. Two overlapping sheets of 36-40 in. width or one sheet of 6 ft. width may be employed. No special weight is to be specified although 60-80 lbs. weight paper has been found excellent.

"Raising and Handling of Flies.—Culture jars are prepared by filling cylindrical battery jars or other satisfactory containers measuring approximately 6 in. in diameter by 9 in. high, three-quarters full of the synthetic medium prepared as follows:

For 1 jar:

400 g. soft wheat bran (coarse)
200 g. alfalfa meal
Mix thoroughly, place in jars, and then add 900 to 1,000 cc. of an aqueous liquid suspension containing:
16 ml. malt extract
10 g. compressed yeast

"Mix the suspension throughout the bran-alfalfa to give a loose mixture. The proportion of liquid ingredients to dry ingredients may be varied slightly to prevent mold growth. Dry ingredients and suspension may be mixed before adding it to the battery jar.

"Horse manure with added water, malt, and yeast may also be used as the rearing medium. The manure, which must be fresh, is preferably pasteurized for 2 hrs. at 160-165° F. After the manure is cooled, it is packed loosely into battery jars, leaving just enough room on top to fit the covers. To each jar is added 100 cc. of yeast cells suspended in water taken from the formula of 1 # yeast to 1,700 cc. water. The volume of suspension added may vary, dependent on the moisture content of the manure, but should be such as to maintain a slight excess of liquid in the bottom of the jar with a thin dry layer of medium on the top at the time of pupation. Other satisfactory breeding media may also be employed such as powdered milk, mixtures of alfalfa meal, bran and brewers' grains or oat hulls, etc.

"Eggs are deposited in the food dishes or other containers suitable for oviposition, in cages containing 4 and 5-day-old flies. It is preferred that eggs be collected from at least two cultures. About 2,000 eggs or sufficient to give 1,500 to 1,800 flies per jar are transferred from the moist cotton to the jar after the medium has been mixed. The number of eggs to use may be determined by gently shaking the eggs in water for a minute, allowing them to settle out and then estimating volumetrically from a calibrated tube. One-tenth ml. of settled eggs contains about 500 eggs.

"When the larvae have migrated to the top inch of media and pupated, usually on the eighth or ninth day after preparation, the pupae are separated from the medium by lifting off the top ½ in. of the medium in each jar, loosening the exposed pupae, and then pouring the pupae with adhering particles of medium on a cafeteria tray. The pupae-medium mixture is placed in a fan blast until it is of such dryness that the fan blast will effect a clean separation of the pupae and medium when the mixture

is sprinkled over an inclined tray placed in the air stream. Other procedures such as the use of funnels may be employed for separating the pupae from the medium. The pupae must be handled gently to avoid injury.

"The separated pupae are thoroughly mixed and weighed into groups as test units and each group is placed in a shallow dish which is, in turn, placed in a cage. If the large group procedure is used the test unit consists of approximately 500 pupae. If the small group procedure is used, 1,200 to 1,500 pupae are placed in stock cages of 1,500 cu. in. or larger size. The series of test units is then kept for 5 days after peak emergence or until the second day of oviposition (usually the 15th day after the culture was prepared). Each cage is supplied with a dish containing a 50 per cent dilution of milk with water (or other satisfactory food), so prepared as to prevent the flies from drowning. A 40 per cent formalin solution at the rate of 1/1500 delays souring of milk for several hours. Satisfactory food must be available to the flies at all times.

"Test Procedure.—On the second day of oviposition the series of cages from one culture is ready for testing. In the large group procedure all flies in one cage are transferred to the thoroughly cleaned Peet-Grady chamber, the floor of which is covered with a satisfactory type of paper. In the small group method approximately 100 flies are used in each test. Samples may be taken by liberating 100 flies directly into the chamber and continuing until about 10 per cent of flies remain in the stock cage. These should be discarded. The order of spray treatments must be randomized as discussed later. Samples may be taken also by discarding the first 100 flies and then counting 50 flies into each of 9 small cages. 100 flies are counted into the 10th cage and then, starting with the 9th small cage and working backwards, 50 flies are added to each. Flies remaining in the stock cage are discarded. The cages for each test are picked at random and the number of flies should be within 5 of 100.

"All ports and entrances are closed, the windows are equally shaded, and a total of 12 ml. of insecticide is applied at 12.5 lb. pressure in approximately equal quantities through each spray hole, care being taken to assure that the insecticide is uniformly distributed throughout the chamber. The chamber is kept closed at a constant temperature in the range of 80-85° F. for 10 min. from the time the spraying is started. At the end of 10 min. the ports are opened and the chamber is ventilated while the flies are being picked up.

"The paralyzed flies are picked up and transferred immediately to clean cages meeting the specifications. These flies may be counted when they are picked up or later, depending upon which time is the most convenient.

"During the subsequent 24-hr. recovery period, the cage is placed in the rearing room and supplied with a gauze-wrapped ball of cotton saturated with 10 per cent sugar solution or suitable food made available by other satisfactory means provided it is near the floor of the cage and flies cannot drown in it.

"The few unparalyzed flies in the chamber at the end of the 10-min. exposure period are counted and removed.

"Before a new cage of flies is liberated for the next test, the chamber walls are cleaned or wiped with a clean cloth saturated with alcohol containing 10 per cent acetone, or with other satisfactory solvents. It is recommended that the chamber be periodically cleaned with soap and water, to make sure that all toxic residues are completely removed. Special precaution should be taken to clean the inside walls of the chamber with the proper solvent after a test with a new chemical compound so as to eliminate the possibility of a toxic residue.

"Assembling the Data.—The number of unparalyzed flies must be counted and recorded at the end of the 10 min. exposure period. The dead flies are counted 24 hrs. ($\pm$ 1 hr.) later, preferably by removing them from the recovery cage. Only flies that show no sign of life upon being touched may be counted as dead. If paralyzed flies were counted as they were collected, the sum of paralyzed and unparalyzed flies yields the total flies in the test. If paralyzed flies were not counted as collected, the recovered flies are killed by placing the cage in an oven at 170° F. for a few minutes, after which they are counted. The sum of recovered and dead flies yields the paralyzed flies and this sum added to the unparalyzed flies yields the total flies used in the test. The mortality is the per cent dead of total flies and the knockdown is the per cent paralyzed of total flies.

"Conditions for Official Evaluation.—1. The tests shall be conducted in accordance with the procedure previously described.

"2. An unknown insecticide to be officially rated shall have a knockdown percentage not lower than that of the O. T. I. with a tolerance of minus 2.

"3. Cages showing a natural mortality greater than 5 per cent on the day scheduled for testing shall not be used.

"4. Evaluation of an unknown shall be based on the difference of the average mortalities obtained with the O. T. I. and the

unknown sample. The O. T. I. kill shall fall between 30 and 55 per cent. Denote the difference between kills by the appropriate grade according to letter: U. S. Department of Commerce Cs-72-38.

Grade AA—excellent	+16 or higher
Grade A —good	+6 to +15
Grade B —equal to O. T. I.	—5 to +5

"5. In the small group procedure no more than 2 unknowns may be tested, in conjunction with one O. T. I. in any one series. Ten tests are run on the O. T. I. and on each of the unknowns in parallel; that is, test each sample of the series the same number of times with flies of the same batch and test every member of the series the same number of times on any one day. The three samples of a series are to be randomized in order of testing until 30 tests have been run. When only one unknown and the O. T. I. comprise the series, the order should likewise be randomized. For example, 1,2; 2,1; 2,1; 1,2; etc., until twenty tests have been completed. The standard error of the mean difference between the average O. T. I. kill and the average unknown kill must be less than 3. If it is 3 or greater, the differences between pairs were too variable and to make the results valid additional paired tests must be run to bring the standard error of the mean difference down to 3 or lower. Calculate the mean difference between kill obtained with the unknown and that obtained with the O. T. I.

"The following example illustrates the arrangement of tests and calculations described in the preceding paragraphs. When two unknowns and the O. T. I. are tested in series, the first table should consist of differences between unknown No. 1 and the O. T. I., the second table should show differences between unknown No. 2 and the O. T. I.

Pair	Date	Batch	Unknown No. 1 % Kill	O. T. I. % Kill	Difference	Deviation from Mean Difference	Deviation Squared
1	12/8	12/3	58	49	+ 9	+2	4
2	12/8	12/3	62	50	+12	+5	25
3	12/8	12/3	50	47	+ 3	—4	16
4	12/8	12/4	52	46	+ 6	—1	1
5	12/9	12/4	60	52	+ 8	+1	1
6	12/9	12/4	65	50	+15	+8	64
7	12/9	12/5	54	48	+ 6	—1	1
8	12/9	12/5	56	57	— 1	—8	64
9	12/10	12/5	51	44	+ 7	0	0
10	12/10	12/5	57	52	+ 5	—2	4
			56.5 M	49.5 M	7.0 M.D.	0	180 Sum d^2

$$\text{Mean difference} = 7.0; \quad \text{Standard error of M.D.} = \frac{\sqrt{\frac{\text{SUM } d^2}{n-1}}}{\sqrt{n}} = \frac{\sqrt{\frac{180}{9}}}{3.16} = 1.42$$

"The standard error of the mean difference is less than 3, thus indicating the test has been properly conducted.

"The letter n (in formula above) denotes the number of paired tests. This number is always 10 except when it is necessary to run additional tests to bring the standard error of the mean difference down to 3 or less.

"The unknown in the example above tests 7 points (units of difference between the percentage kill of O. T. I. and the percentage kill of the unknown) better than the O. T. I.; therefore, unknown No. 1 is an 'A' grade insecticide.

"6. In the large group procedure the evaluation is carried out as follows:

"The evaluation is based on the difference in mortality of the O. T. I. and the unknown as determined by one comparison on each of three cultures. Each culture used in determining the evaluation must show an O. T. I. kill falling between 30 and 55 per cent. Replicated O. T. I. tests on one culture shall agree within 10 points.

"The following example illustrates the order of testing and the computation of the evaluation.

Culture	E		F		G	
Date	11/21		11/22		11/23	
Cage No.	Sample	% Dead	Sample	% Dead	Sample	% Dead
1	O.T.I.....	36	Unknown B.....	55	O.T.I.....	40
2	Unknown A.....	42	O.T.I.....	46	Unknown F.....	54
3	Unknown C.....	32	Unknown C.....	32	Unknown E.....	58
4	Unknown B.....	50	Unknown F.....	55	Unknown A.....	46
5	Unknown E.....	65	Unknown A.....	46	Unknown C.....	30
6	Unknown D.....	55	Unknown D.....	60	Unknown D.....	58
7	Unknown F.....	48	Unknown E.....	70	O.T.I.....	38
8	O.T.I.....	36	O.T.I.....	44	Unknown B.....	55

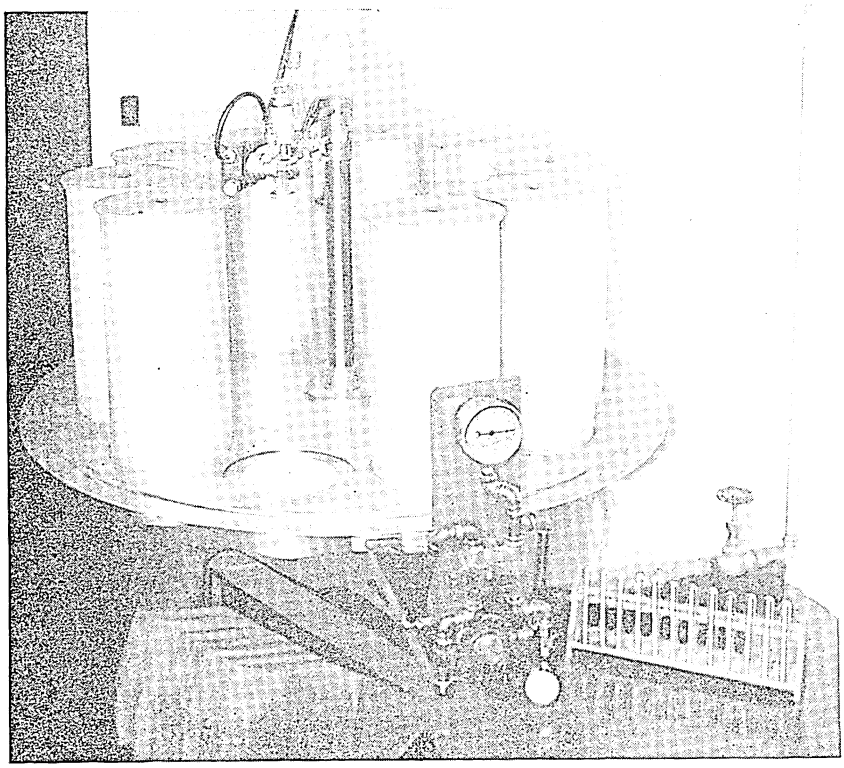
	Culture Mortalities	Average	Rating	Grade
O.T.I.	36; 45; 39	40		
Unknown A	42; 46; 46	44.7	+ 4.7	B
Unknown B	50; 55; 55	53.3	+13.3	A
Unknown C	32; 32; 30	31.3	— 8.7	Below B
Unknown D	55; 60; 58	57.7	+17.7	AA
Unknown E	65; 70; 58	64.3	+24.3	AA
Unknown F	48; 55; 54	52.3	+12.3	A

"The order of testing shall be random, except the O. T. I. shall be replicated within each culture with the random order restricted to the first two and the last two tests in any series."

It has been suggested that the O. T. I. be made approximately equal in toxicity to an AA grade spray instead of a B grade insecticide. This would necessitate changing the pyrethrin content of the O. T. I. from about 100 mg. per 100 cc. to 160 mg. per 100 cc. Such a change can probably not be made before 1946. An excellent description of the preparation, composition and use of the Official Test Insecticide has been published *(1127)*.

Other Methods Using Flies as Test Insects

A metal turn-table, employing the "settling-mist" principle has been developed by Campbell and Sullivan for testing contact insecticide sprays on flies and other insects *(1268)*. This is an improvement of Campbell's turn-table method (page 112). The



Turntable apparatus of Campbell and Sullivan.
(Courtesy E. R. McGovran, U. S. D. A.)

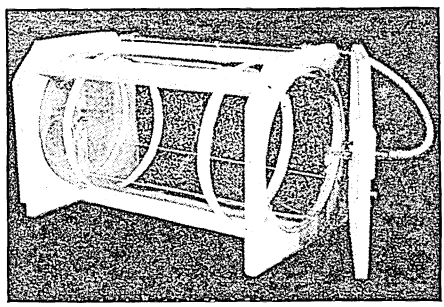
construction is given in detail and the method of operation is described. The method can be used for testing pyrethrum sprays on flies, but it gives lower kills than the Peet-Grady method. Some changes are made in the customary method of rearing and handling flies.

Badertscher *(1149)* has found that the Campbell turn-table method yields lower kills than the Peet-Grady method.

Sullivan and his associates *(2196)* modified Campbell's turn-table method in order to obtain data on speed of knockdown as well as kill. This was done by focusing a camera on the bottom of the cage so that photographs could be taken at desired intervals during the test. Flies that were not knocked down were out of focus. Knockdown counts were then made from the successive photographs.

McGovran, Sullivan and Phillips *(1879)* discovered that houseflies that had been paralyzed with ether, acetone or weak solutions of pyrethrins in kerosene were more resistant than normal flies to pyrethrin kerosene sprays containing 2 to 4 mg. of pyrethrins per cc., applied 5 minutes after the paralyzing treatment.

Zermuehlen and Allen *(2363)* used the "settling-mist" principle as the basis for a method for testing fly sprays.



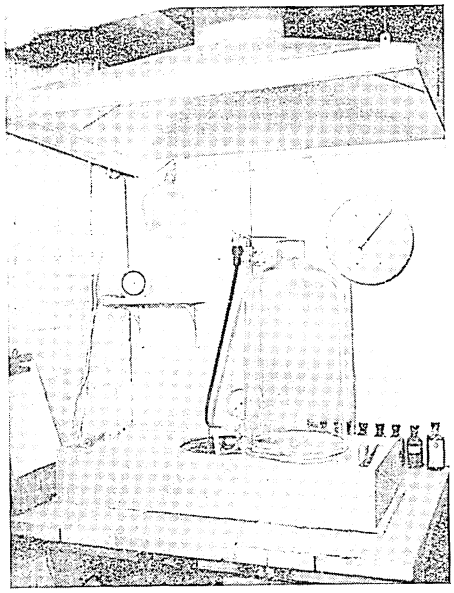
SMALL CHAMBER APPARATUS OF KEARNS AND MARCH.
(REPRODUCED FROM "SOAP" BY PERMISSION)

Allen, Dicke and Brooks *(1004)* have also described a modification of the Campbell turn-table method.

For the evaluation of the toxicity of organic compounds of unknown insecticidal value, Kearns and March *(1722)* developed a small chamber method for testing sprays on houseflies. This method was not proposed to replace the Peet-Grady method, but

is intended to save time, money and labor in making preliminary tests for insecticidal properties.

A rapid method for determining the toxicity of livestock sprays has been described by Eagleson *(1393)*. Housefly larvae are reared in crimped oats from which they are separated by a

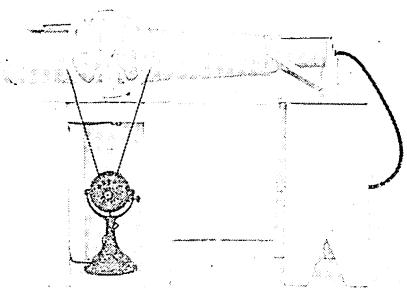


SPRAY TESTING APPARATUS OF ALLEN, DICKE AND BROOKS.
(COURTESY T. C. ALLEN)

sieve when fully grown. They pupate in damp excelsior. The pupae are graded for size and placed in emergence cages. The adults are fed a mixture of banana pulp, sugar, milk and gelatin. The procedure is as follows:

> "Flies to be sprayed are placed in 9 by 17 centimeter cylinders of 14 mesh screen wire. The ends are screen disks soldered into metal screw bands obtained from wide-mouthed Mason jars. The ends fit snugly into the cylinder, screen side inward, so that no protection from the direct spray beam is afforded the insects.
>
> "A cylinder is filled with 50 to 75 flies by darkening the aging cage and holding the cylinder against the port in its end. Experience makes possible rather uniform filling of the cylinders. All the insects are driven from the cage into the cylinders, so the entire population is used. After being filled the cylinders are randomized to provide for a still greater

uniformity in the sample. A spray lot usually consists of ten cylinders. Spray cylinders are always washed in benzene or aviation gasoline after each use.



EAGLESON'S APPARATUS FOR TESTING LIVESTOCK SPRAYS.
(COURTESY CRAIG EAGLESON)

"The spray tunnel is a tapering reinforced sheet-metal tube. It is open at the smaller end and discharges into a hood and filter-column packed with fine steel wool. A constant, controllable stream of air is drawn through the tunnel and filter by a vacuum cleaner. The spray mist is effectively absorbed by the filter and the dust bag on the cleaner. The flies receive only the insecticide that contacts them as a traveling mist, and after the measured dose has been atomized, they are bathed in clean air. An opening in the center of the tunnel permits insertion of the spray cylinders into a rotating mechanism which turns the cage at 80 revolutions per minute. A sliding glass plate closes the opening during the spray discharge. Spraying the flies in a rotating cylinder improves the chances of uniform spray application to the entire surface of each fly.

"The atomizer produces a fan-shaped spray. The air and liquid feed tubes are holes 0.8 millimeter in diameter, diverging at 30 degree angles, bored in a solid block of brass. The insecticide is pipeted into a funnel on the feed tube of the atomizer and flows by gravity through the two apertures, which exactly intercept the air jets. The delivery of the usual dose (2 cc.) requires about 2 seconds. The spray produced is a non-fluctuating mist, uniform throughout its cross section. Air under a pressure equal to 75 centimeters of mercury flows continuously through the atomizer. Eddy currents,

which result when a jet of air is suddenly turned on or off in the tunnel, are avoided by the continuous flow."

After spraying the flies are placed in recovery cages at constant temperature and humidity and with continuous ventilation, to stimulate as nearly as possible actual conditions of use. The mortality is much lower (14% to 20%) when the sprayed flies are kept under forced ventilation.

Eagleson *(1392)* found houseflies more resistant to pyrethrum than horn flies *(Haematobia irritans)* or stable flies *(Stomoxys calcitrans)*.

Eagleson *(1396)*, using his tunnel method, subjected houseflies to a median lethal dose and a hypnotic dose of oil-pyrethrum spray. From these tests he obtained dosage-mortality and dosage-torpor curves and concluded, "that the use of hypnotic doses applied to houseflies in an aerated spray tunnel, with observations of torpor made during their recovery in an aerated recovery cabinet, is an advantageous and satisfactory method of assaying livestock fly sprays."

Nelson *(1943)*, as chairman of the NAIDM committee on cattle spray testing, found no satisfactory method for testing cattle sprays. Nelson observed that results obtained by testing for repellency with houseflies cannot be correlated with repellent action in the field against blood-sucking flies. Since horn flies, stable flies and horse flies, the principal blood feeders, cannot readily be raised in the laboratory, repellency tests should be made in the field. Nelson discusses a number of methods in current use for testing repellency and toxicity and appends a bibliography of cattle sprays containing 73 references.

Mailen and Fenton *(1832)* use a modification of the "one-half cow method" for determining repellency in the field. This method consists of spraying half of the cow with the material to be tested, leaving the other half unsprayed as a control.

Fryer and his associates *(1469)* in summarizing their study of fly repellency tests state: "Testing cattle sprays on the cattle themselves is complicated by the number of variables present in such research. That situation logically requires objective and standardized techniques in planning the experiment, in carrying it through, and in the final interpretation of the data obtained. A review of the literature on cattle fly spray tests indicates that, although considerable progress has been made in developing general techniques and in discovering the variables involved, very little data and few statistical analyses have been presented in support of the conclusions drawn. Periodic counts of the flies

present on cows are distributed in a Poisson-like manner that is not even approximately normal. This circumstance forbids the use of statistical techniques based on normal distributions unless the data can be normalized to a satisfactory degree before the analysis is undertaken.

"Statistical research has led to a general method for determining a transformation which usually will normalize a set of such data satisfactorily. The application of that procedure to the data obtained from a comparison of six repellent sprays and a check produced a new transformation, the reciprocal square root transformation,

$$y = \frac{1}{\sqrt{x+10}}$$

"This transformation was successful under the peculiar circumstances met in fly spray research whereas other previously used transformations failed to normalize the distributions of counts satisfactorily.

"From research at this station, it appears that a randomized latin square design on balanced groups of cows and different periods of time during the summer is well-adapted to cattle fly spray research. A general design for a half-cow method of testing sprays is suggested. Such a design would standardize the test and make a statistical analysis of the data so obtained possible; but, if practical difficulties make it impossible to compare properly two different sprays on the same cow, the newer design would not make the half-cow technique satisfactory."

A method for rearing stable flies, *Stomoxys calcitrans,* has been described by Doty *(1380.)* They are considerably less resistant than houseflies, but they are repelled by the same sprays and in about the same degree as houseflies.

McGovran, Fales and Goodhue *(1871)* have described a method for testing aerosols against houseflies. A special dispenser is necessary since the usual pyrethrum-dichlorodifluoromethane mixture is under about 80 pounds pressure. "A diagram of this dispenser is shown in Fig. XIII. It consists essentially of a heavy-walled graduated glass test tube 1, which is held tightly against the valve 2 in the framework 3, by the adjustable threaded plug 4. The rubber washer 5 prevents leakage, and the rubber plug 6 cushions plug 4 from the glass tube 1. The frame 3 is made from a piece of ½-inch brass pipe 7 inches long and having two slots about ½-inch wide and 5 inches long cut in opposite sides so that the glass tube will be clearly visible. A ⅜-inch pipe

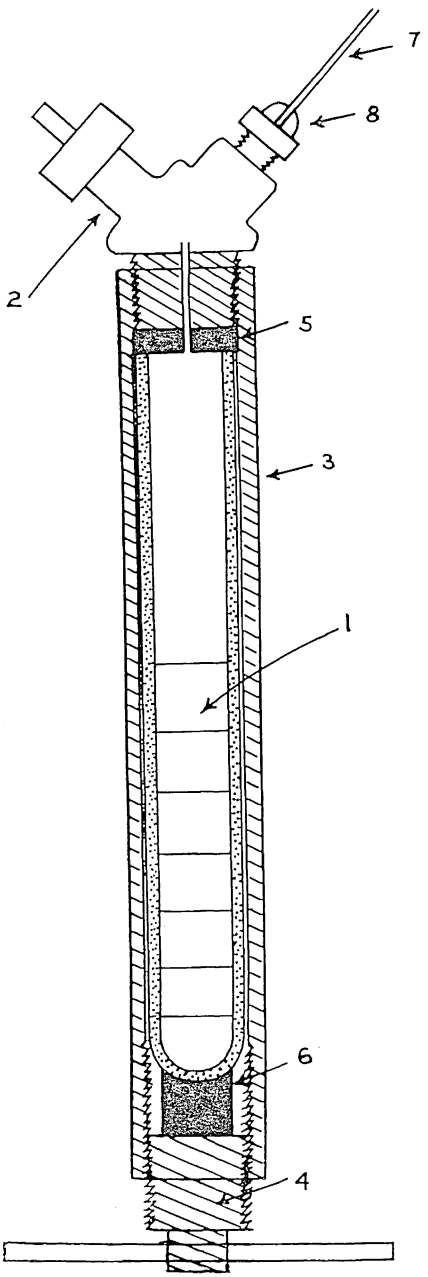


FIG. XIII. AEROSOL DISPENSER FOR BIOLOGICAL TESTS: ACTUAL SIZE.
(MCGOVRAN, FALES AND GOODHUE)

thread is cut inside each end of the pipe. The valve 2 is a standard 3/8-inch Y-valve with a 1/8-inch female pipe connection such as is commonly used on small cylinders containing liquefied gas. The screen, with which the valve 2 is equipped, is removed, and the lower part of the male-threaded portion is ground flat so that the rubber washer 5 will produce a gastight joint. The glass tube may be conveniently calibrated to measure grams of dichlorodifluoromethane solution by weighing successive amounts of orthodichlorobenzene, which has the same density, into the tube and making marks at the desired intervals on a paper glued to the side. Either an oil-burner nozzle (not shown) or the 0.017-inch diameter capillary 7, mounted in the nipple 8, may be used to spray the liquid. The capillary is superior to the nozzle.

"The amount of pyrethrum left in the dispenser after a test was determined colorimetrically for 0.5, 1, 2, and 4 grams of solution, and the corrections noted. The concentrating effect on the solution caused by loss of solvent to the vapor phase was also determined colorimetrically. The difference in these two corrections was applied to the calibration. These corrections range from 6 to 25 per cent, depending on the size and shape of the glass tube, the type of nozzle, and the amount of solution taken. They must be determined for each dispenser and each solution, if the kind and concentration of material in the dichlorodifluoromethane vary widely."

The tests were made in a Peet-Grady test chamber, exposing the flies for 15 minutes. Curves were constructed showing the corrections to be applied in preparing and spraying the solutions.

A sprayer for testing aerosols has also been described by Batt *(1180)*, Fig. XIV. "It consists of an aluminum reservoir (R) having a 100 gm. capacity and closed by two 1/8 in. needle valves (A) and (F). The lower valve is connected to a glass pressure chamber which contains a calibrated glass tube (T) made from a 2 mm. pipette. The aerosol mixture from the reservoir is allowed to drip into the tube (T) by opening valve A slowly. Then the measured amount in tube (T) is sprayed through the capillary tube by opening valve B. It is quite important to fill the voids in valve B with solder, leaving only a small hole to conduct the aerosol.

"To fill the sprayer it is first thoroughly cleaned to remove any foreign material that might plug the nozzle, and evacuated. The reservoir is filled with 100 gms. of aerosol from a container, then the entire sprayer is tipped sideways about 45° and valve

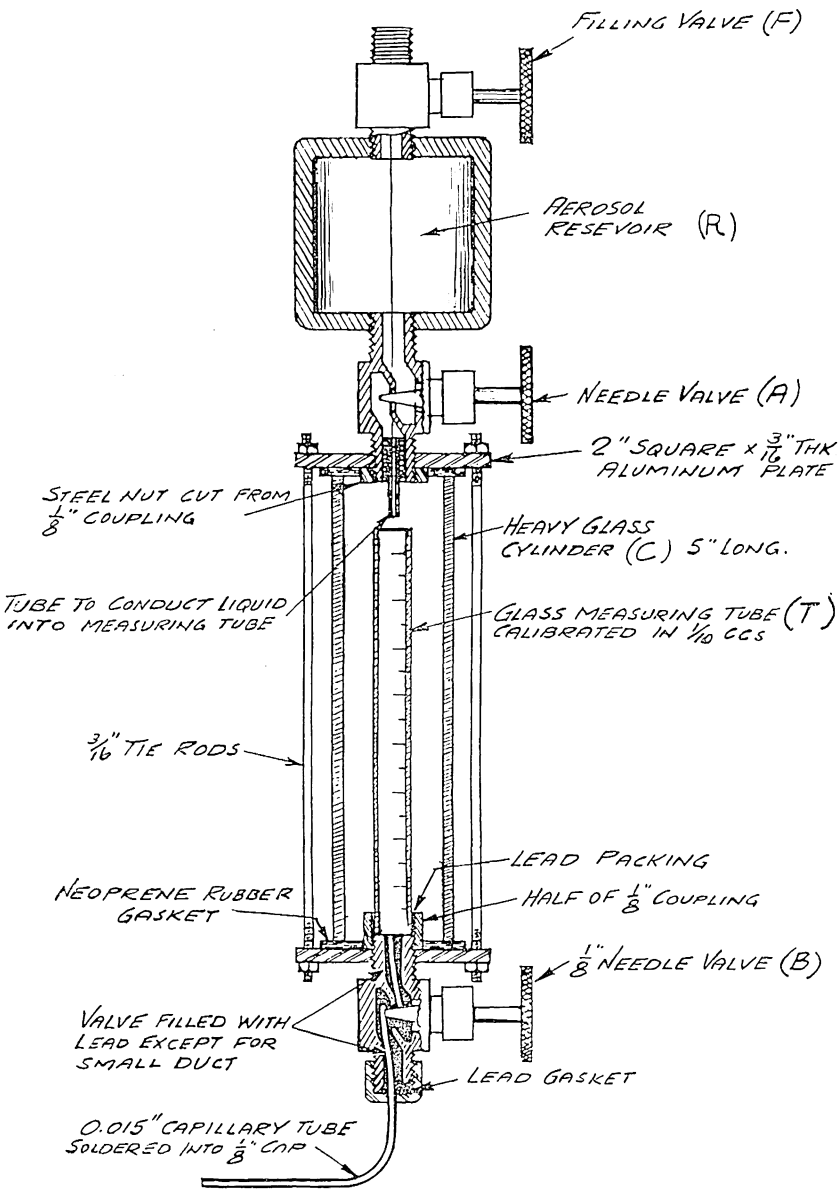


FIG. XIV. SPRAYER FOR TESTING AEROSOLS (BATT).

A opened for a short time. This allows some of the mixture to run into the area between the heavy glass cylinder C and the small measuring tube T. A few grams is sufficient since its only purpose is to maintain a pressure equal to the aerosol mixture,

about 70 lbs. per sq. in., in the measuring device. The sprayer is now tipped back to the vertical position and ready to use.

"Valve A is opened and the desired quantity admitted into tube T. Should too much enter it can be removed by opening valve B and releasing the aerosol through the capillary tube. After the proper amount is in tube T, the capillary is pointed into the Peet-Grady room, and the measured volume sprayed in. As the volume in the measuring tube diminishes, the aerosol between the glass cylinder and the measuring tube vaporizes and pushes out the measured amount at a constant pressure. When the liquid in tube T is gone, only gas escapes from the capillary. This causes the aerosol between the glass cylinder and tube to boil and foam indicating that the entire measured quantity has been sprayed."

Tests for Insecticides Used Against Crawling Insects

It has long been recognized that the Peet-Grady test on houseflies does not necessarily indicate the relative toxicity of household insecticides to cockroaches, bedbugs and other household insects. Woodbury and Barnhart *(2346)* point out that a spray which is excellent for houseflies when tested by the Peet-Grady method may be a poor one for roaches. Campbell *(1265)* states that "synthetics that are better than the Official Test Insecticide against houseflies by the Peet-Grady method are often poorer than the Official Test Insecticide against roaches and bedbugs by the settling mist method. In general the samples tested have a lower rating against roaches and bedbugs than against houseflies." Rather crude methods for testing pyrethrum sprays on cockroaches have been referred to on page 109.

Woodbury *(2344)* has described a method for rearing large numbers of German cockroaches *(Blatella germanica)*, and a modified settling mist method for testing household insecticides against them. Woodbury obtained the most consistent results using nymphs 2 days after their first molt. The percentage of dead roaches at the end of 24 hours was not a measure of the effectiveness of pyrethrum sprays, but the percentage of dead and moribund roaches 24 hours after spraying was proportional to pyrethrin content. Adult female roaches are much more resistant to pyrethrum than adult male roaches. Females with egg capsules dropped them when sprayed with oil-pyrethrum insecticide. Adult male and female roaches are more resistant to pyrethrum than second instar nymphs.

Tuma *(2245)*, in a study extending over three years and involving experiments with 2,000,000 roaches, concluded that roaches *(Blatella germanica)* 17 weeks old are the most resistant to pyrethrum sprays and should be used for test purposes. He describes detailed methods for rearing colonies of roaches of controlled age and a method for testing insecticide sprays on roaches. Natural foods are preferable to synthetic foods and frequent variations in diet are desirable. The percentage of dead and moribund roaches 48 hours after spraying indicates the effectiveness of a spray. Tests on roaches of mixed ages were not reliable.

Woodbury and Barnhart *(2346)* later elaborated Woodbury's cockroach method and described a method for rearing bedbugs and using them as test insects by subjecting them to a settling mist.

In 1937 the NAIDM established a research project for evaluating liquid household insecticides against crawling insects. A final report on this project was made by Campbell and his associates *(1265)* in 1941. Procedures for rearing roaches and bedbugs were developed and three methods for testing household sprays against these insects were devised. The rearing procedures and the settling mist method preferred by Campbell are given herewith in detail.

> "*Rearing roaches.*—Stock cultures of the German cockroach may be maintained in any containers from which the roaches cannot escape. To save space we have generally used chests of drawers, the inside walls of which are lined with celluloid having smooth, rounded corners. The celluloid is lightly coated with a heavy mineral oil to make a slippery surface that the roaches cannot climb. Trays, cans, drums, jars or cages may also be used as containers. Any container having smooth walls may be oiled to prevent escape of roaches and left open at the top. However, as mice may invade open containers and reduce production by eating egg capsules, it is better to cover open containers with cheesecloth or wire screen where mice are present. One should also guard against invasion of the cultures by mites or dermestid beetles.
>
> "Shelter, food, and water should be continuously available to the roaches. For shelter loose coils of corrugated paper (codling moth bands) are placed in the containers.
>
> "It is most convenient to use a standard dry food that can be scattered over the bottom of the container. We have always used a mixture having the following composition by

weight: Ground whole wheat, 50; dried skim milk powder, 45; and dry baker's yeast, 5. Before use this mixture is moistened with water until it forms a crumbly mass which is allowed to dry. The composition just mentioned is not essential, but in our experience it has certainly given good results. Others recommend the use of dog biscuits.

"Water is supplied in vials carefully plugged with a loose roll of cellulose wadding. This plug prevents the water from running out when the vial is placed on its side and provides a soft, wet drinking surface. This wet surface remains as long as the water lasts in the vial, because air pressure forces the plug into the vial as the water recedes. Several such vials are placed in large containers. Because the food does not mold and the water supply lasts a long time, the containers need cleaning and replenishing of food and water only at infrequent intervals.

"Newly hatched German cockroaches are obtained for tests or for dated rearing as follows: Roaches of all sizes are removed from the stock culture containers by shaking the shelter bands over a large, oiled crystallizing dish. This dish is then placed in the freezing compartment of a household electric refrigerator until the roaches are immobile (about 8 minutes). Adult females bearing egg capsules are then picked out of the mass of roaches with light forceps and are placed in a screen bottom tray (maternity ward) shown in Fig. XV. The other roaches are returned to the stock containers. The maternity ward, in which the adult females are isolated, is provided with food in lumps and water in vials as in the stock containers, but the females must do without shelter in order that the newly hatched roaches may be automatically segregated and concentrated.

"The maternity ward rests upon four tight coils of codling moth bands (4 inches diam.) which are placed upon the solid, white-painted floor of a larger similar tray. When roaches hatch, they cannot find shelter in the maternity ward and therefore pass through the meshes of the screen bottom and enter the corrugations of the codling moth bands below. Thus at any time practically all the young roaches are to be found in the bands in the lower tray. To obtain young roaches of known age, it is necessary only to suspend the maternity ward above the lower tray (Fig. XV) and remove the bands containing the young roaches. By tapping the bands above

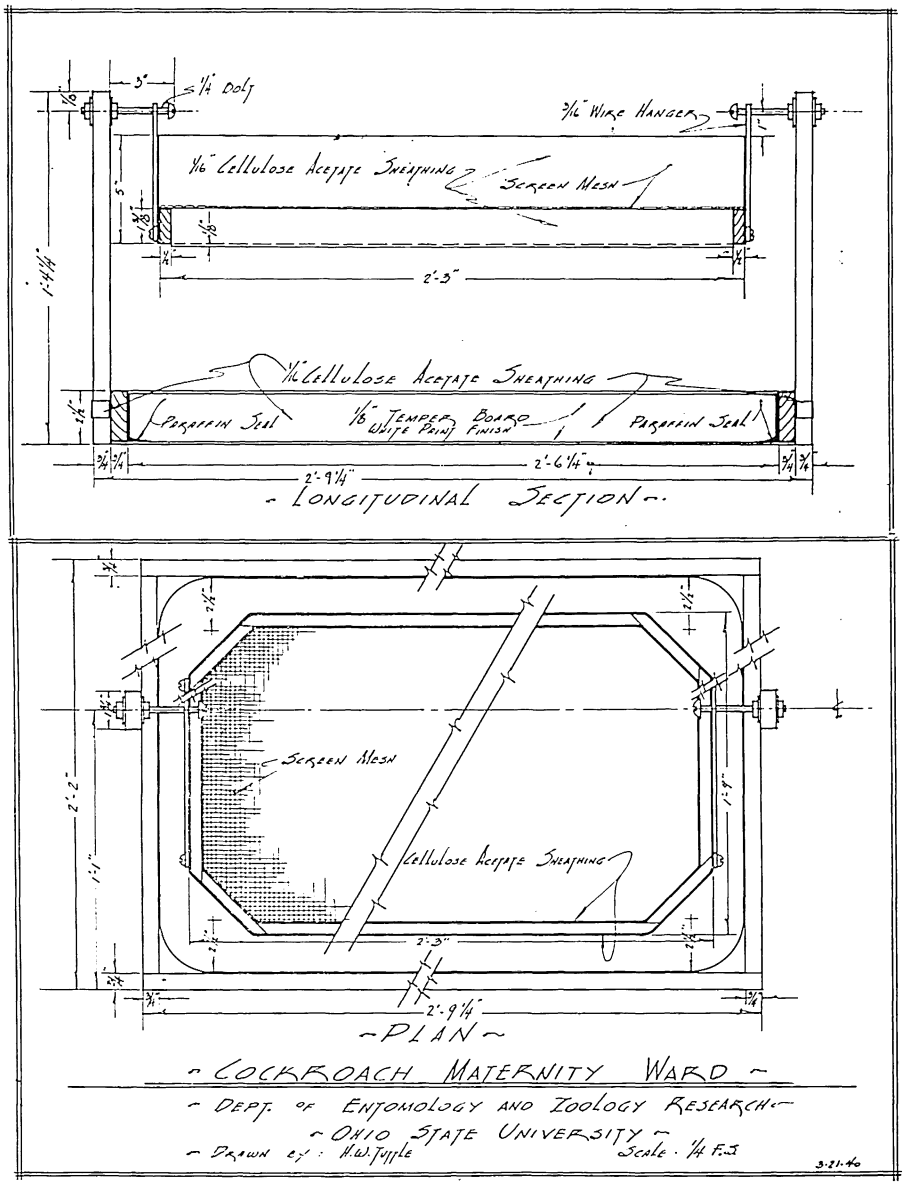


FIG. XV. EQUIPMENT FOR REARING COCKROACHES.
(CAMPBELL, BARNHART AND HUTZEL)

an oiled crystallizing dish, the young roaches can be easily collected and counted out for tests.

"If older roaches of known age are needed for tests, the bands containing young roaches are simply placed in one-

half gallon oiled glass jars containing food and a vial of water, one band per jar. These jars are dated, covered with cheese cloth and set on a shelf where they need no further attention except to replenish water when necessary. There, if desired, the roaches can be allowed to develop to maturity (a period of about 5 to 6 weeks at about 80° F.) or they can be used at an earlier age.

"The age of roaches placed in the jars can be controlled within any desired limits by the frequency of collection from the maternity ward. As we usually collect young roaches every day and make sure that no young roaches are left in the maternity ward after the bands are removed, we can state that the individuals in each culture are not over 24 hours old at the start.

"It is desirable that the roach rearing room contain plenty of shelves and drawers and be held at a controlled temperature of about 80° F. and 60 per cent relative humidity.

"*Rearing bedbugs.*—All rearing operations are done in a small room on a 4 ft. by 6 ft. white painted table which is surrounded by a water-filled moat. The cages containing the bugs are kept upon this table within the moat, which escaped bugs cannot cross.

"Only one type of cage is used; i.e., a 5½-inch half Petri dish covered with 60-mesh white organdy cloth stretched over and cemented into a 6-inch metal embroidery hoop. This cover is held tightly upon the dish by a pair of heavy rubber bands. Pieces of blotting paper 3 inches square are placed in all dishes of bedbugs to serve as shelters, to provide a place for egg laying, and to absorb the liquid that is copiously excreted after feeding.

"All bedbugs, except the first instar nymphs that are to be used in tests, are fed upon the bellies of rabbits once a week. Six large female rabbits are used for this purpose, two each week, so that a given rabbit comes up for a blood transfusion every three weeks. Female rabbits are preferred because they are more amenable to handling than males.

"On a feeding day the first step is to tie a rabbit upon its back on the rack shown in Fig. XVI. Two racks which always stand upon the bedbug table are used at the same time. A rabbit is brought into the bedbug room, held by the scruff of the neck, and seated upon one end of the rack. Looped cords attached to small windlasses are slipped over the hind legs with the free hand and tightened by winding

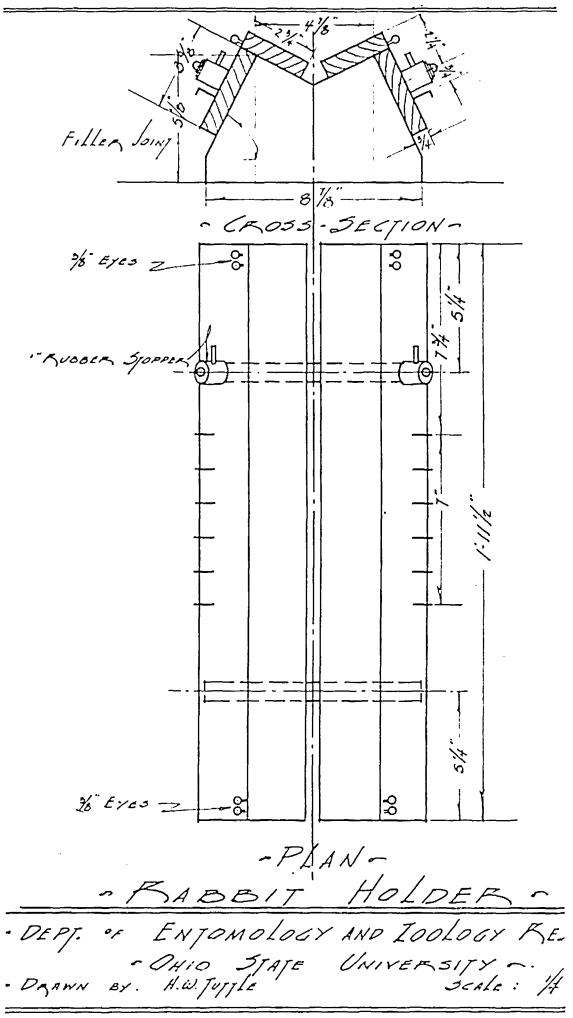


FIG. XVI. RABBIT HOLDER.

them on the windlasses. Then the rabbit is lowered upon its back and cords from the eyelets at the other end of the rack are secured to its front legs. The next step is to clip the hair from the bellies of the rabbits. Barber's hand clippers have been used satisfactorily, but electric clippers do a better and faster job. After having been clipped, the rabbits are ready to receive the bedbugs.

"The bugs are fed without removing them from the cage, which is simply laid on the belly of the rabbit with the organdy cloth in contact with the skin. The cage is held firmly

against the belly by a large rubber band stretched across it and attached to hooks on both sides of the rack. Bedbugs of all instars are quickly attracted to the warm skin of the rabbit and insert their beaks through the meshes of the cloth into the skin, pumping themselves full of blood in a remarkably short time—not more than 15 minutes. On a large rabbit, two cages each containing from 500 adult bugs to 2,000 young bugs can be fed at the same time. As many as 20 cages of bugs have been fed on one rabbit in a two-hour period without adverse effect on the animal.

"The remainder of the rearing procedure may be described as follows, starting with egg laying cages each containing about 500 adult bugs: Just preceding the weekly feeding the contents of all the egg laying cages are dumped into a single large crystallizing dish. The squares of blotting paper on which the eggs have been deposited are arranged in a pile on one side of the dish. These squares are held with forceps one at a time, supported on an inverted 3-inch Petri dish within the same crystallizing dish, and eggs are loosened with a stiff bristled brush and swept into the crystallizing dish. As the papers are cleared of eggs, they are laid in another pile in the dish. Thus when the last paper has been freed of eggs, the bugs have nearly all crawled back onto the papers, which are then distributed among the individual cages. The eggs are then separated from the dead bugs, trash, and the few remaining live bugs by pouring the remaining contents of the large crystallizing dish onto a 16-mesh sieve through which the eggs fall into a clean dish. These eggs, or the nymphs hatching from them, may be used for testing purposes. If not needed, they may be added to the stock culture. Newly hatched bedbugs become adults in about five weeks at 80° F.

"A stock culture of bugs is kept in the regular cages. The only attention these cages of bugs receive is a weekly feeding, an occasional renewal of the blotting paper squares, and transfer to clean cages with thinning out to avoid overcrowding. Since eggs are not separated from these dishes, bugs of all ages are present. The adults are transferred to egg laying cages only when their eggs are needed to produce newly hatched test insects.

"*Settling mist method.*—The spray tower used for the controlled application of a settling mist to the test insects is shown in detail in Fig. XVII. Section B-B gives the best

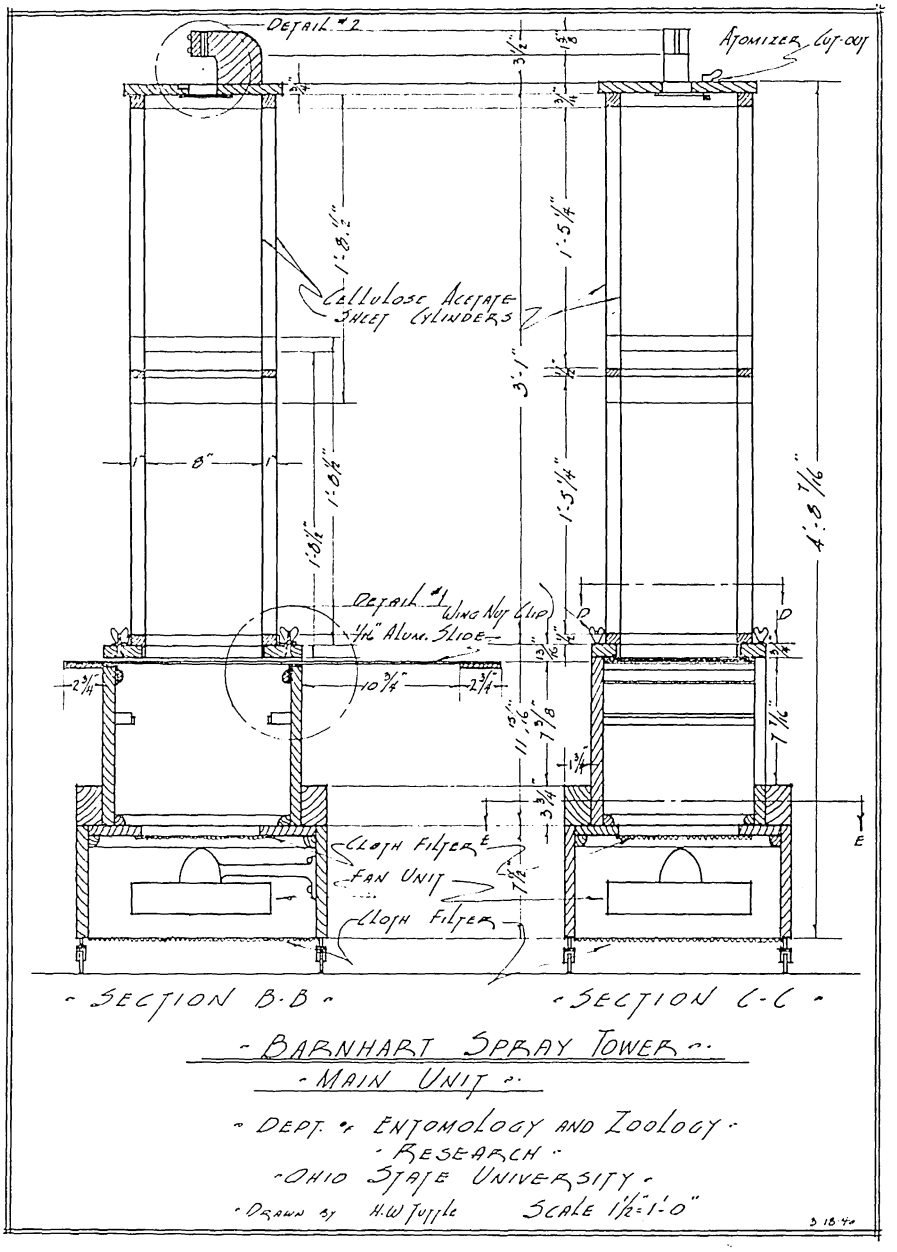


FIG. XVII. BARNHART SETTLING MIST SPRAY TOWER — MAIN UNIT.

general picture of the equipment. The following description of the features of this spray tower is based on this section, beginning at the top:

"The atomizer holder mounted upon the cover of the cylinder carries a DeVilbiss Numograph artists' air brush, type AEN-601 (not shown in Fig. XVII). The ¾" x ⅜" recess next to the central hole in the cover of the cylinder accommodates the cup of the atomizer, permitting the nozzle of the atomizer to be set flush with the surface of the cover. Under the central hole in the cover is a circular aluminum plate or shutter connected with the wing lever shown to the right of the atomizer holder in section C-C. By moving the lever the aluminum plate is made to cover or uncover the central hole.

"The spray cylinder consists of two concentric celluloid (cellulose acetate) cylinders with an annular dead air space between them. This double wall construction is primarily for insulation of the inner 8-inch cylinder. Without such insulation, temperature gradients outside a single walled cylinder may cause convection currents in the mist filling the cylinder and prevent it from settling uniformly. Moreover, the double wall construction makes a more rigid spray cylinder.

"When set up for operation the cover is never removed from the cylinder nor is the cylinder removed from its base. The fit of the cover on the cylinder and of the cylinder on its base is airtight. For convenient transportation, provision is made for removal of the cylinder from its base by removing the wing nuts and lifting the cylinder.

"Immediately below the spray cylinder is the compartment for a drawer holding the cage or dish containing the insects to be treated. Between this compartment and the base of the cylinder is an aluminum slide. When the slide is pushed all the way over to the right, the cylinder is closed and the insects below are protected from mist in the cylinder. When pushed all the way over to the left, the hole in the slide comes under the cylinder, exposing the insects to the settling mist.

"The drawer for holding the insect container is shown in detail in Fig. XVIII. It is much deeper than needed for the small container now being used and was made oversize to accommodate larger containers, if desired. For this reason the drawer has a false bottom, shown in Section A-A.

"The insect container now used is assembled from two parts: (1) a base of window glass cut in octagonal form and covered with a layer of flannel and a circle of filter paper;

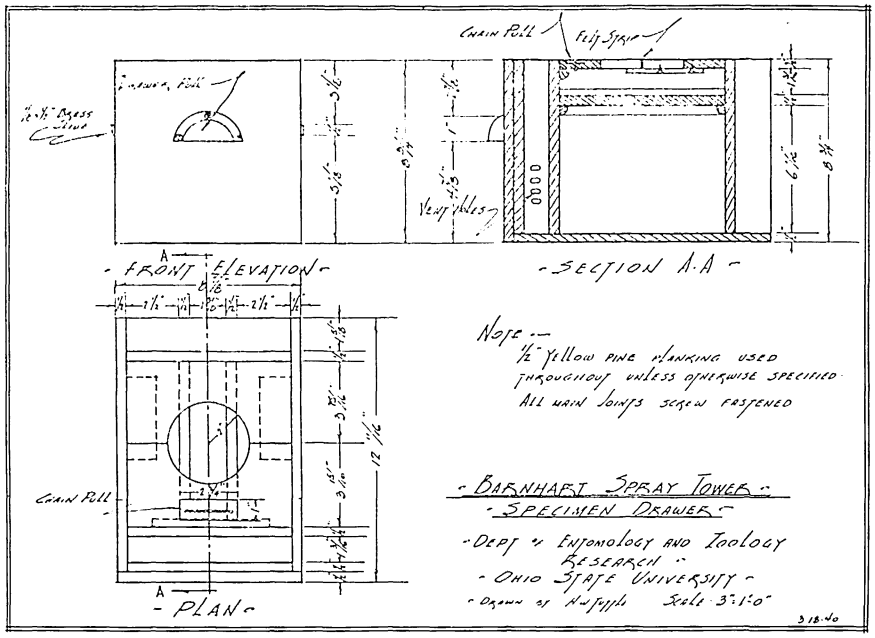


FIG. XVIII. BARNHART SPRAY TOWER — SPECIMEN DRAWER.

(2) a low cylinder consisting of a ring of celluloid, 1 inch wide, inserted in a 4-inch metal embroidery hoop. The embroidery hoop is placed on the circle of filter paper and is held snugly against the paper by special metal clips extending from the hoop around and under the glass plate. The resulting assemblage has the general appearance of a crystallizing dish.

"When the celluloid ring is lightly oiled, young roaches placed in this container cannot escape and it is therefore unnecessary to cover the container with wire screen.

"In order to center the container just described under the spray cylinder, the drawer is provided with a cover having a central hole of the same diameter as the celluloid ring of the container. The cover is cut through the middle and the front half of it is removable. To insert the container the drawer is pulled out, the front half of the drawer cover removed, the container placed on the false bottom and pushed forward against the half circle of the hole in the fixed back half of the cover, and the front half replaced. Looking at the top of the drawer, one then sees the container as a shallow well in the cover of the drawer. The top of the con-

tainer is flush with the surface of the cover. The aluminum slide moves directly upon and across this surface.

"Below the drawer compartment is a ventilating and mist filtering compartment, covered on top and bottom with a cheesecloth filter and containing a household type fan for ventilation. When the spray cylinder is opened, the drawer pulled out, and the fan turned on, mist in the cylinder is quickly pulled into the filter compartment where it is retained. One can therefore rear insects and make tests in the same room, if desired. This ventilation also tends to evaporate oil on the inner walls of the spray cylinder, preventing accumulation and run off of oil. Because of ventilation and evaporation of oil, and because no oil but a fresh settling mist ever reaches the insect container, it is not necessary to wipe any part of this apparatus no matter how frequently it is used.

"*Roach procedure.*—The following is a description of the procedure used for evaluating a series of, let us say, ten samples of liquid insecticides vs. the O. T. I. against first instar German cockroaches.

"Newly hatched roaches are collected in a crystallizing dish as described above. They are brought to the testing room and distributed in lots of approximately 100 in each of 11 insect containers described above. Transfer of roaches from the crystallizing dish to the small containers is easily accomplished by inserting a piece of paper into the dish and allowing roaches to crawl upon it. They are counted as they are brushed from the paper into the container. Each sample of insects is therefore a random sample from a large population. The containers with roaches are set out on the table to be used in random order.

"The reducing valve of the air line is set so that the gauge will show 20 pounds pressure when the atomizer is spraying. The performance of the atomizer is then checked by attaching a 1 cc. graduated pipette horizontally to the liquid inlet of the atomizer. The open end of the pipette is bent down at a right angle so that it can be dipped under the surface of the O. T. I. in a small beaker. The valve of the atomizer is opened and the O. T. I. is drawn into the pipette, filling it. Then with stop watch in hand the contents of the pipette are sprayed into the cylinder and the delivery timed between the 0 and 1 cc. marks on the pipette. The atomizer is set so as to deliver 1 cc. in 5 seconds when operating properly at

20 pounds pressure. Performance of the atomizer is checked frequently in this way.

"After ventilation of the spray cylinder, the first roach container is placed in the drawer, the drawer closed, the slide closed, and the shutter under the top hole is left open. From 3 to 4 cc. of the sample to be tested is poured into the cup of the atomizer and the cup is attached to the liquid inlet. One is then ready to make the application. With stop watch in hand, the liquid is sprayed into the cylinder for five seconds and then the shutter is swung under the top hole closing the cylinder. A pause of 15 seconds is allowed for the air currents in the mist-filled cylinder to subside. Then the slide is pushed over, exposing the insects below to the settling mist. Their behavior can be easily observed through the cylinder. At the end of seven seconds exposure the slide is closed again, the drawer pulled out, the roach container removed, the cylinder opened again top and bottom, and the fan turned on to ventilate the cylinder. The liquid remaining in the atomizer cup is poured out, the cup rinsed in carbon tetrachloride, dried, and filled with the next liquid to be applied. One is then ready to go ahead with the next application as just described. The time required to carry out all steps in a single application is about two minutes. In this case 11 applications would be made one after the other.

"The O. T. I. and any other reasonably good insecticide containing a paralytic toxicant will paralyze the roaches. After becoming paralyzed the roaches in a test container are transferred to a circle of cheescloth held taut in a 7-inch embroidery hoop laid cloth down upon a table. The transfer is easily accomplished by taking the test container apart, lifting over the cheesecloth the circle of filter paper bearing the roaches, and tapping them from the paper to the cloth, distributing them well over the surface. The roaches on the cheesecloth are then covered with a 6-inch half Petri dish and are counted, the number being recorded on the dish. They are then set aside without food or water for about 24 hours in an incubator at 77° F. and 40 per cent relative humidity. At the end of this time mortality counts are made without disturbing the roaches. Those that walk upon the cheesecloth are called alive; those that do not walk, whether dead or not, are called dead, because our observations have shown that roaches incapable of locomotion will soon die. Moribund and

dead roaches are usually found upon their backs. The cheesecloth circle is halved with a pencil line to facilitate counting.

"The number of tests that can be run in a day is usually limited only by the insect supply. It is important to run in succession all samples in a series to be compared with the O. T. I. and with each other and to replicate these tests on the same or on subsequent days. In our tests an effort was made to test each sample at least 10 times and to use about 1,000 roaches for each sample. Results with the O. T. I. vary considerably from day to day, but as pointed out in the paper by Woodbury and Barnhart, we believe that our variation is not greater than that generally encountered in the Peet-Grady method and we know that we have gotten consistent average results with respect to concentration and composition of samples that were 'unknown' at the time of testing.

"The time figures given above for spraying, pause, and exposure are the combination that should usually give about a 50 per cent kill with the O. T. I. against first instar cockroaches. In terms of deposit of O. T. I. per sq. cm. on the bottom of the roach container, this combination is equivalent to 0.08 mg. of O. T. I. per sq. cm. as determined by reweighing microscope slides exposed to the settling mist. (To prevent error from evaporation of oil, the slides are stacked immediately after exposure with the oil deposit sandwiched between slides and are then weighed.) Very smooth curves have been obtained showing the relation between period of exposure and deposit when the time of spraying and pause are held constant. When it is desired to raise or lower the dose of O. T. I., it is done by changing period of exposure only.

"It should be borne in mind that the deposit obtained with a given concentration of time of spraying, pause, and exposure will depend on the viscosity of the oil. Most oil bases used in liquid household insecticides are of about the same viscosity and will spray out of the test pipette in the same time as the O. T. I. To be safe it would be well to check the spraying time of 1 cc. of all samples. A sample differing markedly in viscosity from the O. T. I. should be given a different exposure time that will yield the same deposit as that of the O. T. I., or the atomizer may be adjusted to deliver the same volume of the more viscous liquid in the same time.

"*Bedbug procedure.*—Newly or recently hatched nymphs are removed from the cage in which they hatched in the same manner as were the newly hatched roaches; i.e., by permitting them to crawl up upon paper. The bedbugs are counted as they are brushed from the paper into the test container. This container is a 1⅝" (inside diameter) Petri dish with the bottom snugly covered with a circle of 4.25 cm. filter paper. As the bugs tend to cling to this filter paper, no oil need be used on the walls of the dish to prevent them from climbing out. Not more than 50 bugs are placed in each dish, the number per dish depending on the number of bugs available and the number of samples in the series to be tested against the O. T. I. All dishes for a series of tests are made ready before the applications of the insecticides are begun. Applications are made in the spray tower by the procedure previously described, one or two of the small bedbug containers being placed in a cockroach container in the drawer of the spray tower. As first instar bedbugs are more susceptible to the O. T. I. than first instar German cockroaches, a dose of only .04 mg. per sq. cm. is needed. This was obtained by spraying five seconds, pausing 40 seconds, and exposing five seconds. After treatment the glass covers are replaced on the dishes, which are placed in the same incubator where the treated cockroaches are kept overnight. It should be noted that the bedbugs are kept for observation in the dish in which the mist settled on them. This is done purposely to add the effect of additional contact with the treated filter paper to that of the direct effect of mist on the bugs.

"Mortality counts are made the next day in a very simple manner. A dish is inverted over a piece of paper and tapped. The dead bugs fall out upon the paper while the live bugs remain clinging to the filter paper in the bottom of the dish. As the total number of bugs in the dish is already known, either the dead or the living may be counted.

"The rating of an unknown is represented by the average kill produced by the unknown minus the average kill caused by the O. T. I. In our roach and bedbug tests, the number of replications is greater and the total number of insects smaller (about 1,000 for roaches and 500 for bedbugs) than in the large group Peet-Grady method."

The two other methods suggested by Campbell were the "lard can" method of Barnhart and the "chamber" method of

Hutzel. The former method is designed to take into consideration the drive-out of roaches from a standard place of concealment as well as the effect of direct spray, settling mist and spray residue. The latter employs the Peet-Grady equipment but the roaches are confined in dishes placed at fixed intervals on the floor of the chamber.

McGovran and Fales *(1869)* have described a method designed to measure the effect of the spray that hits and adheres to the roaches and to reduce the fumigating effect, the effect of residue on sprayed surfaces which the insects might touch and the effect of spray droplets floating in the air.

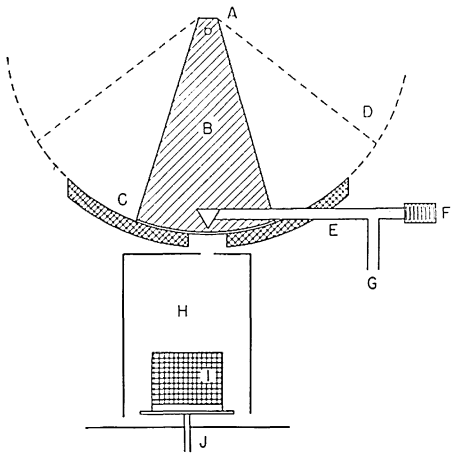


FIG. XIX. APPARATUS FOR TESTING ROACH SPRAYS (MCGOVRAN AND FALES).

"The apparatus (Fig. XIX) produces a direct spray from a nasal atomizer (E) that strikes a group of roaches confined in a circular metal pen (I) 2½ inches deep and 3½ inches in diameter (tin cups with the handles removed). The inside surface of the wall of the pen was covered with a thin film of heavy medicinal-grade petroleum oil to prevent the roaches from escaping or clinging to the sides during treatment. The bottom of the pen was covered with filter paper. To reduce the variation due to uncontrolled air movement, the pen was covered with a glass cylinder (H) 9 inches high and 6 inches in diameter, having a solid top except for an opening ¾ inch in diameter through which the spray was directed. Preliminary tests had shown that the pattern of the spray deposit directly below the atomizer was a heavy circle with lighter deposits inside and outside it. Consequently, this

opening was slightly to one side of the center and the pen was rotated to obtain a more uniform deposit. The bottom edge of this cylinder was raised ¼ inch above the floor of the spray chamber to allow the stream of air and spray that entered the cylinder to escape without returning through the hole in the top. Between the nozzle of the atomizer and the opening in the top of the cylinder was mounted a partition (C), which swung back and forth like a pendulum. This partition was 2 inches wide, 14 inches long, and crescent-shaped. In the center was an opening 1 inch wide and 2 inches long. When the partition was swung back and forth, this opening passed below the atomizer nozzle, allowing spray to pass into the cylinder and onto the insects. The length of the pendulum was 10 inches. Pieces of paper towel and blotter held on the upper side of the partition at each side of the opening absorbed the spray that struck the partition and prevented it from dropping into the cylinder.

"Two pounds of air pressure was used to operate the atomizer. This low pressure gave a wet spray of relatively large drops and produced very little fog. The air pressure was measured with a mercury column and controlled by a reducing valve and a Bunsen valve.

"The pendulum was pushed to one side until the atomizer nozzle was directed at the partition about 1 inch from one end. The air pressure was turned on and the atomizer began spraying on the partition. The partition was held in this position for a second or two until the mercury gage indicated that the correct air pressure had been established. The pendulum was then released and allowed to swing uniformly the number of times required to give the desired deposit. This period ranged from 5 to 25 seconds. The pendulum was then stopped in the same position as at the start and the spray turned off. During the spraying the pen of roaches was rotated at about 40 r.p.m. The glass cylinder around the pen of roaches was removed 30 seconds after the spraying started, and 90 seconds later the roaches were transferred to a 6-inch Petri dish with a screen cover and provided with food and water. Knockdown and mortality counts were made in these Petri-dish recovery cages.

"Observations with oil containing a dye indicated that three or four swings of the pendulum past the atomizer were needed to give a uniform distribution of spray over the floor

of the pen holding the roaches. Weighings of glass slides covered with filter paper the same size as the slide showed that the average amount of spray delivered by each passage of the opening below the atomizer was 0.104 mg. per square centimeter. The maximum average deposit that was measured was 0.109 mg. and the minimum 0.098 mg. per square centimeter. The averages were obtained from dosages deposited by 24 or 70 swings of the pendulum. To reduce evaporation, a glass slide was laid over the oil-sprayed filter paper while it was being weighed."

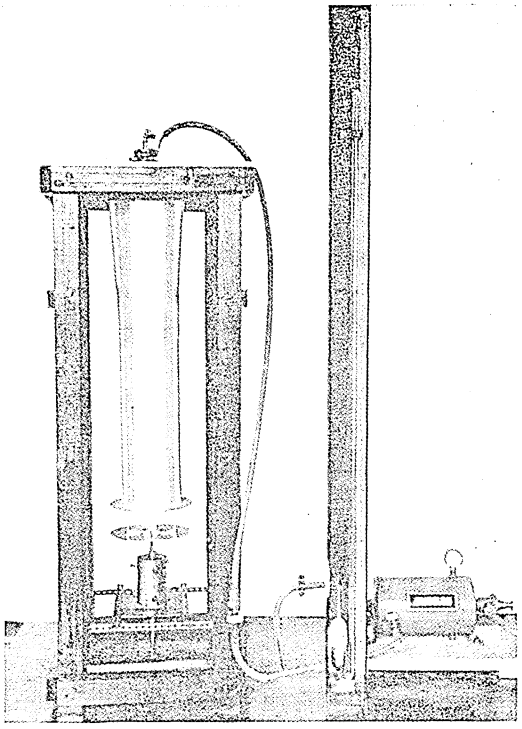
McGovran, Fales and Piquett *(1872)* compared the effect of pyrethrum-oil spray containing 5 mg. pyrethrins per cc. on *Blatella germanica* and *Periplaneta americana,* using the pendulum method. The former species was knocked down more rapidly than the latter, but *B. germanica* was more resistant to the lethal action of the spray than *P. americana.* Twice the deposit applied to *P. americana* caused only slightly higher mortalities when applied to *B. germanica.*

Parker and Campbell *(1967)* applied pyrethrum-kerosene sprays to adult female German cockroaches by the settling mist method. "Under this treatment the majority of the insects dropped their oothecae prematurely, no doubt because of muscular contractions induced by the pyrethrins. By comparing the per cent hatch of oothecae so detached with that of oothecae manually removed from untreated females, it was shown that the pyrethrins exert some insecticidal action on the oothecae, but that the per cent mortality of oothecae was not so great as that of the females to which they had been attached. It was concluded that when a high kill of females is obtained by pyrethrum sprays, the females are more susceptible to this insecticide than are their oothecae. With the possible exception of the large nymphs, the ootheca is the most resistant stage of the German cockroach to pyrethrum sprays."

Potter's Method

Potter *(2006)* lists the following attributes desirable in laboratory apparatus for testing insecticide sprays:

1. It should be possible to deposit a given quantity repeatedly on the sprayed area.
2. The deposit should be evenly distributed over the area sprayed.
3. The sprayed area should be large enough to accommodate fairly large and active insects.



POTTER'S EQUIPMENT FOR PRECISION SPRAY-TESTING.
(REPRODUCED FROM ANNALS OF APPLIED BIOLOGY BY PERMISSION)

4. It should be possible to vary the quantity of spray deposited within wide limits.
5. The apparatus should be usable with a wide variety of sprays.

Potter concluded that the methods of Tattersfield (page 88), Campbell and Sullivan (page 112, 470), O'Kane (page 104) and Hartzell and Wilcoxon (page 90) do not meet all of these requirements.

Potter's apparatus consists of a reservoir and a specially designed atomizing nozzle mounted on a small circular plate carried on three bars, each at an angle of 120° with the others. The end of each bar rests on the top of the metal tower through which the spray is directed on to the spray plate or dish. The spray plate or dish is carried on a table formed by the bottom plate which has a universal adjustment, the whole being mounted on a wooden stand.

The atomizing nozzle (Fig. XX) consists essentially of two parts:

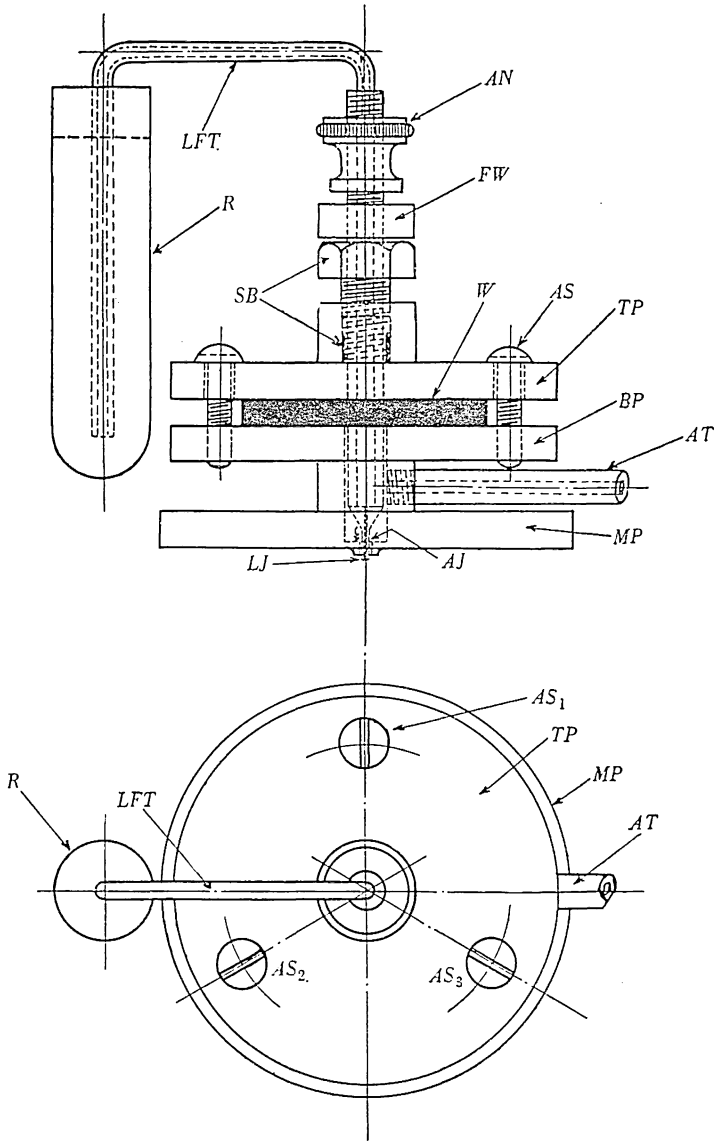


FIG. XX. ATOMIZING NOZZLE, POTTER'S APPARATUS.
(REPRODUCED FROM ANNALS OF APPLIED BIOLOGY BY PERMISSION)

"(a) a top circular brass plate (TP) 2 in. diam. and ¼ in. thick, on which is mounted a liquid jet (LJ) with an adjusting nut (AN) working against a friction washer (FW) for moving the jet up and down, the liquid feed tube (LFT) and the liquid reservoir (R); (b) a bottom circular brass plate (BP) 2 in.

diam. and 3/16 in. thick joined by a narrow brass neck to a mounting plate (MP) in which is bored the air jet (AJ). The tube to the compressed air supply (AT) is led into the brass neck.

"When assembled, the top plate is screwed to the bottom plate by means of three adjusting screws (AS) and there is a compressible rubber washer (W) in between. The two plates, screws and washer together form a universal joint. The liquid feed tube must form an air tight joint in the top plate, but at the same time be capable of movement up and down; this is done by having a stuffing box (SB), through which the tube passes, mounted on the top plate.

"In order to adjust the nozzle for working, the liquid jet is first roughly centralized by means of the three adjusting screws (AS); it is then pushed through the liquid jet as far as it will go and adjusted to the required depth by tightening the adjusting nut (AN); finally, it is accurately centred by means of the adjusting screws (AS). The following are the essential dimensions of the various parts: air-jet orifice, 0.0635 in.; liquid jet orifice, 0.0135 in.; internal diameter of compressed air tube, 1/16 in.; thickness of rubber washer, 5/32 in.; internal diameter of liquid feed, 1/16 in.; capacity of reservoir, 10 cc.

"This nozzle worked well in practice; the accurate centring of the liquid jet in the air jet helps to obtain a symmetrical and even distribution and the absence of any form of needle valve in the liquid feed reduces the risk of blocking. The normal function of the needle valve is to alter the degree of atomization, but this purpose can be achieved in the nozzle described by altering the air pressure and by adjusting the position of the tip of the liquid jet relative to the orifice of the air jet.

"The atomizing nozzle is mounted on a plate of the same diameter as the mounting plate on the nozzle, i.e., 2⅛ in. This plate carries three bars extending out radially, each at an angle of 120° with the other. The bars are 3⅜ in. long measured from the circumference of the plate, ½ in. wide and 3/16 in. thick. 2 in. from the end they carry adjusting screws which work against the inside of the spray tower, by means of which the nozzle is centred in the tower. 5/16 in. from the end are adjusting screws which work against the top of the tower and by means of these, the cone of spray can finally be adjusted to spray down the centre of the tower.

"The spray tower consists of a metal tube which is grounded. In the apparatus described this is of galvanized iron 1/32 in.

thick, but it would be preferable if constructed in some form of thicker rustless material. The tube is 27 in. long, both the top and the bottom having a 1 in. flange. The internal diameter of the top is 6¼ in. tapering to 4¾ in. in 12 in., the rest of the tower being of uniform 4¾ in. bore. The flange at the top of the tower is fitted with three adjusting screws working against the top of the stand, by means of which the tower is levelled.

"In order that the correct deposition and distribution shall be maintained, the bottom of the tower must be covered by a plate adjusted so that it is centrally placed and at a given distance from it. Since this gap is too narrow to allow the insects to be placed in position at the bottom of the tower, it must be possible to lower the plate, put the insects on it, and raise it again to the correct position quickly and easily. The following arrangement is used (Fig. XXI): The spray plate (T) on which the insects are placed, either in a dish or otherwise, is a metal circle of 6¾ in. diam., thus fitting exactly over the bottom of the spray tower. The spray plate carries four adjustable angle-brackets (AB) to hold the spray dish, and adjustments are present for centring the dish under the bottom of the tower. From the centre of the spray plate, a pillar (P) of ¼ in. brass rod runs through a hole bored in a solid metal cylinder (⅝ in. diam.) which is itself fitted in a hollow tube (Tu_1) of the same diameter. The pillar can be adjusted to the requisite height in the solid metal tube then fixed with the Grub screw (g); by this means the gap between the bottom of the tower and the plate can be accurately adjusted. The tube (Tu_1) is fitted into a second tube (Tu_2) ¾ in. ext. diam. in which it is a sliding fit. The tube Tu_2 fits inside a wide tube or cylinder (Tu_3) 2½ in. ext. diam. and 4 in. high and is held in position by six centring screws (S_1-S_6) arranged to project radially into Tu_3, three screws at the top and three screws at the bottom. The tube Tu_2 is prevented from falling through tube Tu_3 by the flange (FL). The tube Tu_3 is mounted on a base plate (BP) which has a ½ in. hole, to allow the passage of tube Tu_1 and Tu_2.

"The base plate is fixed to struts on the stand holding the tower so that the spray table is as nearly as possible directly underneath the tower. The final centring of the spray plate beneath the tower is done by means of the screws S_1-S_6. In spraying practice, the tube Tu_1 is allowed to slide down to its full extent in tube Tu_2, leaving a wide gap between the bottom of the tower and the plate. The insects are put on the plate and the latter then raised by pushing up the tube Tu_1 by means of

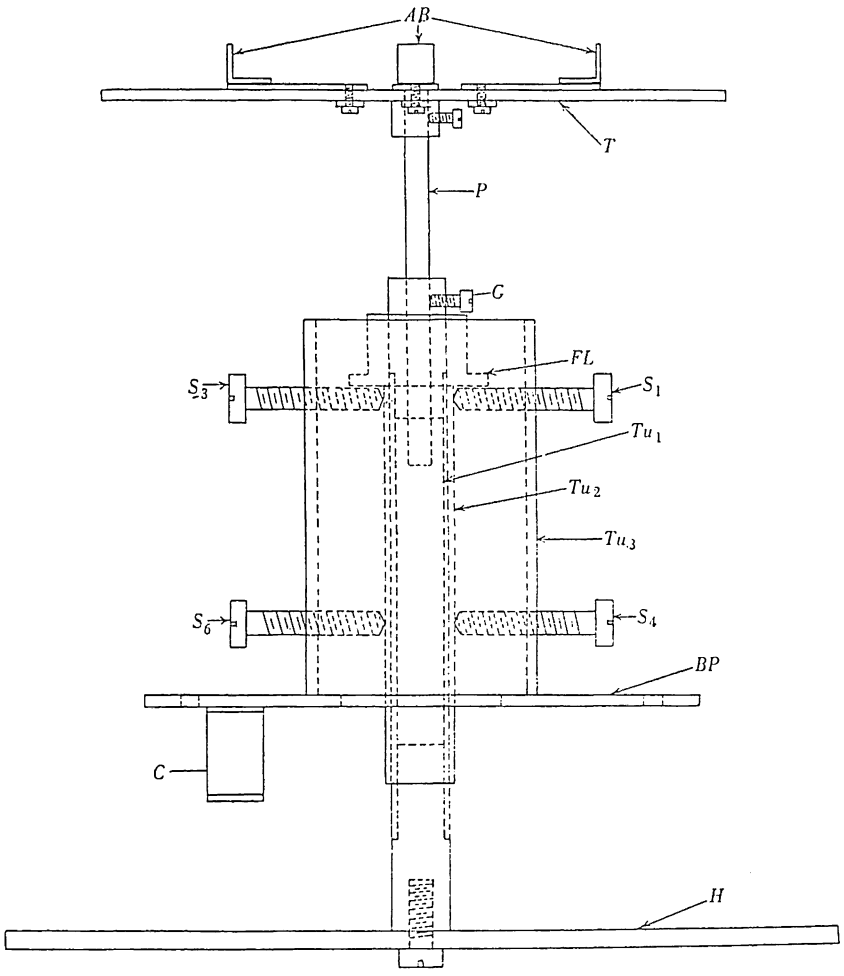


FIG. XXI. ADJUSTABLE SPRAY PLATE, POTTER'S APPARATUS.
(REPRODUCED FROM ANNALS OF APPLIED BIOLOGY BY PERMISSION)

the handle H, which, when it is raised to the full extent is engaged by a turning movement in the rectangular catch C. Thus with any given adjustment the plate is locked in a position with the gap always the same between the bottom of the tower and the spray plate. With a gap of ½ in. with the table up, there is a gap of 2½ in. with it down, i.e., there is a 2 in. movement. Any necessary levelling of the spray table can also be done with the screws S_1-S_6.

"The whole apparatus is mounted in a rectangular wooden stand 41 in. high with sides 15½ in. long. It consists of four

wooden uprights of 2 in. cross-section. At both top and bottom there are four cross-pieces of the same material; on the top of the stand is a platform of five-ply with a hole in the centre through which the tower fits; the adjusting nuts through the top flange of the tower rest on brass pieces on this platform. The struts to take the base plate of the spray table mounting are fitted to the four uprights.

"Compressed air is supplied by an air compressor and is led to the nozzle through a system of filters and pressure regulators and stabilizers. Pressure gauges are inserted in order to check the pressure. Elaborate arrangements for stabilizing the pressure are necessary when low pressures of the order of 20 cm. of mercury and less are used; also the slightest particle of dust or other matter is liable to stick in one side of the nozzle and interfere with the distribution and replication of the spray deposit."

To use the apparatus, the tower is first levelled by means of the three adjusting screws in the top flange so that the plummet of a plumb-line suspended from the center of the top falls in the center of the bottom of the tower. The nozzle is then centered in the top of the tower and roughly levelled. The spray plate is centered over the bottom of the tower and levelled, so that the gap between it and the bottom flange is equal all the way round, this gap being then adjusted to the required distance. For final exact adjustment of the nozzle, it is necessary to do distribution tests with cover-slips, but the rough adjustment normally gives a fairly good distribution.

To test the apparatus for replication of deposit, a glass Petri dish 8.6 cm. diam. with walls 1.5 cm. high and covered with a ground plate lid is weighed and the Petri dish then placed on the spray table. 5 cc. of the liquid to be tested is placed in the reservoir of the atomizer and sprayed through, the dish wiped, the lid put on and the whole reweighed. The deposit on the dish is thus obtained.

To test for distribution of deposit, a glass plate of 9 cm. diam. is covered by a circle of paper which is marked out with a circle of 2.3 cm. diam. at the center and three other similar circles equally distributed round the circumference. They are numbered 1, 2, 3, 4. Four small brass washers, each approximately 1 cm. diam., are placed at the center of each of the circles. Four cover-slips each of 2.3 cm. diam. are placed in numbered weighing bottles and weighed, and put on top of the washers in the circles on the glass plate. The glass plate is then placed on the spray platform and 5 cc. of liquid sprayed on to it; it is

then removed to a table, the cover-slips carefully lifted off with curved forceps, placed in their appropriate weighing bottles and reweighed. The plate is always put on the spray table so that no. 3 cover-slip on the circumference is toward the front of the apparatus. The washers serve the purpose of raising the cover-slips so that none of the deposit is drawn off the surface by the paper and they also facilitate lifting with the forceps. After each spraying the bottles and cover-slips are dried and cleaned carefully. The technique is laborious and subject to error.

In Potter's apparatus, turbulence in the spray stream is promoted by using a long, narrow spray tower. Equalization of air pressure over the sprayed surface is made possible by the adjustable gap at the bottom of the tower. The spray tower is grounded to prevent the accumulation of a static charge.

The problem of evaluating pyrethrum extracts in non-volatile mineral oil has been studied by Tattersfield and Potter *(2209)*. Such extracts are used to control insects attacking foods in warehouses and a method was devised which measured the effect of the insecticide as a direct spray and also as a protective film. The adult flour beetle, *Tribolium castaneum,* was found satisfactory as test insect and detailed instructions are given for rearing this beetle. The apparatus used was Potter's, previously described.

For the direct spray test the beetles were sprayed in Petri dishes as described by Potter. The film technique involved spraying the insecticide on a circle of hardened filter paper, Whatman 544, on which the beetles were then confined for five days. The treated insects were classified as unaffected, slightly affected, badly affected, moribund and dead. The results were statistically analyzed. The biological evaluation agreed well with the results of chemical analysis.

Other Methods

Lowman and Sullivan *(1817)* employed mosquito larvae as test insects for determining toxicity of samples of pyrethrum flowers. Alcoholic extracts of the flowers were prepared and the toxicity was determined by the method of Campbell, Sullivan and Smith *(1269)*. An alcoholic extract of known pyrethrin content was used to measure the variation in resistance of the larvae from day to day. The pyrethrin content of the flowers was determined by Seil's method. The results of the tests on mosquito larvae were sufficiently accurate to indicate samples of high or

low pyrethrin content and the method was valuable in testing large numbers of samples in connection with the development of strains of flowers of high pyrethrin content.

Rosen and Thompson *(2076)* suggest the use of frogs as test animals for determining the toxicity of pyrethrum flowers. A dilute alcoholic extract of pyrethrum is injected in the ventral lymph sac and mortality is determined after 24 hours. A second method, based on the action of pyrethrins on isolated rabbit intestine, is also described. The results obtained by the two methods were not always in good agreement with chemical assays by Seil's method.

Parkin *(1970)* applies oil pyrethrum spray to filter paper under controlled conditions and confines flour beetles, *Tribolium castaneum,* on the sprayed paper, to test pyrethrum sprays.

Franzen *(1462)* used silkworms for testing the effectiveness of pyrethrum dusts.

Belleuvre *(1203)* suggests the use of the crawfish heart and the dorsal vessel of the wax moth, *Galleria mellonella,* for the biological testing of pyrethrum.

Aczel *(998)* devised a method for the biological evaluation of pyrethrum-talc dusts, using the corn weevil, *Calandra granaria,* as test insect.

Robinson *(2066)* employed the tick, *Ornithodorus moubata,* for determining the toxicity of pyrethrum in white oil. The insecticide was either applied to the tick or to a surface over which the tick crawled.

Doehlert *(1372)* proposed the use of the blunt-nosed leafhopper as a test insect for evaluating pyrethrum dusts used on cranberries.

Fifty-two papers on laboratory procedures for the study of chemical control of insects have been collected in one volume by the American Association for the Advancement of Science *(1107)*. This publication is valuable to anyone interested in insect control or the testing of insecticides.

CHAPTER XXI

CORRELATION OF CHEMICAL ASSAYS AND BIOLOGICAL TESTS

The belief that a determination of pyrethrin I is sufficient to indicate the insecticidal value of a given sample of pyrethrum, regardless of the pyrethrin II content, was first expressed by Tattersfield *(880)*, in 1929. This view was supported by Wilcoxon and Hartzell *(968)*, Richardson *(711)* and others; it was opposed by Gnadinger and Corl, who held that the total pyrethrin content is a better index of toxicity. The study of the relative toxicity of the two pyrethrins has been made easier by the development of improved methods of isolating them in fairly pure form. Improvements in chemical and biological assay methods have permitted more accurate examination of the relation between pyrethrin content and toxicity to insects.

Ripert and Gaudin *(748)* isolated pyrethrin I, said to be 96 per cent pure, and pyrethrin II, said to be 98.9 per cent pure, and determined the relative toxicity of the pyrethrins to frogs by intraperitoneal injection. Pyrethrin II was slightly more toxic than pyrethrin I and a mixture of equal parts of pyrethrin I and pyrethrin II was more toxic than either pyrethrin I or pyrethrin II alone. The possibility of synergistic action between the pyrethrins has not, in general, been considered.

Hartzell and Wilcoxon repeated their earlier work on the relative toxicity of pyrethrins I and II, using extracts purified by a modification of the LaForge and Haller method (page 37). Extracts high in pyrethrin I were compared with extracts high in pyrethrin II; the ratio of pyrethrin I to pyrethrin II varied from 4.0 to 0.047. When acetone extracts high in pyrethrin I were compared with similar extracts high in pyrethrin II, on *Aphis rumicis,* diluting with water, pyrethrin I was considerably more toxic than pyrethrin II. This confirmed the conclusions of Tattersfield *(880)* and the earlier work of Wilcoxon and Hartzell *(968)*. When a miscible oil such as Penetrol was used as a solvent, instead of acetone, the difference in toxicity of pyrethrins I and II tended to disappear. When kerosene extracts high in pyrethrin I were compared with kerosene extracts high in pyrethrin II, on houseflies, by the Peet-Grady and Nelson methods, no significant difference was found in the toxicity of the two

pyrethrins. This confirmed the conclusions of Gnadinger and Corl (page 123).

Hartzell and Wilcoxon concluded that the physical condition of the pyrethrins at the time of application is a determining factor in the relative toxicity. When aqueous sprays are used, made by diluting acetone solutions of the pyrethrins with water, the pyrethrins are thrown out of solution, forming emulsions of varying degrees of stability. In kerosene extracts the pyrethrins are, of course, in solution. "The relative toxicity of pyrethrins I and II depends almost entirely upon the method of application." These results obtained by Hartzell and Wilcoxon reconcile the divergent views hitherto held by different investigators regarding the relative toxicity of the two pyrethrins. They also indicate that the determination of the total pyrethrin content is an index of the toxicity of a pyrethrum product, at least in the case of pyrethrum-oil sprays, and that determination of pyrethrins I and II separately is not essential in oil sprays.

Lowman and Sullivan *(1817)* assayed 112 samples of flowers from individual plants by the Seil method. The highest ratio of pyrethrin I to pyrethrin II was 1:0.30 and the lowest ratio was 1:2.02 (see also page 118). Alcoholic extracts of these flowers were tested on mosquito larvae. Pyrethrin I was more toxic to the larvae than pyrethrin II. "The correlation between the toxicity and the percentage of pyrethrin I and total pyrethrins was about equal except in samples with unusually high or low proportions of pyrethrin I."

Woodbury *(2344)* found that the percentage of roaches killed in 24 hours, in his test method, is not proportional to pyrethrin content, but the percentage of dead plus moribund roaches is proportional to pyrethrin content.

Sullivan and his associates *(2196)* compared the toxicity of pyrethrum sprays high in pyrethrin I content and low in pyrethrin II with similar sprays high in pyrethrin II content and low in pyrethrin I. The tests were made on houseflies by the Campbell-Sullivan method. Pyrethrin I was about twice as toxic as pyrethrin II but the latter was far superior to pyrethrin I in knockdown effect.

Eagleson *(1393)* concluded that "the chemical assay of toxicants and determination of specific gravity, distillation range and unsulfonatable residue of the carrier are not an adequate index to the toxicity of livestock sprays."

Haller and Sullivan *(1582)* showed that both pyrethrin I and pyrethrin II have good knockdown effect against houseflies when

tested by the Campbell-Sullivan method. Pyrethrin sprays in which pyrethrin I predominated were more toxic to flies than those in which pyrethrin II predominated. Hydrogenation destroyed the knockdown effect of both pyrethrins and lowered the toxicity of pyrethrin I; the toxicity of pyrethrin II was not materially changed by hydrogenation. Green and his associates *(1560)*, however, noted a marked reduction in both knockdown and kill of pyrethrin II after hydrogenation.

Green, Pohl, Tresadern and West *(1560)* prepared nearly pure pyrethrin II and a concentrate containing 73 per cent pyrethrin I. These were compared for knockdown and mortality to flies by the Peet-Grady method. Pyrethrin II was comparable in toxicity to pyrethrin I and superior to pyrethrin I in knockdown effect. The results of chemical analyses by the Seil and Wilcoxon-Holaday methods were not in conformity with the biological activity of commercial kerosene extracts of pyrethrum.

Tattersfield and Potter *(2209)* prepared three oil-pyrethrum extracts to be used as standards in testing insecticides used for control of insects in warehouses. The effects of the method of preparation on toxicity were investigated. Of the constituents examined, other than oil, only the pyrethrins had a measurable effect on toxicity. The biological tests were made on flour beetles by Potter's method and agreed well with the results of chemical analyses, but correlation was closer with the percentage of total pyrethrins than of pyrethrin I. Tattersfield states "the determination of the pyrethrin I content alone is not a satisfactory index of the toxicity of pyrethrum-in-oil preparations."

Martin *(1850)* investigated the relative toxicities of the pyrethrins in heavy mineral oil and in aqueous sprays. Solutions relatively high in pyrethrin I and low in pyrethrin II were compared with solutions high in pyrethrin II and low in pyrethrin I. In the heavy mineral oil the toxicity of pyrethrin II to the flour beetle, *Tribolium castaneum,* closely approached that of pyrethrin I. In aqueous sprays, the toxicity of pyrethrin II was many times less than that of pyrethrin I. Attempts to detect synergistic or antagonistic action between the pyrethrins were not successful.

McGovran, Mayer and Acree *(1875)* applied measured volumes of kerosene solutions of pyrethrins to adult cockroaches, *Periplaneta americana,* with a micropipette. A solution rich in pyrethrin II (95 per cent pyrethrin II, 5 per cent pyrethrin I) caused more rapid and higher knockdown than a similar solution

rich in pyrethrin I (85 per cent pyrethrin I, 15 per cent pyrethrin II). Pyrethrin I caused higher mortalities than pyrethrin II at concentrations giving 17 to 77 per cent kill, but at concentrations that caused 80 to 83 per cent mortality, the two pyrethrins were about equally toxic. Female roaches were about twice as resistant to pyrethrins as male roaches.

CHAPTER XXII

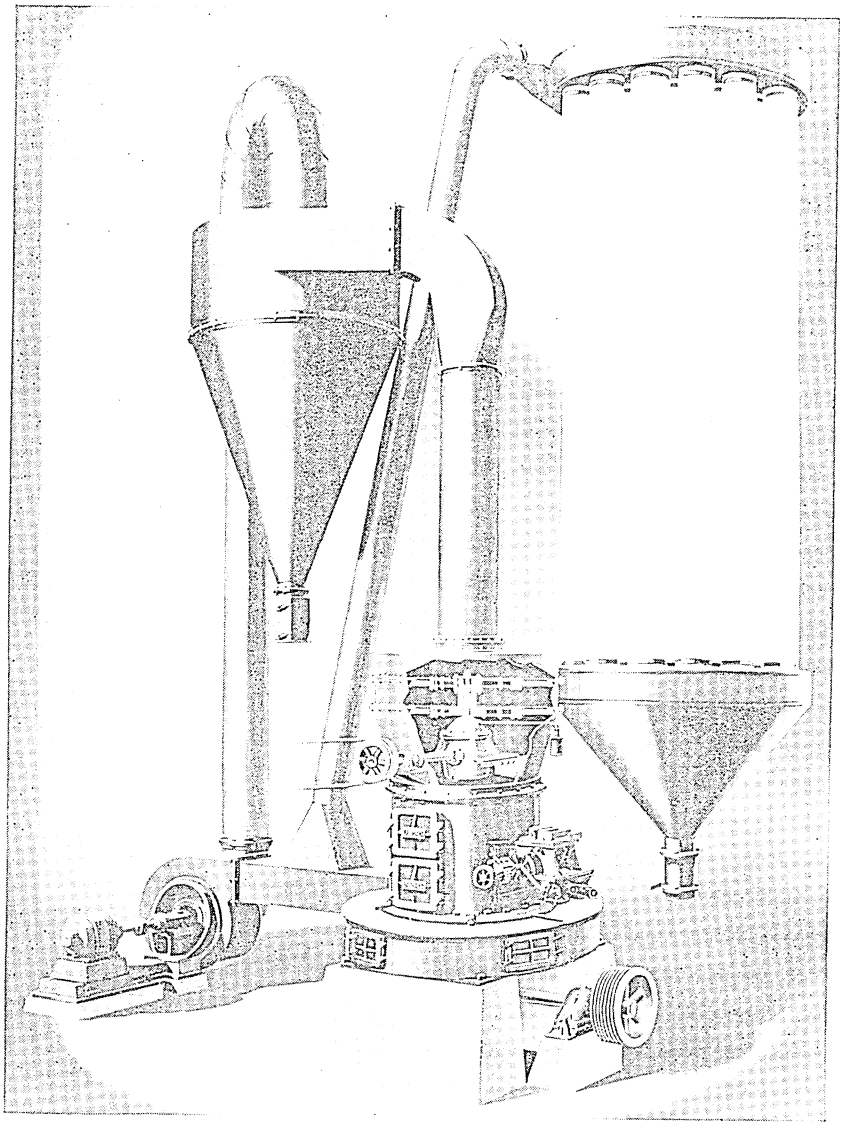
MANUFACTURE OF PYRETHRUM INSECTICIDES

In the last ten years more attention has been given to the production of finely ground pyrethrum flowers. Smith *(2145)* showed that the finer the powder is ground, the more rapid is the rate of paralysis of mosquito larvae and the rate of mortality of bean aphids. When tested in water suspension against fourth instar of mosquitoes, the finest powder required 10.5 minutes to paralyze 50 per cent of the larvae in one series of tests, whereas the coarser powder produced this effect only after 277 minutes. When tested in aqueous suspension against *Aphis rumicis,* the coarsest powder killed 56 per cent and the finest 73 per cent. When applied in dust form against the same insect, the coarsest dust killed 66 per cent compared with 79 per cent for the finest. However, the greater the surface area exposed to light and air, the more rapid is the deterioration of the pyrethrins.

Smith and Goodhue *(2146)* suggest that pyrethrum would probably be more effective if more finely ground.

Pyrethrum powders having a fineness of 95 per cent through 200 mesh can be produced on mills like the Raymond Roller Mill and the Williams Roller Mill, Fig. XXII. A mill operating on a different principle is the jet pulverizer described by Lissman *(1809)*. This mill is known as the Micronizer* Reduction Mill (U. S. Patent 2,032,827) ; in it grinding is done by suspending unreduced material in the form of a rapidly circulating stream or sheet and continuously projecting, by means of high velocity air jets, a stream of particles moving at high speed at an angle to this moving sheet, the velocity being of the order of 1000 feet a second or higher. By this means, a terrific impact is obtained, which is important in extremely fine grinding. As this grinding is being performed, there is a continuous sweeping action which removes sufficiently reduced material out of the grinding and classifying chamber to a product collector (Fig. XXIII). This mill is especially adapted for grinding in the sub-sieve range, 625 to 5000 theoretical mesh, or 20 to 2.5 microns. It is said to have been used successfully for pulverizing pyrethrum.

*Registered Trade Mark.



RAYMOND ROLLER MILL WITH WHIZZER SEPARATOR.
(COURTESY RAYMOND PULVERIZER DIVISION, COMBUSTION ENGINEERING CO.)

PYRETHRUM EXTRACTS

Haller and LaForge *(1572)* remove the fatty acids from pyrethrin concentrate by dissolving the concentrate in aniline, washing with aqueous potassium carbonate solution and then removing the aniline from the concentrate with hydrochloric acid.

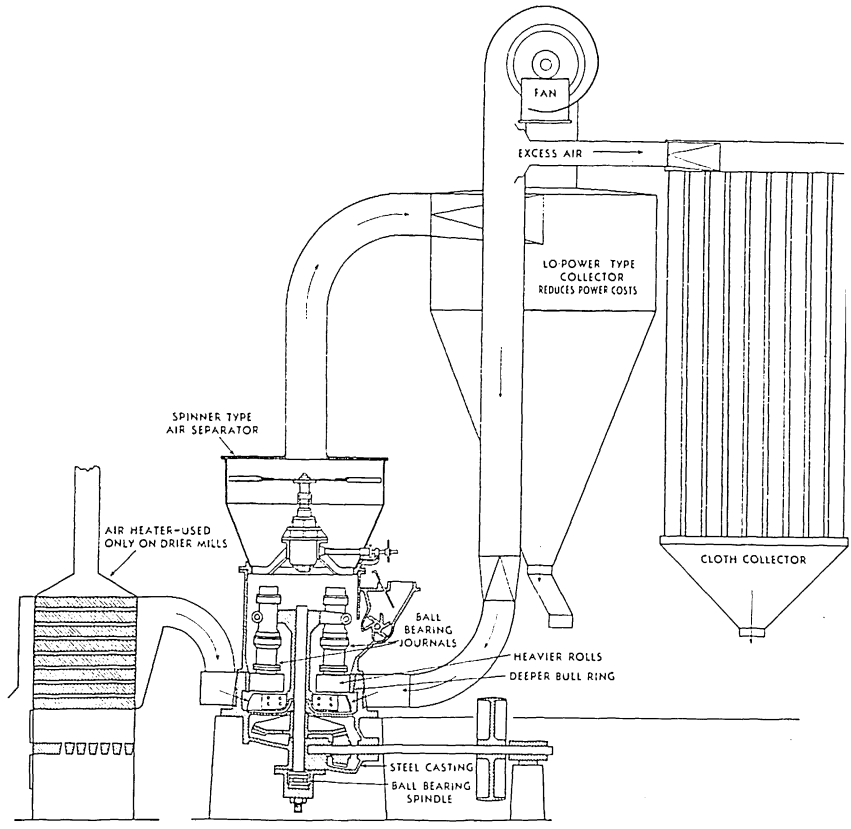


FIG. XXII. THE WILLIAMS ROLLER MILL.
(COURTESY WILLIAMS PATENT CRUSHER AND PULVERIZER CO.)

LaForge and Haller *(1768)* prepare a concentrated pyrethrum extract by dissolving oleoresin of pyrethrum in glacial acetic acid, precipitating fats, waxes and coloring matter by the addition of a small quantity of water, filtering off the precipitate and separating the pyrethrin concentrate from the acid solution by the further addition of water.

Martin and Potter *(1853)* prepared colorless extract of pyrethrum by mixing 12.5 per cent or more of decolorizing charcoal with the finely powdered flowers and extracting with petroleum ether in a Soxhlet extractor. The extract was toxic to insects. Martin *(1848)* states that the pyrethrin I content of extract so prepared is the same as in extract made without charcoal. Martin *(1849)* also used chloroform for the preparation of highly concentrated, colorless extracts, but at least 45 per cent charcoal was necessary.

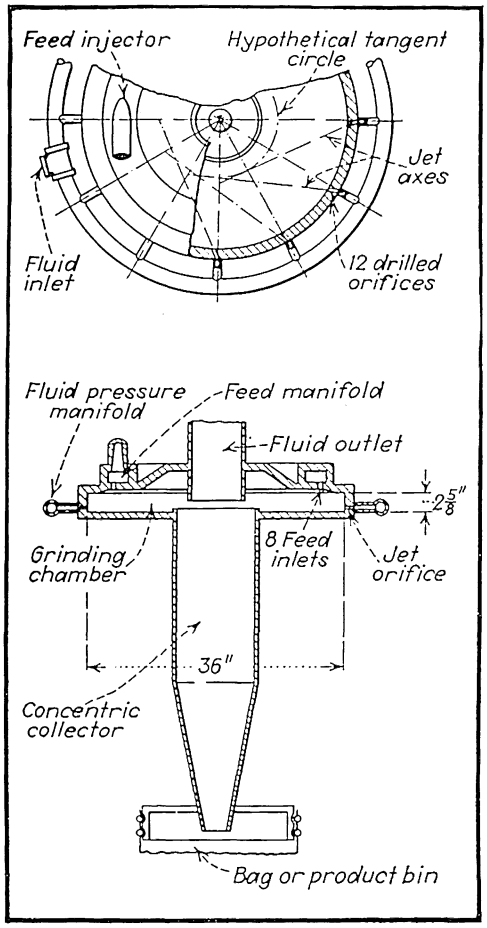


FIG. XXIII. CROSS SECTIONAL VIEWS OF THE MICRONIZER JET REDUCTION MILL. (COURTESY MICRONIZER PROCESSING CO., INC., MOORESTOWN, N. J.)

Fawcett *(1439)* prepares pyrethrins by distillation under high vacuum (10^{-6} mm.) with the distilling and condensing surfaces only 1 to 5 cm. apart. The dried flowers may be treated directly, or fed to the still in suspension, or as an extract.

West *(2176)* produces highly concentrated, pale colored extracts by extracting pyrethrum with acetone, ether, benzene, ethyl carbonate or methyl ethyl ketone, removing the solvent and treating the extractive with 80 per cent methanol. The insoluble residue is rejected and the filtered methanol solution is evaporated. The residue is dissolved in petroleum oil and the insoluble portion is rejected. This is quite similar to the process of Sankowsky and Grant (page 200).

Bataafsche Petroleum Maatschappij *(1179)* has patented a process for making purified pyrethrum extracts, free from resins, by extracting pyrethrum with appropriate volatile organic solvents, cooling at a temperature below zero degrees and separating the precipitate by centrifuging. Organic solvents favoring precipitation of inerts, such as di-isopropyl ether, secondary dibutyl ether or other aliphatic ethers are used. Hydrogenation products of naphthalene, such as decalin, are added.

Blyumberg *(1218)* recommends the use of heat for the extraction of pyrethrum with ethylene dichloride as described by Gnadinger *(341)*.

Toda *(2238)* extracts pyrethrum with an organic solvent, evaporates the solvent and adds calcined magnesium oxide to the residue, which is evaporated to dryness and extracted with fusel oil or camphor oil.

Akita *(999)* extracts pyrethrum with acetone from which the pyrethrins are washed with gasoline or kerosene after the addition of a little water. The oil solution is mixed with creosote, camphor oil, paradichlorbenzene and the oil of the seed of *Zanthoxylum piperitum*.

Schechter and Haller *(2093)* conducted experiments to determine the effect of preliminary aqueous extraction of pyrethrum flowers on the percentage of pyrethrins in the subsequent petroleum ether extracts. Pyrethrum flowers lose about one-third of their weight when extracted with water. The aqueous extract contained a negligible amount of pyrethrins and, although the extract was toxic to goldfish, it was not toxic to the housefly. Extraction of pyrethrum flowers with water followed by drying the marc in a steam cabinet causes a decrease in the amount of petroleum ether extract that is subsequently obtained. The pyrethrin content of this oleoresin remains about the same as that of the untreated flowers, or even less, which indicates that some of the pyrethrins are destroyed by such treatment. Consequently, this process is unsuitable for increasing the percentage of pyrethrins in the flowers or in the petroleum ether extracts.

Muskat *(1931)* states: "I have discovered that when a pyrethrum distillate, such as gasoline or kerosene, is used as the solvent for extracting pyrethrin from pyrethrum flowers and when the extraction is made under conditions preventing as far as possible the extraction of inert non-toxic matter, such as resins, greases, etc., the extract has a much greater toxic effect than when it contains substantial amounts of inert matter. I have also discovered that the pyrethrin is much more readily

soluble in the petroleum distillate than is the inert matter of the flowers and that, by a quick extraction allowing only a short time of contact between the flowers and the solvent, the extraction of inert matter is kept at a minimum, and the extract has a much greater toxic effect. I have found, for example, that by immersing the flowers in a quantity of petroleum naphtha of the order of 1 gallon of distillate to ½ pound of flowers for a period of about 20 minutes and then removing the liquid from the residue of flowers, the toxicity of the extract as determined by the Peet-Grady standard test is nearly equal to the toxicity of an extract made by prolonged treatment of one pound of flowers in a gallon of naphtha. The insecticidal effect obtained per unit weight of flowers is thus doubled in relation to the older extraction methods in which prolonged contact of the flowers with the solvent effected extraction of large amounts of inert soluble matter in the flowers together with the active pyrethrin."

Goodhue and Haller *(1522)* have patented a process for producing a mixture of purified pyrethrum extract and sesamin compounds, comprising the molecular distillation of crude pyrethrum extract with sesame oil, whereby the oil acts as antifoaming agent, and the distillate contains a mixture of pyrethrins and sesamin. Cottonseed oil, olive oil, or other vegetable oil or high boiling mineral oil may be used, instead of sesame oil, to prevent foaming. The preferred mixture for distillation is one part of oil to two parts of crude pyrethrum extract containing 20 to 25 per cent pyrethrins. No special type of molecular or short path still is necessary. A short path still of the falling film type is very satisfactory and in such a still the pyrethrins distill readily at 150° C. under a pressure of 0.1 mm. of mercury. A fraction rich in pyrethrin I can be obtained by conducting the distillation at 100° C. Afterward the temperature is raised to 160° C., yielding a fraction rich in pyrethrin II. A very complete separation of the pyrethrins can be obtained by repeating the fractional distillation. The sesamin has about the same distilling temperature as the pyrethrins and is collected in the distillate with the pyrethrins.

A method for preparing pyrethrum concentrates containing 90 to 100 per cent of pyrethrins has been developed by Barthel, Haller and LaForge *(1176)*. "Five hundred grams of 20 per cent pyrethrum extract in deodorized kerosene was agitated in a separatory funnel with three successive 250 ml. portions of nitromethane. By playing a high-tension spark on a copper wire dipping to the bottom of the funnel, it was found that the emul-

sions sometimes encountered at this stage could be readily dispersed. The separated nitromethane solution was passed through an 8-inch column of activated carbon 1½ inches in diameter. The column of carbon was then washed twice with 100 ml. portions of nitromethane. The nitromethane solutions were combined, and the solvent was removed by distillation under reduced pressure. The remaining concentrate contained 98 per cent of pyrethrins, as determined by the A.O.A.C. method, and weighed 90 grams, which is equivalent to 90 per cent of the pyrethrins contained in the starting material."

This process, which has been patented *(1175)*, is still in the laboratory stage. The yield of 90 per cent, starting with a highly refined concentrate as raw material would correspond to about 84 per cent yield starting with pyrethrum flowers. This is too low for good commercial operation, but part of the remaining 16 per cent might be recovered as by-product. Nitromethane is by no means an ideal solvent. It is flammable and can be detonated; it is somewhat soluble in water and slightly toxic when inhaled. Nitromethane is not chemically inert. Rabideau* treated a concentrate prepared by this method with petroleum ether to remove altered pyrethrins and found that about 8 per cent of the pyrethrin content consisted of altered pyrethrins, whereas in the extract used as raw material, only 2 per cent of the pyrethrin content was altered.

A sample of Kenya pyrethrum was extracted with nitromethane in a Soxhlet extractor and assayed by the Seil method; part of the sample was extracted with petroleum ether and assayed. The ratio of pyrethrin I to pyrethrin II was reversed.

	Pyrethrin I %	Pyrethrin II %	Total %
Nitromethane extraction.....	0.39	0.88	1.27
Petroleum ether extraction....	0.65	0.58	1.23

The extracted flowers and the extractive obtained on evaporating the nitromethane were much darker in color than when petroleum ether was used.

Merritt and West *(1887)* investigated the volatile oil obtained by distilling pyrethrum flowers with steam. The following constants were determined.

Oil from	η_D^{20}	Density 15.5°	Acid Value	Ester Value	$[\alpha]_D^{20}$
Dalmatian flowers...	1.4935	0.9546	95.07	46.22	1.7°
English flowers.....	1.4964	0.9363	44.70	57.20	0.0°
Kenya flowers.....	1.4981	0.9391	48.30	45.61	7.7°

*Unpublished.

The yield of volatile oil was about 0.1%. The pyrethrin content of Dalmatian flowers before distillation was 0.41% pyrethrin I, 0.39% pyrethrin II; after steam distillation, 0.19% pyrethrin I, 0.17% pyrethrin II.

The effect of destructive distillation on exhausted pyrethrum has been investigated by Jacobs *(1689)*.

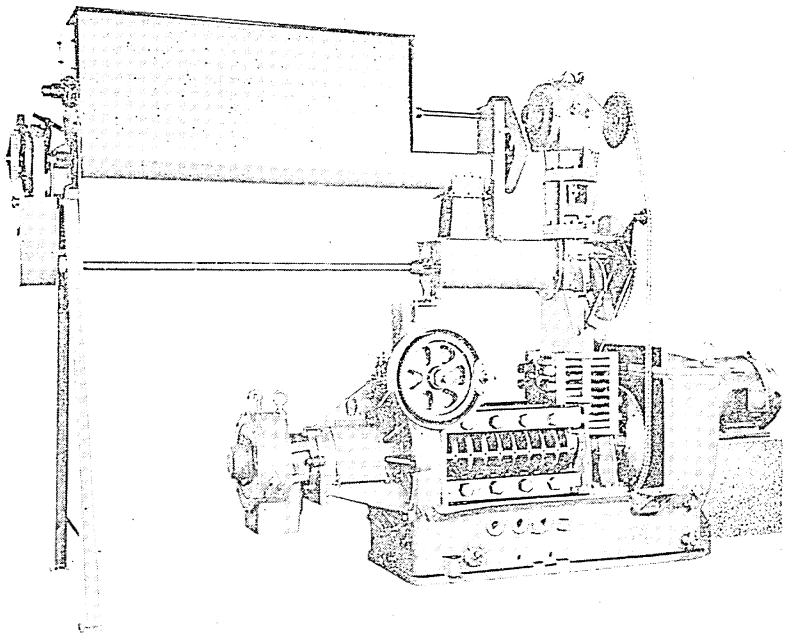
Expeller Process

Several manufacturers use the Anderson Oil Expeller for making concentrated pyrethrum extracts. The process used by one manufacturer is as follows: Pyrethrum flowers are milled to 30 mesh. These flowers are mixed with enough solvent to moisten them and are then packed in percolators. The percolators are covered with a predetermined amount of petroleum distillate and macerated for twelve hours. The free liquid is drawn off and the wet marc is put through the expeller. The pressure on the expeller is regulated most conveniently by means of an ammeter. This relatively weak extract is used as the menstruum for the next lot of fresh flowers. The process is repeated until the extract will test well above standard strength. After preliminary assays, refrigeration and filtration, this extract is then diluted to the standard of 2 grams pyrethrins per 100 cc. and re-assayed.

The volume of liquid used and the number of passes through the expeller are dependent upon the weight and the assay of the flowers to be used and may be varied, but must be so adjusted as to provide a minimum amount of weak percolate and the expulsion of a relatively dry marc. The losses in this process are principally in the marc, which contains some pyrethrins and some solvent.

The process suggested by the manufacturers of the expeller is:

One thousand pounds of ground flowers are placed in a mixer. Fifty gallons of solvent are added and the two are mixed for fifteen to twenty minutes. These flowers are then elevated to the hopper above the expeller and the machine is started. Until the machine is hot and a hard cake is made, the soft cake is returned to the mixer which is operating. When the expeller is functioning properly, the mixer is emptied. Hard expeller cake is discharged into the mixer which does not move until the entire amount is pressed. This is the first cycle and the concentrate produced should be of the required strength—2 grams of pyrethrins per 100 cc. These figures are based on Japanese flowers.



THE ANDERSON EXPELLER (COURTESY V. D. ANDERSON COMPANY).

With Kenya flowers, at least 26 per cent more solvent is used. This concentrate is pumped into Tank No. 1.

Second cycle: fifty gallons of solvent are added to the expelled cake, which is thoroughly mixed and pressed. This concentrate is pumped into Tank No. 2 and contains 1 g. pyrethrins per 100 cc.

Third cycle: fifty gallons of solvent are added to the expelled cake, which is thoroughly mixed and pressed. This concentrate is pumped into Tank No. 3 and contains 0.5 g. pyrethrins per 100 cc.

Fourth cycle: fifty gallons of solvent are added to the expelled cake, which is thoroughly mixed and pressed. This concentrate is pumped into Tank No. 4 and contains 0.25 g. pyrethrins per 100 cc. In this final run the expeller cake is run into drums for disposal.

A fresh batch of 1000 pounds of flowers is placed in the mixer, fifty gallons of new solvent are added, and the mixture is pressed as before in the first cycle. This concentrate is run into Tank No. 1.

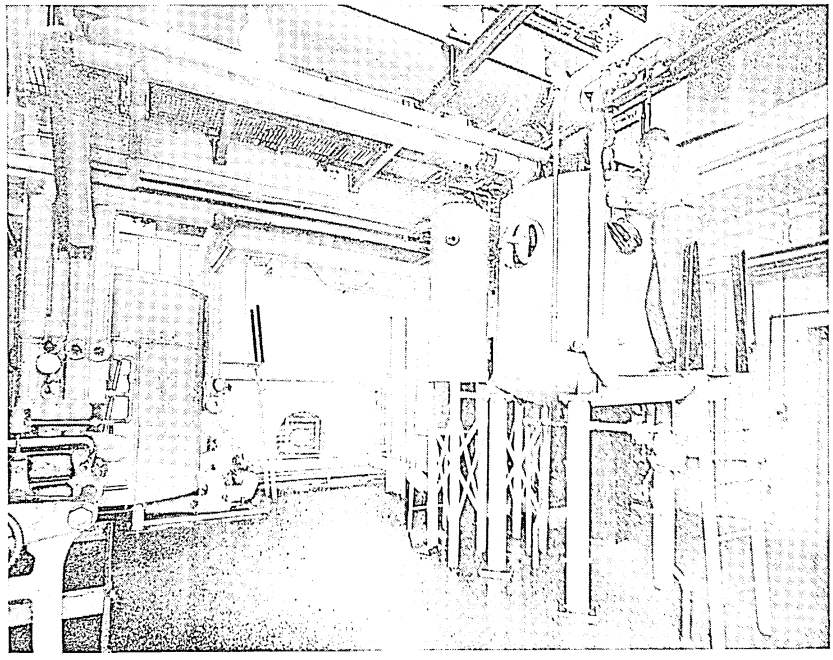
Second cycle: solvent from Tank No. 2 is used and produces a concentrate containing 2 g. pyrethrins per 100 cc., which is run into Tank No. 1.

Third cycle: solvent from Tank No. 3 is used, making a concentrate containing 1 g. pyrethrins per 100 cc., which is placed in Tank No. 2.

Fourth cycle: solvent from Tank No. 4 is used and makes a concentrate containing 0.5 g. pyrethrins per 100 cc., which is placed in Tank No. 3, and so on.

This batch method of counter-current extraction yields extract containing 2 g. pyrethrins per 100 cc. The process can be stopped at the third cycle and the resulting marc, rather rich in pyrethrins and containing 6 to 8 per cent oil, can be powdered, mixed with powdered pyrethrum to adjust the pyrethrin content to about 0.5% and sold as horticultural insecticide.

The demand for more concentrated and less irritating pyrethrum extracts, for use by the Army and Navy in aerosol bombs (page 565), has resulted in a number of improvements in manufacturing processes. Extracts as good as those originally used in the aerosol bomb program can be made by using the ethylene dichloride process (page 191), by using hexane or by the expeller process. However, the best process for producing such extracts



MANUFACTURE OF CONCENTRATED PYRETHRUM EXTRACT.

is that developed in 1943 by Gnadinger and Clark.* This process yields an extract of light amber color, containing 20 per cent pyrethrins. This concentrate, "Pyrocide 175," averages more than 99 per cent soluble in "Freon-12," and the "Freon"-insoluble residue rarely equals 0.75 per cent. It is practically free from moisture, contains no solvent other than mineral oil, and it is non-irritating and nearly odorless when sprayed.

Antioxidants

The advantages of using antioxidants in pyrethrum insecticides to prevent decomposition of pyrethrins have been recognized for some time (Chapter VIII). Faloon *(1433)* has patented the use of lignicol, guaiacol, cresol, thymol, eugenol, resorcinol, pyrogallol, hydroquinone, naphthols, naphthylamine, diphenylamine and benzidine for inhibiting oxidation of pyrethrum.

Smith *(2145)* suggested the use of tannic acid as an antioxidant in pyrethrum powder. Titanium oxide also prevented decomposition of pyrethrins by reflecting or absorbing light of injurious wave length.

Covello *(1320)* suggests the treatment of pyrethrum with a current of sulfur dioxide to inhibit the activity of the oxidase present.

Trusler *(2242)* investigated the value of a number of compounds as antioxidants for pyrethrum-oil sprays, determining their effectiveness by biological methods. Materials having value as antioxidants were benzaldehyde, eugenol, *p*-phenylphenol, beta-naphthol, *o*-cresol and isothymol. Vanillin had an injurious effect on fly spray.

West *(2303)* observed that pyrethrum concentrates stored in the dark for several months were no longer completely soluble in petroleum ether and the insoluble material assayed high in apparent pyrethrin content. He suggested that this change may be due to polymerization involving the pentadienyl side chain. West *(2177)* arrested the natural deterioration of kerosene extracts of pyrethrum by the addition of a solution of hydroquinone or pyrogallol in a high boiling ketone, a terpene alcohol or an ester of a terpene alcohol.

Martin *(1850)* determined the stability of pyrethrum-oil extracts with and without antioxidants, with these results:

*Unpublished.

Pyrethrin Extract in	Antioxidant	Storage in Dark	Loss of Pyrethrin I %
Heavy mineral oil	none	2 years	14
Olive oil	none	2 years	18
Heavy mineral oil	pyrocatechol (0.25%)	6 months	0
Heavy mineral oil	pyrocatechol (0.25%)	19 months	0
Heavy mineral oil	commercial product	11 months	5

Pyrethrum extracts to which antioxidants were added did not retain their toxicity to insects longer when sprayed in thin films on a textile material.

CHAPTER XXIII

PYRETHRUM HOUSEHOLD INSECTICIDES

Liquid Insecticides

According to MacNair, "The first kerosene extract of pyrethrum flowers sold as a household insecticide was 'Walker's Devilment,' developed by a patent medicine manufacturer located at Thomasville, Ga., and sold there first in 1916. In the same year, 'Shepherd's Housefly Driver and Insect Exterminator' was brought out in Wilmington, N. C., by the Shepherd Chemical Co. This product was a pine oil extract of pyrethrum, offered also as a disinfectant. In 1917, 'Komo' was put on the market in Philadelphia by the Komo Chemical Co. It was essentially the same as 'Walker's Devilment,' being a kerosene extract of pyrethrum, as were 'Flyosan,' made in Darby, Pa., in 1919 and 'Fly Flu,' first sold in that year at Ocilla, Ga. From these beginnings, in less than a quarter-century, has grown an American household insecticide industry comprising close to two thousand brands." *(1823)*.

In 1936 the National Association of Insecticide and Disinfectant Manufacturers adopted the specifications for liquid household sprays, reported by Thomas *(2227)*. These specifications have since been slightly revised and are now as follows *(1126)*:

1. A household spray oil type insecticide shall be harmless to man and warm-blooded household animals, when used as directed.
2. When sprayed, as directed, it shall not stain fabrics, wall paper and general household furnishings, that are not stained by dry cleaning fluid.
3. When used in the customary manner it shall not contaminate closed packages of food materials commonly found in homes.
4. It shall not corrode metals.
5. It shall have no objectionable odor, and no particular odor shall be specified.
6. It shall have a flashpoint not less than 125° F. when tested in the Tagliabue closed cup.
7. It is recommended that it be purchased on a direct competitive basis with the Official Test Insecticide of the National Association of Insecticide and Disinfectant Manufactur-

ers, Inc., by using the method of test specified in literature accompanying the Official Test Insecticide.

8. The Association hereby adopts the following grades, the plus or minus figures shown therein designating the points over or under the Official Test Control Insecticide when the "Unknown" and the "Control" are tested at the same time in the same manner:

Designation	Grade	Kill Classification
AA	Excellent	+16 or higher
A	Very Good	+ 6 to +15
B	Equal to Official Test Insecticide	+ 5 to — 5

Federal specifications for Liquid Insecticide (Fly Spray) and Liquid Insecticide (For Roaches and Carpet Beetles) were adopted in 1942 and revised in 1944 *(1118, 1117)*. The specification for fly spray is similar to that for AA spray and does not specify the use of pyrethrins. The roach spray must contain not less than 0.13 g. pyrethrin I in 100 cc. together with the normally accompanying amount of pyrethrin II. There is also a Federal Specification for pyrethrum powder *(45)*. These specifications may be obtained from the Superintendent of Documents, Government Printing Office, Washington 25, D. C.

A light application of pyrethrum-oil fly spray to the body 2 or 3 minutes before bathing is recommended for removal of chiggers, *Eutrombicula alfreddugesi (1082)*.

Occasional light spraying of the clothing with pyrethrum-kerosene spray is said to be helpful in preventing infestation in areas where ticks are prevalent. A similar extract is effective for killing dog ticks in houses *(1098)*.

Pyrethrum fly sprays are recommended for control of wasps, bees, hornets, rat mites, millipedes and other insects *(1102)*.

Ellis *(1419)* uses pyrethrum sprays for killing black widow spiders and eggs. The spray recommended is about twice as strong as the usual household pyrethrum spray.

Eckert and Mallis *(1404)* obtained control of the Argentine ant by first perforating the nests with a stick and then pouring pyrethrum-oil spray into them. Where vegetation is present an emulsion of 1 part household spray in 500 parts of soapy water is used.

Gray *(1556)* has shown that pyrethrum spray, in odorless base oil, gives the most satisfactory control of the spider beetle which infests cereal products in storage.

Wilson *(2335)* found no loss of pyrethrins nor decrease in toxicity in commercial fly sprays stored for 4 and 7 months in soldered cans in which confectioner's glaze, rubber base and glucose base seaming compounds were used.

Insecticidal Powders and Dusts

The use of pyrethrum in roach powders has been studied by numerous investigators. Replies to a questionnaire sent to one hundred pest control operators and manufacturers of insecticides by the magazine "Soap" *(1078)* disclosed a preference for a mixture of sodium fluoride and pyrethrum as the best all-around roach powder. A majority of those replying considered 75 per cent sodium fluoride and 25 per cent pyrethrum the best mixture; second choice was a mixture of equal parts of pyrethrum and sodium fluoride. Laudini and Sweetman *(1790)* tested a number of the materials discussed in the "Soap" questionnaire and concluded that the preference for pyrethrum-sodium fluoride mixtures is justified.

An excellent review of the use of pyrethrum against roaches has been given by Campbell *(1264)*, who states, "Because pyrethrum offers no hazard to man and animals and is effective against roaches when applied in sufficient quantity to the bodies of the insects, it is believed to hold an important and unique place among insecticides used for roach control."

Klostermeyer *(1735)* conducted extensive tests with various roach powders on *Blatella germanica*. The dusts tested, in the order of their decreasing rates of lethal action were: mixtures of sodium fluoride and pyrethrum; sodium fluoride; mixtures of sodium fluoride and pyrophyllite; mixtures of sodium fluosilicate and pyrethrum; sodium fluosilicate; pyrethrum; mixtures of borax and pyrethrum, and borax.

Hockenyos *(1641)* found that a mixture containing 25 per cent pyrethrum and 75 per cent talc acted faster on roaches than a mixture of equal parts pyrethrum and talc.

Dewey *(1367)* tried 41 different materials in laboratory experiments with roach powders against the German cockroach. His work indicated that "First instar nymphs are least resistant to sodium fluoride and in order of increasing resistance are second and third instar nymphs, fourth and fifth instar nymphs, adult males, adult females, sixth and seventh instar male nymphs and sixth and seventh instar female nymphs.

"Aluminum oxide, bauxite, and Anderson's clay, which have

antidotal properties for sodium fluoride, were found to accelerate the toxic action of sodium fluoride on the German roach.

"A powder containing 1.4 per cent pyrethrum extract (2 per cent pyrethrins), 4 per cent sodium fluoride, 6 per cent lubricating oil, and 89 per cent pyrophyllite mixed in gasoline and dried gave a knockdown of all roaches treated and was nearly as effective as pyrethrum-sodium fluoride, 25:75 or 50:50.

"Pyrethrum-sodium fluoride-pyrophyllite (12.5:25:62.5) was about as effective as pyrethrum-sodium fluoride, 25:75 or 50:50.

"Sodium fluoride and 2, 4-dinitro-6-cyclohexylphenol (50:50) produced leg paralysis of the roaches treated and was as effective as sodium fluoride-pyrethrum.

"Sodium fluoride-borax (50:50) killed the roaches more quickly than sodium fluoride. Sodium fluoride-borax (10:90) was slightly more effective than sodium fluoride alone.

"Freshly hydrated lime greatly accelerates the toxicity of sodium fluoride, but it is of questionable value as an activator for sodium fluoride in roach powders owing to carbonation which takes place readily upon its exposure to air.

"Males are more susceptible than females to all the dusts tested."

Gould *(1537)* concluded, after extensive work on roach control: "Sodium fluoride dust for all species of roaches and phosphorus paste for the large species of roaches are still the standard treatment for these insects. When these materials are not available or cannot be used because of the health hazard, one of the new materials can be successfully substituted. In laboratory tests boric acid was found to give a better kill than borax and with pyrethrum marc added was a satisfactory roach powder. The marc alone killed few roaches, but did improve the performance of boric acid, dinitroanisole and sodium fluoride. A mixture of 15 parts of dinitroanisole with 85 parts of pyrethrum marc or one containing 10 parts of dinitroanisole, 45 parts of pyrethrum marc and 45 parts boric acid showed much promise and should be given further trial."

The pyrethrum marc, referred to by Gould, is the exhausted pyrethrum from which the pyrethrins have been extracted in the manufacture of concentrated pyrethrum extract. Ordinarily it contains from 0.05 to 0.15 per cent pyrethrins. During the period when all pyrethrum was allocated for war uses, the sale of the marc was permitted for civilian uses.

Johnson and Vallee *(1711)* recommend a mixture containing 0.4 per cent pyrethrins and 1 per cent rotenone with alkali-free

sulfur or non-alkaline clay. This is said to be safe and more effective than sodium fluoride. It is also patented *(288)*.

Marcovitch and Stanley *(1840)* have patented a roach powder containing 10 per cent sodium fluoride, 10 per cent pyrethrum, 70 per cent dextrin and 10 per cent peanut flour. This diluent is said to be decidedly superior to those commonly used.

Synergists for Pyrethrum

About five years ago the term "synergist" began to replace the term "activator" in describing those materials which increase, or appear to increase, the insecticidal effect of pyrethrins or other insecticides. Synergism is defined as "co-operative action of discrete agencies such that the total effect is greater than the sum of the two effects taken independently; the opposite of *antagonism*, as in the action of the mixtures of certain salts or drugs."* At the present time the action of synergists of pyrethrum is not fully understood. There are apparently cases of true synergism as well as instances of what may be termed false synergism, in which the apparent synergistic action is due to the peculiar conditions under which the test is made. A further complication is caused by the fact that synergistic action can be used as the basis for patent applications, hence it may sometimes be claimed where its existence is questionable or difficult to prove.

A material that is a synergist for pyrethrum may have no such action with another insecticide. Moreover, a synergist for pyrethrum against the housefly may have no synergistic action against the cockroach or other insect. Some synergists for pyrethrum have insecticidal action of their own, others have little or no value as insecticides when used alone. Lime is antagonistic to pyrethrum since it decomposes the pyrethrins, but under the above definition, it would be synergistic for nicotine sulfate, since it releases free nicotine which is more effective against aphids, than the additive effect of nicotine sulfate and lime when used alone. Lime has been used in nicotine dusts for many years. This type of action is quite different from that in which both materials have a direct physiological action on the insect itself. Clearly, synergism, as it applies to insecticides, needs to be more exactly defined.

Fulton *(288)* has patented an insecticide comprising pyrethrins and rotenone. Woodbury *(2344)* attempted to ascertain whether a combination of rotenone and pyrethrins is more effec-

*Webster's New International Dictionary.

tive than either insecticide used alone. He concluded that the combination is not markedly more toxic to roaches than would be expected if the effect of the components were additive.

Ripert and Gaudin *(748)* found a mixture of pyrethrin I and pyrethrin II more toxic to frogs than either pyrethrin alone.

Weed *(2295)* first reported the synergistic action of isobutylundecylenamide (IN-930) on pyrethrins when tested in fly sprays by the Peet-Grady method.

Eagleson *(1395)* discovered that sesame oil is a synergist for pyrethrum. Sesame oil alone has little insecticidal action on houseflies, but when 5 per cent of sesame oil was added to a pyrethrum fly spray, the toxicity of the spray to houseflies was greatly increased. The discovery of the synergistic action of sesame oil with pyrethrins resulted from trials of 42 animal and vegetable oils, none of which, excepting sesame oil, had any effect on houseflies. Among the materials that showed no synergism with pyrethrum were the following oils: almond, apricot kernel, avocado, butter, babassu, cacao butter, candlenut, castor, chaulmoogra, cherry kernel, coconut, cod liver, corn, cottonseed, grapefruit seed, hazelnut, hemp seed, Japanese wood, oiticica, olive, peanut, perilla, poppy seed, pumpkin seed, raisin seed, safflower, seal, soybean, sunflower, tung, walnut, bitter almond, china, origanum, and pine *(1398)*.

Haller and associates *(1581)* corroborated Eagleson's results. Sesame oil was separated into four fractions by molecular distillation. The first two fractions were highly effective as activators for pyrethrins when used against houseflies. These fractions were combined and the colorless, crystalline compound, sesamin, $C_{20}H_{18}O_6$, was isolated from them. Sesamin proved to be a highly active synergist for pyrethrins, but in itself was nontoxic to houseflies. No other crystalline product could be isolated from this fraction. Sesame oil contains about 0.25 per cent sesamin.

Haller, LaForge and Sullivan *(1579, 1580)* studied the structure and synergistic effect of sesamin and a number of related compounds. Isosesamin, asarinin, pinoresinol, pinoresinol dimethyl ether and diacetyl pinoresinol were prepared and tested with respect to their synergistic effect on pyrethrins. Isosesamin and asarinin were at least as effective as sesamin, but the other three compounds were without appreciable action.

Asarinin is a constituent of the bark of southern prickly ash, *Zanthoxylum clava-herculis*. Pinoresinol is obtained from spruce gum. Sesamin, isosesamin and asarinin have the same chemical

structure but differ with respect to the configuration on 4 carbon atoms, asarinin being the mirror image of isosesamin. These compounds were tested against houseflies by the turntable method (page 470) with the results shown in Table CVIII.

TABLE CVIII. SYNERGISTIC EFFECT OF SESAMIN AND RELATED COMPOUNDS (HALLER ET AL.).

No.	Material	Concentration %	Average mortality after 24 hours %
	Sesamin and its isomers:		
1	Sesamin	0.2	4
2	Sesamin + pyrethrins	0.2 +0.05	84
3	Isosesamin	0.2	5
4	Isosesamin + pyrethrins	0.2 +0.05	87
5	Asarinin	0.2	14
6	Asarinin + pyrethrins	0.2 +0.05	88
7	Pyrethrins (control)	0.05	25
	Pinoresinol and derivatives:		
8	Pinoresinol	0.18	1
9	Pinoresinol + pyrethrins	0.18+0.05	12
10	Dimethyl pinoresinol	0.2	1
11	Dimethyl pinoresinol + pyrethrins	0.2 +0.05	17
12	Diacetyl pinoresinol	0.3	2
13	Diacetyl pinoresinol + pyrethrins	0.3 +0.05	11
14	Pyrethrins (control)	0.05	19

A method for the quantitative determination of sesamin has been described *(1690)*.

McGovran and Fales *(1869)*, in six tests of a spray that contained 10 per cent of sesame oil and 1 mg. of pyrethrins per milliliter in refined kerosene killed 62 per cent of adult female roaches as compared with 68 per cent in six tests of a spray containing 1 mg. of pyrethrins per milliliter in refined kerosene, but without the sesame oil. Similar results were obtained in three tests each with adult males and large nymphs. They concluded that the addition of sesame oil to pyrethrum in petroleum-oil base does not appear to enhance the toxicity of the sprays to roaches.

McGovran and Fales *(1870)* investigated the efficiency of sesame oil and isobutylundecylenamide as synergists for pyrethrum in oil sprays, when used against mosquitoes, *Aedes aegypti*. The synergists alone gave very low knockdowns and kills. Both synergists increased the effectiveness of pyrethrum-oil sprays against mosquitoes "but to a much less degree than has been reported on houseflies."

Madden, Lindquist and Knipling *(1826)* obtained no synergistic action from sesame oil used with pyrethrins in dusts for the control of chiggers.

Lindquist and associates demonstrated that ordinary motor oil is an effective substitute for sesame oil in pyrethrum aerosols used against mosquitoes *(1806)*.

Marcovitch and Stanley *(1838, 1839)* found that sodium fluoride and sulfur are synergists for pyrethrum. Their explanation of the action of mixtures of pyrethrum and sodium fluoride is: "The pyrethrum produces a rapid paralysis, so that the roach is unable to clean itself of the offending powder. In addition, it causes the roach to salivate, with moist droplets appearing over the abdomen and legs. The moisture thus produced appears to dissolve the fluoride, which then penetrates the chitin and causes death. The combination of sodium fluoride and pyrethrum will give a rapid and desirable effect—one being water-soluble and entering through the pore canals of the chitin, and the other being fat-soluble and entering through fatty tissue."

Pierpont *(1994, 1995)* reported the synergistic action of ethylene glycol ether of pinene (D.H.S. Activator) with pyrethrum, but found no synergistic effect on *n*-butyl carbitol thiocyanate (Lethane 384). Pierpont *(1996)* also found terpin diacetate to be an effective activator for pyrethrum against houseflies.

In connection with the use of pyrethrum sprays for mosquito control, David and Bracey *(1343)* examined the efficiency of numerous formulas containing pyrethrins and activators on the mosquito *Aedes aegypti*. Four activators were tested. They caused a delay in knockdown and prolonged the period of flight through the mist. Increasing the activator content increased, within limits, the percentage of kill and also increased the delay in occurrence of knockdown. Delaying the knockdown gives the insects a longer time to pick up a lethal dose of spray. To demonstrate the importance of flight, normal mosquitoes were tested in comparison with mosquitoes whose wings had been removed and with chloroformed mosquitoes. The kill of normal mosquitoes was five times greater than that of walking (wingless) and motionless (chloroformed) insects. Mosquitoes that had been chloroformed and allowed to recover before spraying gave about the same kill as normal mosquitoes.

Period of flight is not the only factor in activation. David and Bracey modified their test by causing the mist from the sprayer to move at known speeds past chloroformed insects.

Increasing the speed of the mist from 0.3 miles per hour to 3 miles per hour increased the kill from 8 per cent to 97 per cent, when the same total volume of mist was used in each test. Sesame oil, isobutylundecylenamide and lubricating oil, all having low vapor pressures, persist when sprayed, after the more volatile carrier oil has evaporated. The particle size of the ultimate droplets that come in contact with the insects is, therefore, larger than when no activator is used. The final droplet size also depends to some extent on the size of the original droplets produced by the spray-gun. Many substances of low vapor pressures such as olive oil, oleic acid, and sesame oil free from sesamin greatly increase the kill of pyrethrin sprays.

David and Bracey mention that their work does not explain Haller's results with pure sesamin. They also point out that their results were obtained on mosquitoes and their conclusions may not be applicable to other insects.

The work of Hartzell and Scudder *(1610)* indicated that both the pyrethrins and isobutylundecylenamide have a direct and different physiological action on houseflies. The pyrethrins caused clumping of the chromatin of the nuclei in all tissues, but the amide caused dissolution of the chromatin. The synergism of the amide for pyrethrins may be due to the interaction of the two types of nuclear destruction.

Harvill, Hartzell and Arthur *(1619)* found piperine more toxic to houseflies than pyrethrins when used at a concentration of 0.1 per cent. Sprays containing 0.05 per cent piperine and 0.01 per cent pyrethrins were more toxic to houseflies than sprays containing 0.10 per cent pyrethrins. Amides of piperic acid were more effective than the amides of cinnamenylacrylic acid in increasing the toxicity of pyrethrum solutions. Lengthening the side chain attached to the methylenedioxyphenyl group increased the effectiveness of the amide. Piperine was more effective than the piperidide of 3,4-methylenedioxybenzoic acid in increasing the toxicity of pyrethrum solutions.

Hartzell *(1608)* investigated the histological effect on houseflies of pyrethrum, rotenone, sesame oil, piperine, β-butoxy-β-thiocyano-diethyl ether, ethylene glycol ether of pinene and DDT. All of these materials, excepting rotenone, produced definite histological changes in the muscles. Pyrethrum and the activators, sesame oil and piperine, produced characteristic effects on the brain. Pyrethrum caused destruction of fiber tracts and separation of the tissue; piperine caused almost complete destruction of the cellular components of the fibers. Sesame oil

affected the nerve cells only at extremely high concentrations. The thiocyano ether and pinene ether produced effects similar to those of piperine. DDT caused degeneration of brain and ganglia nuclei. Activation is due to the destruction of at least two tissue components. The insecticide has an affinity for one component, the activator for another component. Together they operate at lower concentrations than when they are used separately.

Synerholm, Hartzell and Arthur *(2200)* prepared 22 esters and 25 substituted amides of piperic acid and determined the toxicity of these compounds to houseflies. The amides and esters of piperic acid have a synergistic action on pyrethrins, tetrahydrofurfuryl piperate being the most active synergist among the esters. In Peet-Grady tests amides derived from primary or secondary alkyl or cycloalkyl amines containing 3 to 7 carbon atoms in the alkyl group and esters derived from aliphatic or alicyclic alcohols with 4 to 6 carbon atoms were most toxic to flies.

Roark *(2063)* has made the following observations on the work of the U. S. Bureau of Entomology and Plant Quarantine on synergists for pyrethrum. "It is significant that sesamin and the other effective synergists contain a 3,4-methylenedioxyphenyl group, and hence are closely related to piperonal, which is 3,4-methylenedioxybenzaldehyde. Another compound containing this group is piperine, the alkaloid from black pepper. Entomologists of the Bureau have shown piperine to be ineffective against a number of insects, including the southern armyworm and the melon worm, whereas a mixture containing 90 parts of pyrethrum powder and 10 parts of piperine proved more toxic than pyrethrum alone to these species. Piperine is thus a good synergist for pyrethrum, but its value as an insecticide is low.

"Not all compounds possessing the 3,4-methylenedioxyphenyl group act as synergists, however—for example, piperonal, safrol and ethyl piperonylate are ineffective.

"Because certain amides and certain piperonyl compounds are effective synergists for pyrethrum, it was thought worth while to investigate compounds possessing both groupings.

"Twenty-six piperonylamides *(1481)* were prepared and tested. One of these, fagaramide (N-isobutyl-3,4-methylenedioxycinnamamide), occurs naturally in the root bark of *Zanthoxylum macrophyllum*. Fagaramide, 2 mg. per ml., plus 0.5 mg. of pyrethrins per ml. killed as many flies as a solution containing twice as much pyrethrins but without the fagaramide. When used alone at 10 mg. per ml., the diethyl, dipropyl and

isobutyl piperonylamides gave a mortality at least as high as the standard pyrethrin solution (1 mg. per ml.). The same compounds and also six others displayed synergistic action when added to a solution containing 0.5 mg. of pyrethrins per ml. The limiting factor appeared to be solubility. Where as much as 10 mg. per ml. of the synthetic product could be dissolved, good kills were usually obtained.

"Unlike the pyrethrins, however, the compounds did not show a rapid paralytic or knockdown action. Most of the disubstituted aliphatic amides are liquids and are therefore more soluble than the solid amides. The aromatic-substituted piperonylamides, however, also showed some synergistic effect, although concentrations usually were low. The ortho-substituted aromatic amides appeared to be more effective than the corresponding meta or para derivatives."

Gertler, Fales and Haller *(1482)* also investigated the synergistic action of derivatives of benzamide on pyrethrins against houseflies. The N-amyl, N-butyl and N,N-dibutyl derivatives, definitely increased the toxicity of the pyrethrum solution. The N-butyl and the N-amyl derivatives knocked down some of the flies, but this effect was much less than with the pyrethrins alone.

The relative effectiveness of certain N-substituted benzamides and N-substituted piperonyl amides as synergists for pyrethrum has been determined by Gersdorf and Gertler *(1479)*. N,N-diethylpiperonylamide was more effective than N-butylpiperonylamide or N-isobutylpiperonylamide. At 4 mg. per milliliter the three piperonylamides, when mixed with 0.5 mg. of pyrethrins per milliliter, each gave a spray which caused a much higher mortality of houseflies than 2 mg. of pyrethrins and at 2 mg. per milliliter these amides under the same conditions gave sprays equal to or better than the same concentration of pyrethrins.

N,N-dibutylbenzamide was much more effective than N-butylbenzamide but much less effective than the piperonylamides. The comparisons were made on houseflies. The use of the piperonylamide compounds as insecticides has been patented by Gertler and Haller *(1485)*.

The synthetic organic compound hexylpiperonyl cyclohexenone is said to be a synergist for pyrethrum and to have insecticidal properties when used alone.

Simanton *(2131)* patented the use of ethylene glycol monoethylether acetate and diethylene glycol monoethyl ether acetate for increasing the effectiveness of pyrethrum fly sprays.

West *(2301)* compared the toxicity of a number of anils to

blowflies. The anils were prepared from furfuraldehyde, benzaldehyde and citronellal, reacting with cyclohexylamine, monoethanolamine or aniline. West concluded, "Whilst the insecticidal action of these compounds in themselves would not appear to be sufficiently great to justify their use alone as insecticides, they have been found to exert a distinctly enhanced effect on non-aqueous solutions containing pyrethrum or rotenone, and in spite of the basic nature of this type of compound no loss of activity has been observed when such solutions were stored for periods up to twelve months. Large-scale experiments on mixtures of these compounds with pyrethrum insecticides against bedbugs have confirmed the synergistic effect produced."

Ginsburg *(1500)* has patented the use of thiodiarylamines as synergists for pyrethrum in mosquito larvicides.

McGovran and Sullivan *(1878)* report that 3 per cent of methylphenylnitrosoamine has a significant synergistic effect on pyrethrins. This compound has a noticeable odor and its solutions in mineral oil discolor on standing. Five per cent of 2, 4, diamylcyclohexanol is also a synergist for pyrethrum; it has little odor and does not discolor on standing.

Bushland, Eddy and Knipling *(1254)* tested 200 or more materials as synergists in pyrethrum-pyrophyllite powders against body lice. Sesame oil, ethylene glycol ether of pinene and isobutylundecylenamide were effective as synergists, although all were nontoxic to lice. The best synergist for this purpose was isobutylundecylenamide, which increased the toxicity of the pyrethrins about 100 times.

The use of alkoxyalkylene amines as synergists for pyrethrum has been patented by Hester *(1633)*. A patent for a fly spray containing *n*-isobutylundecylenamide, *n*-octyl thiocyanate and pyrethrins has been issued to Guy and Goddin *(1566)*. Hyman *(1682, 1683)* finds methylated naphthalene synergistic for pyrethrum and has patented several types of insecticides containing methylated naphthalene (Velsicol) alone and in combination with other materials.

Synthetic Substitutes for Pyrethrum

There are many substances, for which no synergistic action is claimed, that have been offered as substitutes for pyrethrum. Some of these materials are intended to replace pyrethrum only in part; others are offered to replace it completely. Up to the present time no insecticide has been discovered that has all the valuable properties of pyrethrum. Bowen and Smith *(1237)*

have compiled a catalog of organic compounds mentioned in United States patents from February 1938 to June 1941. Seventy-seven patents are listed covering about 1400 compounds, most of which are intended to be used with pyrethrum in petroleum oil sprays.

Probably more thiocyanates have been proposed as contact insecticides than any other class of organic compounds. Roark and Busbey *(2064)* list nearly 100 organic thiocyanates and 8 isothiocyanates that have been patented and the list compiled by Bowen and Smith contains an additional 339 thiocyanates and 4 isothiocyanates. These lists are by no means complete. Only a few of these compounds are of commercial importance, at the present time.

One of the first thiocyanates to be used in household sprays was β-butyoxy-β-thiocyanodiethyl ether (*n*-butyl carbitol thiocyanate). A 50 per cent solution of this compound in mineral oil is known as Lethane 384. A mixture of β-butoxy-β-thiocyanodiethyl ether and thiocyano ethyl esters of higher fatty acids with 50 per cent of mineral oil is called Lethane 384 Special. A more recently introduced thiocyanate is the terpene thiocyano ester, Thanite, said to be largely isobornyl thiocyano acetate or fenchyl thiocyano acetate. Alpha-naphthyl isothiocyanate was recommended as a household insecticide by Tischler and Viehoever *(2236)* but it caused severe dermatitis and was withdrawn from the market *(2104)*. β, β-dithiocyano diethyl ether has been offered for use in roach powders under the name Lethane A-70 *(1123)*. Dodecyl thiocyanate is a constituent of Loro.

Von Oettingen, Hueper and Deichmann-Gruebler *(2261)* have reported on the pharmacological action and pathological effects of eight organic thiocyanates. The compounds studied were: methyl thiocyanate, ethyl thiocyanate, *n*-butyl thiocyanate, octyl thiocyanate, decyl thiocyanate, lauryl thiocyanate, myristyl thiocyanate and *n*-butyl-carbitol thiocyanate.

The toxicity of these compounds was determined on mice, rats or cats, or rabbits by subcutaneous injection, when administered by mouth in large and small doses, when applied to the skin and when inhaled. It appeared that liver tissue is able to liberate hydrocyanic acid from some thiocyanates.

These investigators concluded that even small doses of the lower homologues cause paralysis of the medullary centers; the higher homologues were effective only in large doses. The higher homologues, however, were found to have a distinct irritant effect on the mucous membranes and the skin. Acute thiocyanate

poisoning is characterized by severe circulatory disturbance. With the higher homologues degenerative changes occur in the brain, liver and kidney.

Cameron and Doniger *(1261)* investigated the toxicity of lauryl thiocyanate and Lethane 384 to animals. They report that undiluted lauryl thiocyanate produces a severe local skin reaction. It is fatal to mice, rats, guinea pigs and rabbits only when introduced into the body in fairly large amounts. Death is due to respiratory failure probably of medullary origin. Dilutions such as are used in field work have no ill effects. According to these investigators, Lethane 384 produces only a slight local skin reaction but is fatal to animals in much smaller quantities than is lauryl thiocyanate. Otherwise it produces the same effects.

Murphy *(1921)* has presented evidence indicating that Lethane 384 presents no hazards to health when used in fly sprays.

Harvill and Arthur *(1618)* obtained rapid paralyzing effect, in Peet-Grady tests, by using γ-thiocyanopropyl and β-thiocyanoethyl phenol ethers. Gamma-thiocyanopropyl ether of 1, 3, 5 xylenol was excellent for use in household sprays because of its toxicity to flies, quick knockdown and lack of odor.

The outstanding synthetic organic substitute for pyrethrum, at this time, is unquestionably 2, 2-bis (parachlorophenyl) 1,1,1-trichloroethane, usually referred to as DDT, an abbreviation of the name dichloro-diphenyl-trichloroethane. The use of DDT in insecticidal compositions has been patented by Müller *(1917)*. This material is now being used exclusively by the armed forces, with very limited quantities released by the War Production Board for research on post-war uses and for civilian use.

For certain uses DDT is almost as toxic to insects as the pyrethrins. Its residual action is more prolonged than that of the pyrethrins. It does not have the rapid knockdown effect of pyrethrins and it is far more toxic to man and animals than the pyrethrins, which are practically non-toxic to warm-blooded animals.

Fleck and Haller *(1448)* found no reaction between pyrethrum and DDT when equal quantities were heated together for one hour at 115°-120° C.

Ginsburg's preliminary tests *(1503)* indicate that DDT is more toxic to roaches than pyrethrum powder.

Lindquist and others *(1803)* allowed bedbugs to feed on rabbits to which DDT and pyrethrins had previously been administered by mouth. "Mortality as high as 100 per cent

occurred when the bugs were allowed to feed 3 to 5 hours after DDT was administered in dosages from 228 to 400 milligrams per kilogram of body weight. Because of the method of administration, the mortality can be attributed only to ingestion by the bug of the insecticide present in the blood or tissues of the host. Some dosages showed toxic effect on the rabbit.

"Pyrethrum extract gave similar results, but the knockdown of the bedbugs was faster. With pyrethrum 100 per cent knockdown usually occurred, but some insects recovered in a few hours. In some cases paralysis set in before the end of the 5-minute feeding period and the bugs did not engorge completely. Stableflies, which fed on a few of these rabbits, showed typical pyrethrum paralysis in less than a minute. Dosages of 250 to 400 milligrams of pyrethrins per kilogram of body weight did not appear to be injurious to the rabbits. The addition of *n*-isobutylundecylenamide, a compound known to synergize pyrethrum on some insects, did not increase the kill of bedbugs."

Preliminary tests of DDT in household sprays against flies, by Gersdorff and McGovran *(1480)* indicate that it is very effective either alone or in conjunction with pyrethrins or organic thiocyanates.

McGovran, Richardson and Piquett *(1877)* did not find DDT as effective as pyrethrins in laboratory tests against roaches, when applied as a spray or as a dust.

Madden, Lindquist and Knipling *(1828)* made laboratory tests on a large number of organic compounds, including many commercial insecticides, to find a material for the control of bedbugs that would retain its toxicity for a long time. DDT and pyrethrum were the only insecticides satisfactory for this type of treatment and DDT had much longer residual toxicity than pyrethrum. The concentrations of DDT used were much higher than the concentration of pyrethrins.

Lindquist and others *(1807)* investigated the effect of temperatures on the knockdown and kill of houseflies exposed to DDT and pyrethrum. "Flies exposed to the residue of pyrethrum in treated cages showed a faster knockdown at 95° F. than at 70°, and a greater recovery when held at the lower temperature. Furthermore, greater mortality occurred after 24 hours when exposure was made at 95° than at 70°, irrespective of holding temperature. In all respects these results are the reverse of those obtained with DDT."

Several 2, 2-bis (*p*-alkoxy phenyl) - 1,1,1 trichloroethanes have been synthesized by Prill, Hartzell and Arthur *(2012)* and tested against houseflies by the Peet-Grady method. Pyrethrum

extract was added to the sprays to provide satisfactory knockdown. The methoxy analog gave the best knockdown, but the ethoxy analog gave the best kill, and was about two-thirds as effective as DDT against flies. The *n*-propoxy and *n*-butoxy analogs were only slightly toxic to flies.

Dichloro-diphenyl-dichloroethane and phenyl-chlorophenyl-trichloroethane are also said to be very effective insecticides. Difluoro-diphenyl-trichloroethane (Gix) is said to be more effective than DDT, but much more expensive. Other chlorinated compounds reported to be effective as insecticides are ω-chloromethyl-4-chlorophenyl sulfone (Lauseto neu), ω-chloromethyl-phenyl sulfone and 3,4-dichlorobenzyl alcohol.

Among other synthetic organic compounds recommended as substitutes for pyrethrum are monohydric or polyhydric phenols containing an allyl or propenyl group *(1617)*; primary and secondary amines, especially dioctylamine *(2017)*; polymethylated naphthalenes *(1683)*; indandiones *(1730)* and dinitroanisole *(1537)*. Roark *(2062)* has suggested that certain compounds that have been used as horticultural insecticides may be of value as household insecticides. Among these are: phenothiazine, xanthone, phenoxathiin, pentaerythrityl bromide, styrene dibromide, paraiodoazobenzene, paraiodonitrobenzene, phenazine, 4,6-dinitroorthocresol, 2-chlorofluorene, phthalonitrile, 2-furanacrylonitrile, paraiodoacetanilide, paraminoacetanilide, and 2-furaldehyde semicarbazone. Additional compounds of this type have been listed by Smith *(2160)*.

The compound hexachlorocyclohexane, known as 666 because of its formula $C_6H_6Cl_6$, is said to be more toxic to flies and less toxic to mosquitoes than pyrethrins. The pyrethrins also have quicker knockdown. This compound exists in various isomeric forms. The alpha and beta isomers are relatively inactive. The insecticidal activity is due almost entirely to the gamma isomer, which is present to the extent of 10 to 12 per cent in the crude product. This isomer, "Gammexane" has proved to be highly toxic to many kinds of insects *(1125)*.

Botanical Substitutes for Pyrethrum

The use of rotenone and derris in combination with pyrethrum has been restricted by a patent covering the use of such combinations *(288)*.

Lightbody and Mathews *(1801)* have shown that rotenone administered by mouth, in oil, is much more toxic than when given as a solid or in suspension. They state: "It was soon found

that rotenone in olive oil solution fed to white rats had a toxicity comparable to that of strychnine." This is about 20 times the toxicity previously reported.

Mathews and Lightbody *(1856)* found that the toxicity of an acetone extract of derris to rats was five times as great as expected from the rotenone content, indicating the presence of physiologically active compounds other than rotenone.

Ambrose and Haag *(1007)* concluded that rotenone is not an index of the toxicity of derris to warm-blooded animals and that derris is much more toxic when inhaled than when taken by mouth. Derris acts upon the respiratory system.

All of these results indicate that rotenone and derris are much more toxic to warm-blooded animals than hitherto supposed, especially in oil solutions or when inhaled. Millers of derris and pyrethrum have known for years that men employed in the milling of derris are frequently made ill, while in milling pyrethrum, no discomfort of any kind is experienced.

The use of sabadilla seed in household insecticides has been studied by Allen, Dicke and Harris *(1003, 1005)*. Kerosene extracts of sabadilla were highly toxic to houseflies and retained their toxicity for two years when stored in brown glass. Exposure to light caused rapid deterioration. The application of heat and treatment of powdered seed with soda ash prior to extraction increased the toxicity. The optimum temperature was 150° C.

Sabadilla seed has long been used as an insecticide for body lice and as a source of the alkaloid veratrine. It is strongly sternutatory. Veratrine is an intense local irritant and a powerful muscle and nerve poison.

Kerosene extract of the ground seed of *Mammea americana,* the mamey tree of Puerto Rico, was found by Plank *(1998)* to be toxic to roaches, ants and mosquitoes.

The oleoresin of male fern, *Aspidium filix-mas,* was reported by McCallan and Wilcoxon *(1859)* to be toxic to mosquitoes and aphids. Combined with pyrethrins in mineral oil it was toxic to houseflies.

The petroleum ether extract of the bark of southern prickly ash, *Zanthoxylum clava-herculis,* is said by LaForge, Haller and Sullivan to be toxic to flies *(1775)*. The seed of *Zanthoxylum piperitum* have been used as an insecticide by Akita *(999)*.

The powdered rhizome of sweet flag, *Acorus calamus,* is said to be an effective insecticide against mellophaga on fowls and against bedbugs *(2190)*.

Household Aerosols

The aerosol bomb, used by the armed forces for mosquito control, has been restricted by the War Production Board to use in combat areas, hence little work has been done with this type of pyrethrum spray on household insects. Monro *(1902)*, using an aerosol containing 1 per cent pyrethrins, 2 per cent sesame oil and 97 per cent dichlorodifluoromethane, obtained control of adult Indian meal moths, *Plodia interpunctella,* but concluded that it would not be satisfactory for use in holds of ships or in railroad cars for control of other insects infesting grain. This aerosol was effective against the German cockroach.

Billings, Goodhue and Sullivan *(1210)* determined the effectiveness of a similar aerosol against adults of the cheese skipper, *Piophila casei.* Almost complete mortality was obtained.

A more complete discussion of pyrethrum aerosols is given in Chapter XXVI.

Labelling Pyrethrum Insecticides

The following is a notice to the trade, issued by the U. S. Department of Agriculture, on the labelling of pyrethrum powder and preparations containing it *(1068)*:

"The Insecticide Act of 1910 provides (Section 8) that if an insecticide consists in part of an inert substance (or substances) which in itself does not prevent, destroy, repel, or mitigate insects, its label must bear a plain and correct statement of the names and percentage amounts of each and every inert ingredient or, in lieu thereof, a plain and correct statement of the names and percentage amounts of each and every active ingredient together with the total percentage of the inert ingredients.

"In the case of plant materials the actual constituent, or constituents, of the plant material that is toxic to insects is held to be the active ingredient, and this provision of the law has, in general, been applied to such products in the past. One exception has been pyrethrum powder or insect powder, the reason being that until comparatively recently, the ingredients to which the powder owes its activity as an insecticide had not been identified, and after their identification a considerable time elapsed before satisfactory methods for their quantitative determination had been developed. The active ingredients have been found to be two closely related esters, which have been designated

Pyrethrin I and Pyrethrin II, and methods have been worked out for their determination.

"In view of these developments the Administration is now in a position to put into effect the provision pertaining to a statement of ingredients as outlined in Section 8 of the Insecticide Act of 1910.

"Since Pyrethrin I and Pyrethrin II are so closely related chemically, it will not be required at present that the percentage of each be stated separately. The following form of statement, which should appear prominently on the front or main panel of the label, will be accepted as in compliance with the law.

Active Ingredients	
Pyrethrins	———— %
Inert Ingredients	———— %
Total	100 %

the correct values being inserted in the blank spaces indicated.

"The same principles of labeling will apply to insecticides containing pyrethrum powder or pyrethrins as to pyrethrum powder itself."

The methods of analysis used in determining the pyrethrin content, under the above regulations, are the official and tentative methods of the A.O.A.C. (page 448).

CHAPTER XXIV

PYRETHRUM LIVESTOCK SPRAYS

On the basis of experiments conducted during a period of about 80 days in summer, Davis *(1352)* observed the following effects of fly sprays on dairy cows. "During the first few weeks of the tests and before flies were abundant, the sprayed animals showed a decrease in milk production. In other words spraying seemed to have a definite adverse effect on the animals. However, as the animals became accustomed to the spray and as the flies became increasingly abundant, the treated animals returned to their normal or near normal milk production, while the unprotected animals showed a gradual but distinct decline in milk production. The net result was that the check or unsprayed lot showed a total net rate of decline of 32 per cent for the entire time of the experiment, while the average total net rate of decline for all sprayed lots combined was only 13.4 per cent, or a difference of 18.6 per cent in favor of the sprayed cows. Factors, such as lactation, were considered."

Shaw and Atkeson *(2115)*, in experiments extending through July and August, observed no abnormal decline in milk flow when good sprays were properly applied. There was a normal decline in production of both sprayed and unsprayed cows. No skin injuries or other ill effects were observed. The sprays were made with colorless, odorless mineral oil base and contained pyrethrum extract and pine oil, or pyrethrum extract and ethylene glycol ether of pinene (D.H.S. Activator), or fenchyl thiocyanoacetate (Thanite) alone. The amount of spray applied daily to each animal was 50 cc.

Searls and Snyder *(2107)* consider a solution of 0.1 g. pyrethrins per 100 cc. of mineral seal oil the most effective spray for keeping cows reasonably free from flies from milking time in the morning to after milking time in the afternoon. The oil had a viscosity of 45 seconds and 1⅓ ounces of spray were applied to each cow. Stable flies, which annoy the animals, seldom enter the barn and are seldom active before nine in the morning.

Howell and Fenton *(1663)* investigated the repellent action of a spray containing 0.04 g. pyrethrins per 100 cc. plus 3 per cent Lethane 384 and 1 per cent Lethane 384 Special. This spray

applied to cows at rates of 0.5 cc., 1 cc. and 2 cc. per 3.23 sq. ft. of body surface area was repellent to the hornfly for a period up to 10.5 hours after spraying. Similar application rates were less repellent to the stablefly, both as to amount and duration. Little repellency against this species was observed after four to five hours following spraying. As the time interval following spraying increased, the amount of repellency to both species decreased. In parallel tests, 2 cc. of spray per 3.23 sq. ft. of surface area was more repellent to the hornfly than 0.5 cc. for 7 hours after the morning sprayings. At other times no significant differences were noted. The heavier application was more repellent to the stablefly only for 4 hours after morning spraying, and for 2 hours after the evening spraying. The hornfly infestation was greater in the morning than during the afternoon, but the stablefly infestation was greatest in the evening.

Adams *(5)* has patented the use of alkyl phthalates in combination with pyrethrum sprays, an improvement on the patent of Moore and Buc *(1906)* covering insect repellents comprising alkyl phthalates. Simanton *(2132)* has patented oil solutions of diethylene glycol monobutyl ether or diethylene glycol monoethyl ether acetate as insect repellents.

Law *(1792)* states pyrethrum sprays are useful in fur farming for control of fleas, ear mites, flies and other pests.

Haseman and Roland *(1621)* consider pyrethrum effective for killing warble fly larvae on cattle.

Cory, Harns and Anderson *(1312)* investigated the use of pyrethrum dusts for controlling flies on cattle. They concluded that impregnated dusts were more effective than dusts made by mixing pyrethrum powder with fillers. Impregnated dusts were also more effective than sprays. Pine oil added little to the efficiency of the dusts. Stable flies were more susceptible to the dusts than houseflies.

Shaw *et al. (2116)* have reported the results of a two-year study of repellents for stable flies. Ethylene glycol ether of pinene (D.H.S. Activator) and pine oil were not effective repellents, either alone or when added to pyrethrum sprays. Fenchyl thiocyanoacetate (Thanite) was the most effective of the several repellents tested.

Atkeson *et al. (1136)* tested the effects of seventeen livestock spray oils on cattle. These oils were applied at the rate of 10 cc. on 2 sq. ft. of skin surface daily, for 30 days. The specific gravity, viscosity, flash point, neutralization number, distillation range

and unsulfonatable residue were determined on each oil. The unsulfonatable residue was the best index of the effect of an oil on the skin. Oils containing 92.5 per cent or more unsulfonatable residue caused the least injury; those containing 87.5 per cent or less caused soreness, sometimes accompanied by thickening and cracking of the skin and loss of hair.

Atkeson and his associates *(1138)* also determined the toxicity of sixteen fly sprays to houseflies. The applications were made in a barn, at the rate of 1 cc. of spray per 36 cu. ft. The following conclusions were drawn: "Thanite was the best in killing power of any single toxicant used in base oil." "Pyrethrum and Lethane were among the least efficient toxicants in killing power, but when D.H.S. Activator was added to a pyrethrum spray containing 3.75 per cent or more of a 20 to 1 concentrate, the pyrethrum sprays ranked among the most efficient." "Pyrethrum was the least efficient in knockdown . . ."

These conclusions of Atkeson, Smith, Borgmann and Fryer are unusual, to say the least. Inspection of their data shows that pyrethrum was used alone in one base oil, "A," but the combinations of pyrethrum and D.H.S. Activator were used in a second base oil, "E." Furthermore, base oil "A" had a specific gravity of 0.8275 and distilled at 506° to 590° while the specific gravity of base oil "E" was 0.7809 and it distilled at 392° to 488°. Thanite was used in three oils, "B," "C," and "D," but it was not used in oil "A." It is, of course, well known that the knockdown and kill of pyrethrum sprays are greatly influenced by the type of base oil used.

Numerous counts of flies in dairy barns by Atkeson and others *(1137)* indicated that nearly 100 per cent of the flies were house flies. Spraying the barn gave effective control, even in bedded barns, if the windows were screened.

CHAPTER XXV

PYRETHRUM HORTICULTURAL POWDERS, DUSTS AND SPRAYS

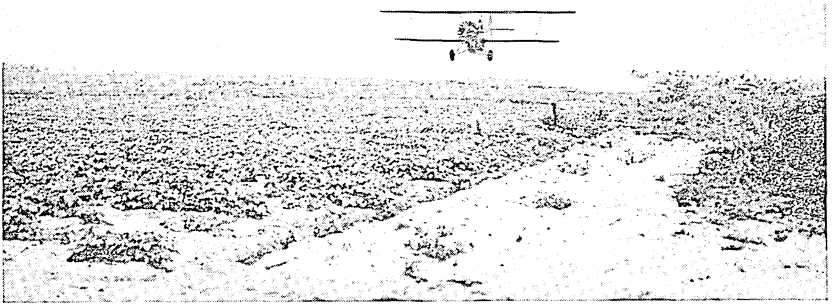
Within the last ten years, the use of pyrethrum insecticides for horticultural purposes has greatly increased. This has been due largely to the development of more efficient and less costly pyrethrum dusts and to the demand for insecticidal materials that offer no hazard to public health.

When supplies of pyrethrum for civilian use were cut off by the War Production Board in 1943, the extent to which agriculture had depended on pyrethrum was made evident. In April 1945, limited amounts of pyrethrum that did not meet military specifications were released for the control of insects attacking beans, cole crops, sugar beet seed-stock, grapes, cranberries, celery, mushrooms and certain orchard crops. These were uses of pyrethrum for which no satisfactory substitutes existed.

Pyrethrum Dusts

The most effective method of utilizing the pyrethrin content of pyrethrum flowers for horticultural purposes is that patented by Gnadinger *(1512)*. So effective and economical is this material, Dry Pyrocide, in utilizing pyrethrins that its manufacture was permitted by the War Production Board until August 1943 in order to provide pyrethrum dusts for crops on which substitutes could not be used. In Dry Pyrocide the pyrethrins are formulated in such a manner that a finished dust containing 0.1 per cent pyrethrins will frequently control insects for which 0.3 to 0.6 per cent of pyrethrins is required when powdered pyrethrum flowers are used. Dry Pyrocide was the first of the so-called "impregnated" dust concentrates. It should not be confused with dust concentrates which are by-products of the manufacture of concentrated pyrethrum extract.

The marc, or exhausted pyrethrum, resulting from the manufacture of pyrethrum extract, may contain from 0.05 to 0.15 per cent pyrethrins which cannot be extracted economically; the amount of pyrethrins in the marc depends on the extraction process used. This exhausted pyrethrum has some slight insecticidal value and it has been used in mixtures with pure pyrethrum and other insecticides. It has also been finely powdered and



AIRPLANE APPLICATION OF PYRETHRUM DUST TO BEANS.

fortified with concentrated pyrethrum extract, thus forming a solid concentrate, usually containing about 0.5 per cent pyrethrins. In some cases synergists have been added.

The inert filler used in horticultural pyrethrum dusts is of considerable importance. Certain materials such as clays are entirely unsuitable and some forms of talc give much better results than others. Pyrophyllite forms efficient dusts, but is somewhat abrasive on the metal parts of dusters. This abrasiveness can be avoided if the pyrophyllite is micronized.

Wilson, Dieter and Burdick *(2329)* correlated the frictional electrostatic charges carried by dusts with their insecticidal efficiency. "Dusts producing comparatively high charges generally give higher mortality than dusts producing low charges. Pyrophyllite, flaky talcs, calcium carbonate, and gypsum produce comparatively high charges and give high mortality. The clays and other talcs studied produce low charges and give low mortality. When oil is added to dusts the electrostatic charge may be increased or decreased according to the diluent used."

The density and flowability of insecticidal and fungicidal dusts have been investigated by Wilson and Vogel *(2332)* and by Wilson and Irons *(2331)*.

When materials of widely different densities are mixed together in dusts, there may be some separation of ingredients when the dust is applied to a crop, the heavier materials falling near the nozzles of the duster and the lighter materials falling

farther away. This is likely to occur in mixtures of derris and talc or powdered pyrethrum flowers and talc. It does not occur in a dust made from Dry Pyrocide.

A dust containing 10 per cent Dry Pyrocide and 90 per cent pyrophyllite was discharged from a duster nozzle into a long tunnel. Samples were collected from settling stations at intervals of 8 feet and were assayed for pyrethrin content. There was little variation in pyrethrin content, as the following analyses show:

Distance from Nozzle Feet	Pyrethrins %
0*	0.20
8	0.19
16	0.19
24	0.21
32	0.22
40	0.22
48	0.23

*Original sample.

Huckett *(1667)* was one of the first to observe that pyrethrum dusts made with clay are less effective than those made with talc, gypsum or infusorial earth.

Thirty-seven different talcs, pyrophyllites and other diluents were examined by Wilson and Janes *(2330)* to determine their suitability in rotenone dusts. These materials came from the following states:

California—five deposits of talc and one of pyrophyllite.
Georgia—four deposits of talc.
Maryland—two deposits of talc.
New York—three deposits of talc.
North Carolina—three deposits of talc; two deposits of pyrophyllite; one deposit of mica.
Vermont—one deposit of talc.
Virginia—two deposits of talc.
Washington—one deposit of a magnesite.
Wisconsin—one deposit of talc.

A pyrophyllite and a calcium carbonate were superior to talcs for rotenone dusts. High alkalinity of some diluents is believed to have caused immediate deterioration of rotenone.

Turner *(2248)* has reviewed the effect of diluents in various dusts and has confirmed the work of Wilson and Janes on diluents for rotenone dusts.

Control of the squash bug, *Anasa tristis*, with pyrethrum dust was first obtained by Hoerner *(1648)*. A dust containing 0.33

per cent pyrethrins (Pyrocide Dust) gave practically 100 per cent kill of all bugs hit. Applications were made at the rate of about 15 pounds per acre.

Hoerner, with reference to experiments on control of squash bugs, states: "Out of 200 individual cage tests with various concentrations of dusts and liquid sprays, Dry Pyrocide, a concentrated stabilized pyrethrum dust, with finely ground gypsum, gave the best control. This insecticide, when mixed 1 part with 5 parts gypsum, gave practically 100 per cent kill on the bugs hit with the dust." *(1647)*. The pyrethrin content of the finished dust was 0.3 per cent.

Beard *(1186)* controlled the squash bug with pyrethrum dust containing 0.2 per cent pyrethrins or with pyrethrum spray made by emulsifying one part of kerosene extract of pyrethrum in 500 parts of water. The pyrethrum extract contained 2 g. pyrethrins per 100 cc.; the emulsifier, Ultrawet, was used in the proportion of 1 to 1000. Control was directed against the nymphs, since they are easier to kill than adult bugs.

Eichman *(1417)* obtained as high as 95 per cent kill of squash bugs, with pyrethrum dust containing 0.2 per cent pyrethrins, applied at the rate of 40 pounds per acre. Reinfestation by migration of bugs from other fields occurred, making frequent applications of dust necessary.

Regarding experiments employing pyrethrum dusts for control of pentatomids attacking tomatoes, Mundinger *(1918)* states: "Dry Pyrocide as used in these tests was much more toxic to this species of pentatomid than were any of the other materials applied. The difference in degree of toxicity of the insecticides tested is so great as to suggest Dry Pyrocide as a most promising material for control of these insects."

The western twelve-spotted cucumber beetle, *Diabrotica soror,* is a serious pest of ripening deciduous fruits in California orchards. The best control for these beetles, according to Michelbacher, MacLeod and Smith *(1891, 1892)*, is a dust composed of ground pyrethrum flowers and talc, containing 0.1 to 0.2 per cent pyrethrins and 1 to 2 per cent of special solvent, and applied at the rate of 50 pounds per acre. Pyrocide Dust containing 0.1 per cent pyrethrins gave equal fruit protection, but mortality was not so high as that obtained with the other dust.

The western spotted cucumber beetle causes serious injury to beans in Oregon. Mote and Thompson *(1914)* controlled this beetle with pyrethrum dusts and sprays.

Pyrethrum has been widely used for the control of insects attacking cranberries. At first, finely powdered pyrethrum containing 0.9 per cent pyrethrins was used but this was later replaced by the more economical dusts containing 0.2 to 0.3 per cent pyrethrins. The blunt-nosed leafhopper, *Ophiola striatula,* carries the virus that causes the disease known as false blossom. Pyrethrum dust is very effective against this insect and its use for this purpose has been studied by Franklin *(1456)*, Doehlert *(1372, 1373, 1374, 1375)*, Beckwith *(1193, 1196, 1197)*, Wilcox *(2322)* and Kelsall *(1724)*. Beckwith *(1196)* states: "Impregnated dust containing from 0.3 to 0.4 per cent pyrethrins appears to be as effective as the pure pyrethrum flowers which analyzed 0.9 per cent." Doehlert *(1376)* observed no serious destruction of bees in dusting cranberries with pyrethrum. Doehlert *(1377)*, seeking a substitute for pyrethrum, found Lethane dusts less effective than pyrethrum against blunt-nosed leafhopper. Dinitro-*o*-cyclohexylphenol (DN) dusts gave results similar to those obtained with Lethane. Pyrethrum dusts have also been used successfully on cranberries by Franklin for control of the black-headed fireworm, *Rhopobota naevana,* gypsy moth, *Porthetria dispar,* and the cranberry girdler, *Crambus topiarius, (1456)*. Franklin *(1458)* has suggested the use of pyrethrum-cryolite dusts for control of the black-headed fireworm. Pyrethrum dusts are applied at the rate of 30 to 50 pounds per acre.

Pyrethrum dusts were successfully used by Pepper and Haenseler *(1983)* for controlling *Macrosteles divisus.* Control of this leafhopper, which transmits the virus of lettuce yellows, resulted in a reduction of the disease on lettuce.

Linn *(1808)* also obtained a significant decrease in the yellows disease of lettuce by controlling this leafhopper, using a pyrethrum-sulfur dust containing 0.15 per cent pyrethrins, applied at weekly intervals at the rate of 35 pounds per acre.

The use of pyrethrum dusts for the control of the potato leafhopper, *Empoasca fabae,* has been investigated by Mader and his associates *(1829, 1830)*. Pyrethrum dust besides killing the leafhoppers seemed to have a stimulating effect on the growth of potatoes and caused an increase in the blooming of the plants *(1831)*. Rinke has patented a plant stimulant containing petroleum distillate and exhausted pyrethrum *(2047)*. Skaptason *(2138)* has expressed the opinion that if any stimulation of potato plants occurs following application of pyrethrum dusts, it is probably due to the irritating effect of the oil which the

APPLYING PYRETHRUM DUST FOR CONTROL OF BEAN LEAFHOPPER.

dusts contain, rather than to any effect of the pyrethrins on the plants. Skaptason and Blodgett *(2140)* noted a decrease in the fungicidal efficiency of cuprous oxide when used with pyrethrum dusts on potatoes. The composition of the pyrethrum dusts is not stated. Pyrethrum-sulphur dust is recommended by Batten and Poos *(1181)* for controlling heavy infestations of potato leafhopper on peanuts. Watkins *(2281)* found pyrethrum dusts effective for the control of the clover leafhopper, *Aceratagallia sanguinolenta.*

Coon and Wakeland *(1309)* prevented the feeding of the beet leafhopper on tomatoes in the greenhouse by applying a dust containing 0.23 per cent pyrethrins. In the field, however, such applications were only partially effective.

Pyrethrum dust containing 0.5 per cent pyrethrins was among the most effective of the materials tried by Morrill *(1910)* against the potato flea beetle on shade-grown tobacco. A similar dust was found to be practicable for controlling the tobacco thrips *(1911).*

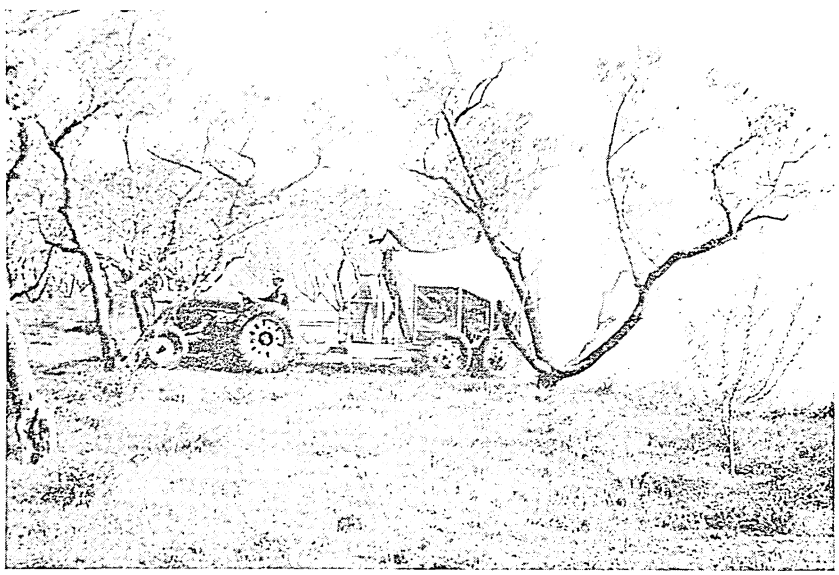
Lacroix *(1760)* controlled thrips and flea beetles on shade-grown tobacco with pyrethrum dusts. Morrill and Lacroix *(1912, 1913)* finally concluded that pyrethrum dusts gave better control of tobacco thrips, in terms of wrapper damage, than any other treatment.

Several species of *Lygus* have caused severe damage to peaches in the Pacific Northwest. Moore and Fox *(1905)* controlled this damage using a Pyrocide Dust containing 0.2 per cent pyrethrins. Three dusts were applied at the rate of 40 to 50 pounds per acre. The first dust was applied when the buds were in the pink stage. The second was applied just after petal fall and the third, just after shuck fall. The harvested crop was 98 per cent free from Lygus injury.

Notley *(1951)* controlled *Antestia* and *Lygus* on coffee by dusting with finely ground pyrethrum flowers at the rate of 7.5 pounds of powder per acre of 600 trees. Kills of 94 to 98 per cent were obtained.

A prolonged investigation of the field control of *Lygus* bugs in seed alfalfa was made by Sorenson *(2167, 2168)*. Pyrethrum dust (Pyrocide Dust) containing 0.1 to 0.2 per cent pyrethrins was the most efficient insecticide found. It was applied at the rate of 15 to 30 pounds per acre, but the cost was excessive because of the frequent applications necessary to control reinfestations.

Fisher and Shull *(1447)* obtained good control of *Lygus* bugs in seed alfalfa with pyrethrum-rotenone dust. They also found heavy reinfestations shortly after dusting. Lieberman obtained better control of *Lygus* with DDT than with pyrethrum.



APPLYING PYRETHRUM DUST TO PEACHES FOR CONTROL OF *Lygus*.

Several species of *Lygus* cause severe damage to sugar beets grown for seed. Field tests by Hills and Romney *(1638)* indicated that when *Lygus* can be controlled, an increase in viable seed will result. On light infestations they obtained good control with pyrethrum dusts and with atomized pyrethrum-oil spray but there was some damage to the beets from the latter. Further work was done by Hills *(1635, 1636)* on heavy infestations in 1942 and 1943. The best treatment developed was a pyrethrum-sulfur dust containing 0.2 per cent pyrethrins and 50 per cent sulfur. Two applications of this dust were as good as three applications of the other dusts tested which included dusting sulfur, phenothioxin-sulfur dust and sulfur-arsenical dust. Results with dinitro-*o*-cyclohexylphenol dust alone were markedly inferior. Later, Hills concluded that a dust containing 4.5 per cent DDT gave better control than any other tested *(1637)*.

Manson *(1837)* controlled the sugar beet webworm and the gray and black blister beetle with pyrethrum dust containing 0.15 per cent pyrethrins.

Roney *(2073)* controlled the Hawaiian beet webworm, *Hymenia fascialis*, on beets with weekly applications of pyrethrum-sulfur dust containing 0.1 per cent pyrethrins, at the rate of 25 pounds per acre.

Walker and Anderson *(2272, 2273)* used pyrethrum dusts containing 0.1 to 0.2 per cent pyrethrins to obtain control of Hawaiian beet webworm on spinach; 35 to 40 pounds of dust were applied per acre.

Kelsall *(1724)* and Patterson *(1976)* obtained excellent control of the green apple bug, *Lygus communis*, apple redbug, *Lygidea mendax*, and pale apple leafhopper, *Typhlocyba pomaria*, using a pyrethrum dust composed of 30 per cent pyrethrum powder and 70 per cent gypsum. The pyrethrum powder contained 0.9 per cent pyrethrins and the finished dust had a pyrethrin content of 0.27 per cent; 50 to 90 pounds of dust per acre were applied. Pyrethrum powder was mixed with water at the rate of 3 pounds per 100 gallons and applied as a spray, with good results. Dean *(1355)* also found pyrethrum dusts containing 0.2 per cent pyrethrins very effective against apple redbug.

Gnadinger, Moore and Coulter *(1515)* made an extended investigation of the use of pyrethrum dusts for codling moth control. A dust containing 0.2 per cent pyrethrins was very effective against adult moths and all stages of larvae. Twenty applications of dust were made between May 5 and September 20. Five appli-

cations of oil sprays, containing 0.5 to 0.75 per cent of medium oil, were made for ovicidal action and to control mites.

A control plot in the same orchard, which was very heavily infested, received a calyx spray and 11 cover sprays of lead arsenate. The apples harvested from the plot dusted with pyrethrum had about the same percentage of wormy apples as those from the plot sprayed with lead arsenate, but the latter had five times as many stings. The dusted apples matured earlier, had better color and a much higher proportion of extra fancy grade than those sprayed with lead arsenate. It was not necessary to wash the apples dusted with pyrethrum to remove lead arsenate. Other insects killed by the dust were rosy aphids, leaf rollers, buffalo tree hoppers and several species of *Lygus*. The cost of the pyrethrum dust program was slightly higher than the lead arsenate spray program.

Pyrocide Dust, containing 0.2 per cent pyrethrins, was applied by Beckwith *(1199)* to cultivated blueberries for the control of the fruit worm, *Mineola vacinii*. Two applications, at the rate of 30 pounds per acre, reduced the number of worms on harvested fruit by 94 per cent.

Huckett *(1670)* reported on five years of field experiments with non-arsenical dusts for cabbage worm control. Pyrethrum dusts were more effective than rotenone dusts against the cabbage looper, *Autographa brassicae*. Dusts containing 0.6 per cent pyrethrins gave the best control. Talc was the best diluent.

Neiswander *(1940)* recommends cryolite and pyrethrum dusts for the control of the strawberry leaf roller, *Ancylis comptana*, the pyrethrum dust to be used in the application immediately preceding harvest. Lamerson and Parker *(1780, 1781)* found strong pyrethrum dusts effective against the American strawberry leaf roller, *Ancylis fragariae*, after the leaves were rolled, giving 97 per cent control.

Bryant and Beach *(1251)* recommend pyrethrum dusts for control of strawberry ground beetles, *Harpolus caliginosus*, and strawberry weevil, *Anthonomus signatus*.

Bailey *(1151)* obtained the best control of bean thrips, *Hercothrips fasciatus*, with pyrethrum dusts containing 0.25 per cent pyrethrins, applied to beans at the rate of 25 pounds per acre. This dust killed many of the newly emerged larvae up to 5 days after application in the field under normal summer weather conditions.

List *(1810)* recommends the use of pyrethrum dust containing 0.2 per cent pyrethrins for control of tomato psyllid during

the harvest season, when the presence of excessive sulfur on the fruit is undesirable.

Thomas *(2220, 2221)* found pyrethrum dusts effective for controlling the tomato pin worm, *Gnorimoschema lycopersicella*.

An excellent description of insects attacking mushrooms and a discussion of the use of pyrethrum for the control of some of them has been given by Thomas *(2224)*. Pyrethrum dusts are recommended against Sciarid, Phorid, Cecid and Chironomid flies, sowbugs, loopers and certain beetles.

Pickles *(1991, 1992, 1993)* obtained kills of 80 to 99 per cent of adult sugar-cane froghoppers with a pyrethrum-sulfur dust containing 0.5 per cent pyrethrins and 50 per cent of sulfur. The dust was applied, in large scale tests, at the rate of 10 pounds per acre.

Hamilton *(1584)* recommends pyrethrum dusts for the control of the orchid weevil, *Diorymerellus laevimargo*.

Loucks *(1816)* states dusts containing 0.05 to 0.08 per cent of pyrethrins are effective against grape leaf skeletonizers and aphids.

Kelsell and Stultz *(1726)* found pyrethrum dusts effective against more insects than derris dusts but the latter were effective against some species, including flea beetles and imported currant worm, for which pyrethrum was not satisfactory. Toxicity was less persistent at high temperatures and in sunlight.

Pyrethrum Sprays

The use of light mineral oil for control of the corn earworm, *Heliothis obsoleta*, was suggested by Barber in 1938 *(1162)*. In this method of control, 0.5 to 1 cc. of oil is applied to the tips of the ears of corn by means of a force oil can, set to deliver a definite quantity of oil. Application is made within the husk, several days after the silks are first exposed. Since the oil alone was not sufficiently effective, Barber later tested 67 materials or combinations of materials to increase the insecticidal action. He found that the addition of 1 per cent of pyrethrins to the oil greatly increased the control *(1163, 1164, 1165)*. Pepper and Barber *(1982)* substituted dichloroethyl ether for pyrethrins and obtained good control, but corn treated in September had an off-flavor, probably because of the slower evaporation of dichloroethyl ether in cool weather. The use of dichloroethyl ether was further investigated by Barber *(1166, 1167)*.

Carruth *(1276, 1277, 1278)* obtained relatively good control with oils containing as little as 0.1 per cent pyrethrins or 1 per cent dichloroethyl ether.

Bailey *(1157)* tried to control corn earworm in Puerto Rico by clipping the tips of the ears of sweet corn, as suggested by Emmert *(1422)*, by using mineral oil alone and by using mineral oil containing pyrethrum, or derris or pyrethrum and derris. Oil containing pyrethrum caused less burning and gave satisfactory control of corn earworm, corn silk fly, *Euxesta stigmatias*, and fall armyworm, *Laphygma frugiperda*. Emmert and Price *(1423)* obtained better control by clipping than by the use of pyrethrum-oil.

Because of the war-time shortage of pyrethrum, Barber *(1169)* made an investigation of the advisability of substituting dichloroethyl ether for part of the pyrethrum in sprays used for corn earworm control. Judged by the percentage of larvae killed and the percentage of ears containing no larvae, sprays made from white oil containing 0.1 per cent pyrethrins and 2 per cent dichloroethyl ether gave protection superior to that obtained with 0.2 per cent pyrethrins or 2 per cent dichloroethyl ether alone in oil. A combination of 0.1 per cent pyrethrins and 1 per cent dichloroethyl ether or 0.15 per cent pyrethrins and 0.5 per cent dichloroethyl ether gave protection equal to that obtained with 0.2 per cent pyrethrins or 2 per cent dichloroethyl ether.

Wilcox *(2320)* observed extensive commercial applications of oil insecticides for corn earworm control in California; he concluded: "These tests demonstrated that oil containing 0.2 per cent pyrethrins is superior to oil alone and to oil containing 2 per cent of dichloroethyl ether, when applied at the rate of three-fourths ml. per ear. If not carefully timed, all treatments may damage the tips of the ears. In southern California it is necessary to go over the fields twice, in order to treat the ears at the right stage of development. The first application should be made when about 60 per cent of the ears are pollenized. The treated ears should be marked, so that they may easily be skipped when the balance of the ears are treated after a 4- or 5-day interval. Treatment with the oil-pyrethrum mixture consistently protected 80 per cent or more of the ears from injury. This percentage is necessary in order that the corn may be marketed as 'worm-free'." The cost of the oil-pyrethrum treatment varied from $8.50 to $13.00 per acre. The ears were marked, as they were treated, with lumber crayon or thin paint. Oil of 100 viscosity caused more damage than oil of 180 viscosity. Oil contain-

ing dichloroethyl ether caused more damage than oil containing pyrethrum.

The results of field tests conducted by Barber *(1168)* showed that: "Styrene dibromide (α, β-dibromoethylbenzene) when used at the rate of 0.75 gram or more per 100 cc. of white oil afforded protection to sweet corn ears at a rate that compared favorably with that resulting from applications of oil containing 0.2 per cent of pyrethrins, 2 per cent of dichloroethyl ether, or 2 per cent of ethylene dichloride. The chemical is slowly soluble in oil, imparts no color to it, and the solution causes no injury or change in appearance of any part of treated ears. Persons eating treated ears failed to detect residue on the kernels. The results of feeding treated ears to rabbits showed that the chemical was harmless to them when eaten in small quantities. The results of toxicity tests on earworm larvae showed that oil-styrene dibromide was a contact insecticide but that larvae often lived for several days after treatment, although they usually did not eat during this time."

The use of styrene dibromide for corn earworm control was also investigated by Barber and Wilcox *(1172)*. Davidson *(1345)* reported better control with styrene dibromide than with pyrethrum or by clipping. Barber *(1171)* concluded that oil-pyrethrum was more effective in ears having short loose husks and oil-dichloroethyl ether was more effective in ears with longer, tighter husks.

Barber *(1170)* also studied the effect of insecticide sprays used for corn earworm control on the growth of the tips of sweet corn ears. He concluded: "In commercial and experimental use of the mineral-oil treatment for earworm control in sweet corn, an increase in the length of undeveloped tips of treated ears was the result of (1) inhibition of the development of immature kernels if the oil reached them, and (2) interference with pollination if a secondary growth of the cob occurred, as evidenced by late appearance of a tuft of fresh silk from the tip kernels. Whether oil did or did not reach the kernels was determined by the length of the husk extension and the dosage of oil. No significant difference was found between the increase in length of undeveloped tips caused by the oil alone and by oil containing insecticides. Oil applied at a dosage of 0.5 cc. to ears having a husk extension of at least 2 inches caused negligible increases in the length of the undeveloped tips, but the length of the undeveloped tip was increased when the husk extension was less or the dosage was greater. The evidence showed that it was safer to begin the treat-

ment on the seventh day rather than the sixth day after silk exposure. The average increase in the length of undeveloped tips caused by commercial or experimental oiling of Golden Cross Bantam sweet corn was not more than 0.4 inch per ear. In 7-inch ears this represents a loss of only about 5 per cent of the kernels. This loss is thought to be of very minor importance, as compared with the great improvement in quality, quantity, and value of the crop resulting from the proper application of the treatment."

Among other investigators who have found oil-pyrethrum satisfactory for corn earworm control are Michelbacher *(1889)*, Ditman, Secrest and Cory *(1371)*, Pepper *(1981)* and Fisher and Shull *(1446)*.

Webster and Eichmann *(2291)* obtained good control of corn earworm with oil containing phenothiazine or dinitro-*o*-cyclohexylphenol, applied at the rate of 0.6 to 1.3 ml. per ear.

Fall infestations of the beet leafhopper, *Euttetix tenellus*, transmit a virus disease known as curly top to beets grown for seed.

Douglass, Wakeland and Gillett *(1381)* found pyrethrum-oil spray "the only insecticide specific against the beet leafhopper." The spray contained 20 gallons of kerosene, 10 gallons of light-medium summer spray oil and 1 gallon of pyrethrum extract containing 2 g. pyrethrins per 100 cc.; it was applied at the rate of 6 to 8 gallons per acre with a blower-type vaporizer.

Experiments conducted during a six year period by Romney *(2071, 2072)* showed significant reductions of curly top and increases in yields of seed when the beet fields were sprayed in the fall with pyrethrum-oil spray. The spray was composed of 10 parts white oil, 20 parts kerosene and 1 part pyrethrum extract containing 2 g. pyrethrins per 100 cc. Applications were at the rate of 6 to 9 gallons per acre.

Campbell *(1270)* obtained approximately 98 per cent control of the sugar beet leafhopper with an oil-pyrethrum spray containing 0.1 per cent pyrethrins, applied as a fog.

Boyce and Mabry *(1241)* first recommended the use of pyrethrum extract and light-medium spray oil emulsified in water as a spray for controlling greenhouse thrips on citrus. This insect, *Heliothrips haemorrhoidalis*, is an important pest of Valencia oranges in California.

The aqueous spray recommended by Quayle *(2015)* for control of greenhouse thrips and black scale, *Saissetia oleae*, on citrus consists of 1.67 per cent light-medium or medium spray oil to which is added ¼ to ½ pint of pyrethrum extract containing

2 g. pyrethrins per 100 cc. for each 100 gallons of aqueous spray. This is applied in summer or fall. If a supplemental spring application is necessary, ½ to ¾ per cent of light-medium oil is used.

Ebeling *(1402)* also recommends the addition of one-fourth pint of pyrethrum extract (2 grams pyrethrins per 100 cc.) to 100 gallons of regular spray containing 1.67 per cent light-medium oil, for control of greenhouse thrips on citrus.

In experiments by Bartlett and Persing *(1177)*, pyrethrum proved to be extremely toxic to adult thrips, either as dust containing 0.2 per cent pyrethrins or as aqueous spray containing 0.5 per cent light-medium oil and 0.06 to 0.12 per cent pyrethrum extract equivalent to 0.0012 to 0.0024 per cent pyrethrins.

Watson *(2284)* suggests dipping onion sets in pyrethrum extract to destroy onion thrips, *Thrips tabaci.*

On the Pacific Coast, pyrethrum sprays are used on cranberries for the control of fireworms, tipworms and fruitworm millers *(1329)*. Pyrethrum extract containing 2 g. pyrethrins per 100 cc. is used in the proportion of 1 quart to 100 gallons of water with ½ pint of emulsifying spreader. Two gallons of kerosene may be added if the spreader is increased to one pint. Sprays are used at the rate of 300 to 400 gallons per acre.

A pyrethrum-oil spray program was developed by Gnadinger *(1514)* for the control of over-wintering codling moth larvae. The limbs of apple trees were scraped with the exception of about 6 feet of the trunk. This caused the larvae to migrate to the unscraped trunk to spin their cocoons under the rough bark. After all larvae had hibernated, a pyrethrum-light mineral oil spray, containing 0.07 per cent pyrethrins, was applied to the unscraped bark. Kills of 97 to 99 per cent of larvae in the unscraped portion of the tree were obtained, corresponding to about 88 per cent of all larvae in the tree. This spray caused some damage to trees when freezing weather followed immediately after its application.

Yothers, Carlson and Cassil *(2358)* used a pyrethrum-oil spray to kill hibernating codling moth larvae. The emulsion type spray, which readily penetrated the cocoons under rough bark and in crotches and pruning scars, contained 0.05 per cent pyrethrins, 3 per cent ethanolamine oleate, 1 per cent of a mixture of equal parts ethylene glycol monobutylether and trichloroethylene and 15 to 20 per cent stove oil having unsulfonatable residue of 70 per cent and viscosity of 32 seconds. The kill was 91 to 93 per cent.

It is well known that chemically treated bands of corrugated paper are only partially effective for control of codling moth because many of the mature larvae do not enter the bands, but spin their cocoons elsewhere in the tree. Yothers and Carlson *(2357)* sought a repellent, which could be applied to the trunks and scaffold limbs of apple trees, thus causing the larvae to enter the bands. "Of some 250 formulas tested, the best results were obtained with combinations of 5 per cent pyrethrum extract with either 5 or 10 per cent of cottonseed oil, emulsified with blood albumin. Undiluted pyrethrum extract was highly repellent but the repellent effect decreased with long periods of exposure. Cottonseed oil undiluted was considerably less repellent, although its repellency increased with longer exposure. Kerosene alone was neither repellent nor attractive and when combined with pyrethrum extract reduced the repellency of that material." Whether this promising repellent applied to the trees will cause a larger proportion of larvae to enter the bands has not been determined.

Garman and Townsend *(1476)* noted that pyrethrum apparently has little effect on the enemies of the European red mite, although strong pyrethrum sprays will probably kill the lady-beetle predator.

An oil-pyrethrum spray recommended by Essig and Hoskins *(1424)* contains:

Pyrethrum extract (2 g. pyrethrins per 100 cc.)	1 to 2 pints
Summer grade commercial oil emulsion, emulsive oil or tank mix oil with 4 oz. of blood albumin spreader	2 gallons
Water to make	100 gallons

This is used for the control of artichoke plume moth, thrips on avocado and cotton dauber.

Richardson, Deonier and Simanton *(2039)* used pyrethrum sprays in laboratory and field tests against adult chinch bugs with successful results. Bovey *(1236)* recommended a pyrethrum-soap spray for the control of the pear blossom weevil.

Aqueous dilutions of pyrethrum sprays are used to remove leaftiers from celery before shipping it to market. The crated celery is immersed in a weak solution, such as a 1:400 or 1:600 dilution of a spray concentrate containing 1.4 per cent pyrethrins. This causes the leaftiers to stop feeding and loosen their hold; rinsing with water removes them from the celery. A similar treatment is used to remove aphids and webworms from spinach.

Anderson and Walker *(1014)* controlled tomato pinworm with several pyrethrum and pyrethrum-rotenone sprays. These sprays also gave practically complete control of pinworm eggs, spinach aphids and whitefly.

Hartzell and Wilcoxon *(1613)* used an aqueous spray containing 0.02 per cent pyrethrins and 0.5 per cent Tergitol 7 against adult Japanese beetles. Kills of 85 to 100 per cent were obtained; there was some injury to five of the 26 species of plants sprayed.

The use of pyrethrum in the control of insects attacking agricultural crops has been discussed in a valuable compilation by Essig and Hoskins *(1424)*. Among the insects for which pyrethrum is recommended by these authors are cucumber beetles, greenhouse thrips, bean thrips, beet leafhopper, green peach aphid, whitefly, cabbage aphid, squash bug, green cabbage worm, black citrus aphid, parsley caterpillar, celery leaftier, chrysanthemum aphid, corn earworm, lygus bug, false plume moth, grape thrips, grape leafhopper, alfalfa semilooper, cotton aphid, oak aphids, pear thrips, canker worm, plum aphid, red-humped caterpillar, potato aphid and snapdragon plume moth.

Another compilation has been made by Watson and Tissot *(2287)*, who recommend pyrethrum for bean leafhopper, blister beetle, flea beetle, celery leaftier, corn lantern-fly, pumpkin bug, cotton stainer, onion thrips, young squash bug, pameras, burrower bugs, whitefly and tomato pinworm.

Chupp and Leiby *(1290)* found pyrethrum effective for cabbage worms, Mexican bean beetle, spinach aphid, cucumber beetles, asparagus beetles and corn earworm.

The sod webworms commonly found in bluegrass sod in Kentucky are the bluegrass sod webworm, *Crambus teterrellus*, the striped sod webworm, *Crambus mutabilis*, and the leather-colored sod webworm, *Crambus trisectus*. Jewett *(1709)* tried a number of insecticides against these pests, including pyrethrum, kerosene emulsion, lead arsenate, dichloroethyl ether, barium carbonate, nicotine oleate, derris, Loro, Lethane and poison bait. A spray containing pyrethrum was the most effective for controlling the worms in bluegrass sod. Pyrethrum extract containing 2 g. pyrethrins per 100 cc. should be diluted at the rate of 1 ounce to 4 gallons of water and applied at the rate of 2 gallons to 20 square feet.

Smith *(2155)* recommends the following spray for control of cyclamen mite, *Tarsonemus pallidus*, on chrysanthemums:

Pyrethrum extract (alcoholic, 2 g. pyrethrins per 100 cc.)	4 tablespoonfuls
Derris powder (4 per cent rotenone)	3 tablespoonfuls
Sulfonated castor oil	2 tablespoonfuls

This quantity is diluted with three gallons of water for spraying. Neither pyrethrum nor derris alone gave good control, but the above formula killed 98 per cent of the mites. For heavy infestations 2 or 3 applications, at four day intervals, are necessary.

The black blister beetle, *Epicauta pennsylvanica*, is rather resistant to pyrethrum sprays. Smith and Sullivan *(2156)* obtained only 69 per cent kill of this beetle with an aqueous spray containing 0.01 per cent pyrethrins, although complete kills of cucumber beetles, tarnished plant bugs, and garden flea hoppers were obtained.

A pyrethrum-oil aqueous spray containing 0.004 to 0.005 per cent pyrethrins in 1 to 2 per cent of light-medium oil is recommended by Lange *(1783)* for control of the snapdragon plume moth.

Underhill *(2252)* controlled the green stinkbug, *Acrosternum hilaris*, with pyrethrum sprays.

Hamilton *(1583)* employed a paste made of clay, pyrethrins and water for killing boring insects.

Trappmann and Nitsche *(904)*, in experiments on 18 species of insects, concluded that pyrethrum is clearly superior to rotenone as an insecticide.

Mannitan monolaurate has been used in combination with pyrethrum as a horticultural spray under the trade name NNOP *(2079)*.

Ovicidal Action of Pyrethrum

Breakey and Miller *(131)* reported the toxicity of pyrethrum to eggs of Angoumois grain moth, *Sitotroga cerealella*, and the black blow fly, *Phormia regina*. Maercks *(572)* found pyrethrum toxic to eggs of the codling moth, *Carpocapsa pomonella*, and the grape moth, *Polychrosis botrana*. Encouraging results against the eggs of the plum sawfly were obtained by Jancke and Maercks *(1698)* with pyrethrum. Toxicity of pyrethrum to the eggs of the rice fly, *Agromyza oryzella*, has been observed by Okazaki *(1960)*.

Potter and Tattersfield *(2007)* sprayed the eggs of various insects with aqueous pyrethrum sprays. Concentrations of 5 to

10 mg. of pyrethrins per 100 cc. of spray killed 60 to 100 per cent of the eggs of the cabbage worm, *Pieris brassicae*. Sprays containing 25 to 50 mg. pyrethrins per 100 cc. killed 85 to 100 per cent of the eggs of the Mediterranean flour moth, *Ephestia kuehniella*. Eggs of Angoumois grain moth were also killed by concentrations of 50 mg. of pyrethrins per 100 cc. of spray. Similar sprays were toxic to eggs of the diamondback moth, *Plutella maculipennis*, and to overwintering eggs of *Aphis rhamni*.

Bartlett and Persing *(1177)* found pyrethrum-oil spray effective on eggs of greenhouse thrips causing mortalities of 97 to 100 per cent.

Onoe and Fukuda *(1961)* obtained complete kills of the eggs of the rice borer, *Chilo simplex*, with an aqueous solution containing 0.009 per cent pyrethrins and 0.5 per cent soap.

Mori *(1909)* immersed eggs of the rice borer for 10 seconds in solutions containing 0.01 per cent pyrethrins; the kill of young and old eggs was 97 to 99 per cent.

Nel and Mathew *(1941)* and Naude *(1939)* reported the toxicity of pyrethrins in mineral oil to eggs of the tobacco moth, *Ephestia elutella*.

Richardson's experiments *(2040)* showed that pyrethrins are only slightly toxic to housefly eggs which were immersed for 10 to 12 seconds in an aqueous solution containing 0.04 per cent pyrethrins. Under similar conditions a solution containing 0.007 per cent pyrethrins was extremely toxic to eggs of Angoumois grain moth.

Cupples *(1335, 1336, 1337)* has compiled descriptive lists of commercial detergents, wetting, dispersing and emulsifying agents. Referring to the use of these materials in insecticides, he states: "Soaps have definite disadvantages for use with insecticides, such as incompatibility with lime-sulfur, bordeaux mixture, calcium arsenate, and other compounds containing calcium. Much of the effectiveness of soap is lost in hard water, owing to the precipitation of insoluble calcium and magnesium soaps. . . . Because of these limitations of ordinary soaps, various sulfated and sulfonated organic compounds have lately come into use. These sulfated and sulfonated compounds are extensively used in the textile industry as detergents and wetting-out agents. Many of them show promise of replacing soap as a wetting agent or emulsifying agent for use with insecticides. In addition some of them possess insecticidal value, especially against aphids."

Nelson *(1945)* has made a useful compilation of conversion tables and equivalents for use in work related to insect control.

Among synthetic organic compounds that have shown promise as agricultural insecticides, Haller *(1571)* mentions phthalonitrile, styrene dibromide, nitrostyrene dibromide, 1,4-diphenyl semicarbazide, dimethyl acridan, diisopropyl ketone semicarbazone, pentaerythrityl bromide, dinitrosopiperazine, 4,6-dinitro-*o*-cresol methyl ether, and phenoxathiin.

Swingle, Phillips and Gahan *(2199)* tested the toxicity of 979 organic compounds against a number of species of leaf-eating insects. The 33 most toxic compounds were acetone semicarbazone, *p*-aminoazobenzene, *p*-aminoazobenzene-hydrochloride, *p*-bromoiodobenzene, *p*-chloroiodobenzene, diazoaminobenzene, 4,6-dibromo-*o*-cresol, α, β-dibromoethylbenzene, α, β-dibromo-β-nitroethylbenzene, 2,5-dichloroaniline, 1,4-diphenylsemicarbazide, 4,6-dinitro-*o*-cresol, 2,4-dinitrophenol, 1,4-dinitrosopiperazine, hexachlorophenol, *o*-nitroaniline, *p*-nitrobenzyl bromide, *o*-nitrobromobenzene, *o*-nitrochlorobenzene, *p*-nitrochlorobenzene, *o*-nitroiodobenzene, *p*-phenylenediamine, phthalonitrile, thiocoumarin, xanthydrol, *p*-chloroacetophenone semicarbazone, *p*-chlorobenzenesulfonamide, cyclohexanone semicarbazone, cyclopentanone semicarbazone, 2,4-dimethyl-3-pentanone semicarbazone, ethyl methyl ketone semicarbazone, 2-furaldehyde semicarbazone and 2-nitroresorcinol.

CHAPTER XXVI

MISCELLANEOUS USES OF PYRETHRUM

Mosquito Control

The destruction of mosquitoes was one of the earliest uses of pyrethrum. The powdered flowers were sprinkled on the top of a hot stove or burned in cones or incense sticks containing potassium nitrate and sawdust, thus volatilizing a part of the pyrethrins in the room. As early as 1890, candles containing pyrethrum were burned for insect control. The extreme sensitivity of mosquitoes to pyrethrum has long been known. DDT is said to be only about one fifteenth as toxic to adult mosquitoes as the pyrethrins *(2179)*. With the development of oil sprays, the use of pyrethrum for mosquito control increased (page 272). The establishing of international airlines made it necessary to spray the planes to prevent dissemination of disease-carrying mosquitoes. Pyrethrum was used for this purpose, not only because of its quick action on mosquitoes, but because it is harmless to man. Ramsey *(2018)* states: "More than two thousand aircraft were examined at Khartoum during the period between July, 1935 and August, 1938, and excluding common housefly varieties, the total number of insect specimens was 1960, comprising no less than 146 species. This is the number captured. We do not know how many escaped capture."

At Miami, Florida, during 1938 almost 400 aircraft which had been sprayed half an hour before landing, were inspected for possible mosquito infestation. Of these, 187 planes were found to harbor dead or live insects of various species. A total of 651 insects was recovered, of which 166 were alive. Among the collection were forty-five mosquitoes, of which forty were dead. No yellow-fever carriers were found on any aircraft that year. Most prevalent insects were domestic flies, with midges, gnats and other minute flies next in number *(1064)*.

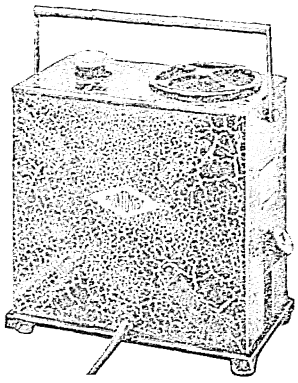
The general specifications for an insecticide for use by commercial airlines have been stated by Mackie and Crabtree *(1820)*. Their ideal insecticide for airplanes is:

1. Highly toxic to insects.
2. Non-flammable.
3. Harmless to passengers.
4. Non-corrosive and non-staining.

5. Stable during storage.
6. Miscible with water.

Davies *(1347)* gives the following specifications for insecticide used by United Air Lines:

1. A liquid insecticide to be used in a sprayer is desired.
2. It shall be highly effective against flies, mosquitoes and gnats.
3. There shall be no after odor.
4. It shall be non-corrosive and non-injurious to aluminum, fabric, leather and rubber.
5. It shall be stored in metal containers.
6. It shall be non-flammable; the flash point should be 200° F. or more, open cup.



PHANTOMYST AEROSOL SPRAYER, AIRCRAFT MODEL.

Two types of sprayers were recommended by Mackie and Crabtree *(1820)* for use on the flying boats of Imperial Airways. In the passenger compartments an electrically driven machine known as the Phantomyst apparatus was used. This disseminated a "dry" mist of particles about 6 microns in size and no condensation of the mist was noticeable, although an aqueous pyrethrum spray was employed. Regarding this apparatus the manufacturer stated, in 1939: ". . . it will turn out into the atmosphere any liquid which may be placed in it in the form of an 'aerosol' or mist, the particles of which are so small that they will not wet or condense on any objects with which they may come in contact." The pyrethrum insecticide used was apparently a concentrated emulsion or water miscible solution known as Deskito. The pyrethrin content of the concentrate is not stated, but it was diluted 1 to 10 with water and the aqueous

spray was applied at the rate of 14 cc. to 480 cubic feet, this application requiring 10 minutes. A second type of apparatus was used for baggage compartments, lockers and all parts other than passenger compartments. This consisted of a simple form of spray gun operated by a Sparklet liquid carbon dioxide bulb (Fig. XXIV). The insecticide container, holding 50 cc. of aqueous spray, was fitted into the operating head by a quick-action threaded collar. This apparatus delivered a wetter spray and used three to four times as much spray as the Phantomyst for a given space. The Phantomyst is said to have been successfully used against insects in barracks, warehouses and homes, with several types of insecticides.

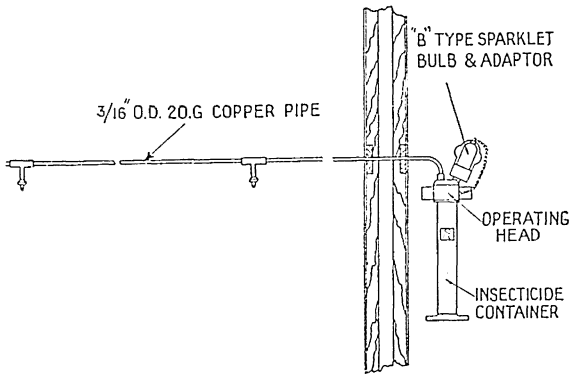


FIG. XXIV. LARMUTH'S AIRPLANE SPRAYER OPERATED BY CARBON DIOXIDE SPARKLET BULB.

Ross was an observer of the tests made by Mackie and Crabtree and held that the aqueous base extract of pyrethrum at 1 to 14 dilution was as inflammable as mixtures of mineral oil extracts with carbon tetrachloride, which Ross preferred *(2078)*. Caldwell *(1257)* preferred aqueous pyrethrum sprays for airplanes because of the lack of fire hazard.

Ginsburg *(1496)* has employed his pyrethrum larvicide (page 274) to protect outdoor gatherings from mosquito bites. The concentrated stock larvicide was diluted with 10 to 12 parts of water and applied, as a fine mist, at the rate of about 53 gallons per acre. Spraying was done about half an hour before the gatherings took place. Sixty-five meetings were treated; the area treated ranged from 1000 square feet to 8 acres. In 72 per cent of the experiments complete elimination of mosquito annoyance was obtained and in 26 per cent partial elimination was accomplished. There was little or no injury to grass or shrubbery.

Ginsburg *(1497)* and Headlee *(1627)* have described the method used to protect an audience of 10,000 to 20,000 people from mosquitoes in Newark Schools Stadium. It was first necessary to kill all mosquitoes in the stadium with pyrethrum larvicide and then it was necessary to produce a wall of larvicidal fog against those attempting to come in from outside areas. This procedure gave very satisfactory results.

Ginsburg *(1498)* concluded that the toxicity of kerosene to mosquito larvae can be increased four times by incorporating 0.01 to 0.04 per cent pyrethrins, thereby reducing the amount of oil required to cover the surface of water, under laboratory conditions, from 12 gallon per acre to 3.

Ginsburg has also given additional instructions on the use of the larvicide *(1499)* and has patented an insecticide and mosquito larvicide composed of petroleum oil, pyrethrum extract, a thiodiarylamine, water and an emulsifier. The amine is said to have a synergistic action on pyrethrum *(1500)*.

Mentzer, Daigh and Connell *(1885)* modified Ginsburg's mosquito larvicide formula by substituting pine oil or ethylene glycol ether of pinene or fenchyl thiocyano acetate for part of the pyrethrum. A preparation containing half the pyrethrum used by Ginsburg with 5 per cent of ethylene glycol ether of pinene was said to be the most satisfactory product tested. McDaniel *(1866)* recommends liquid soap as an emulsifier for pyrethrum larvicide similar to Ginsburg's formula.

Ginsburg *(1501)* later experimented with three types of emulsions in the New Jersey mosquito larvicide formula and concluded that a stable emulsion produces the most uniform surface film with oil droplets averaging 3 microns in size and 100 per cent kill of mosquito larvae. Neither a quick-breaking emulsion with an average oil droplet size of 15 microns, nor a miscible oil, the droplets of which were smaller than 1 micron, produced efficient kills of larvae. In general, there appears to exist a definite relationship between the size of the oil droplet in the emulsion and the efficiency of the oil-pyrethrum larvicide.

King, Bradley and McNeel *(1732)* decreased the mosquito population of uncleared areas more than 75 per cent by spraying with kerosene-pyrethrum extract emulsified in water with soap.

Sinton and Wats *(815)* reported that a mixture of one part of pyrethrum extract (Pyrocide 20) with 19 parts of kerosene made a comparatively cheap and highly efficacious mosquitocide for household use in India.

Russell and Knipe *(2089)* used oil-pyrethrum extract (Pyrocide 20) to control malaria by spraying native dwellings in India to kill adult mosquitoes. The malaria rate was markedly reduced in three years; in one village the parasite rate decreased from 80 per cent to 0 in two years. De Burca *(1356)* also used Pyrocide 20 as a larvicide and as a spray against mosquitoes to control malaria at Quetta Cantonment, India. He states: "Malaria incidence among the troops was markedly low as compared with the figures recorded in previous years. The spraying of barracks with pyrethrum insecticide is thought to have played a considerable part in bringing about this result."

The eradication of *Anopheles gambiae* from Brazil has been described by Soper and Wilson *(2166)*. This mosquito, "the most notorious of the African vectors of malaria," was found in Natal, Brazil in 1930, having been introduced from Africa. By 1938 the infestation had reached gigantic proportions, and in the first six months of that year, it was officially estimated that a minimum of 100,000 cases of malaria and 14,000 deaths had occurred. The essential elements in the anti-gambiae campaign were Paris green and pyrethrum extract. All possible breeding places were treated with the former and buildings in the infested areas were subjected to frequent spraying with pyrethrum-oil insecticide to control the adults. In November 1940, the last evidence of gambiae infestation in Brazil was found, less than two years after the eradication campaign was begun. More than 11,000 gallons of pyrethrum extract (2 g. pyrethrins per 100 cc.) were used, equivalent at 1:20 dilution to 220,000 gallons of finished spray.

Moreau *(1907)* found that an aqueous base pyrethrum extract containing 5.5 per cent pyrethrins was stable in storage and formed homogeneous suspensions when diluted with water. These suspensions gave good control of mosquitoes.

The Pyrethrum Aerosol Bomb

For many years some producers of pyrethrum insecticides in the United States had foreseen the necessity for a domestic source of supply of pyrethrum in the event of war, and efforts had been made to establish such a source. Prior to the war, efforts to interest Government officials in the use of pyrethrum for malaria control met with little success with the exception of some in the U. S. Public Health Service, who recognized the value of pyrethrum for that purpose. About 1934, the British

HOW PYRETHRUM IS USED IN NATIONAL DEFENSE

THE FOLLOWING ARE A FEW OF THE WAYS THAT PYRETHRUM PRODUCTS ARE USED IN THE NATIONAL DEFENSE PROGRAM

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PYROCIDE 20

- The original standardized pyrethrum concentrate.
- The standard of the industry since 1929.
- A solution of pyrethrins in mineral oil. Contains 2 grams pyrethrin per 100 cc. (Seil method).
- Deodorized and clarified grade for odorless sprays.
- Regular grade for cattle sprays.
- Pyrocide 20 can be used in making any of the pyrethrum-oil sprays described.

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U. S. ARMY—Uses Pyrethrum-oil spray for mosquito control in airplanes and for control of insects in camps.

U. S. NAVY—Also uses Pyrethrum-oil spray for destruction of insects.

U. S. PUBLIC HEALTH SERVICE—Uses Pyrethrum-oil spray for control of mosquitoes which transmit malaria, yellow fever and other diseases.

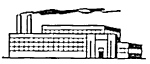
BRITISH ARMY—Employs Pyrethrum-oil spray for control of malaria and blackwater in Africa, the Middle East and Far East.

CIVILIAN AIRLINES—Use Pyrethrum-oil sprays for preventing introduction of insect-borne disease by planes arriving from foreign countries.

MEDICAL RESEARCH FOUNDATION—Used Pyrethrum-oil spray for eradication of the Anopheles gambiae mosquito, transmitter of a virulent strain of malaria in Brazil.

INSECTICIDES USED TO COMBAT INSECTS THAT ATTACK MAN AND ANIMALS
Pyrethrum-oil sprays are used to eradicate bed bugs, flies, mosquitoes, fleas, lice. These insects are said to spread typhoid fever, typhus fever, sleeping sickness, infantile paralysis and many other serious diseases. Such sprays are used in the home, in theatres and other places of assembly, in Pullman cars, in kennels and barns.

INSECTICIDES USED IN PRODUCTION OF DAIRY FOODS — Pyrethrum-oil sprays are extensively used to combat insects that attack dairy cows. They are applied to the cattle and also to the premises.



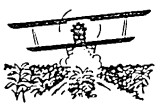
INSECTICIDES USED IN FOOD FACTORIES AND FOOD STORAGES— Pyrethrum-oil sprays are used extensively in flour mills, bakeries, creameries, packing plants, wholesale groceries and many other places where foods are made or stored. They are effective against roaches, grain weevils, flies and many other insects.

DRY PYROCIDE

First concentrated stabilized Pyrethrum dust for making dust insecticides.

Conserves Pyrethrins.

Saves Freight.



INSECTICIDES USED IN THE PRODUCTION OF FOOD CROPS

Pyrethrum-oil sprays are used to control citrus thrips, grape leafhoppers, codling moth on apples, lygus on coffee, sugar-beet leafhopper.

Pyrocide Dust, the original impregnated Pyrethrum dust, is recommended by:

Colorado Experiment Station, for control of squash bug, harlequin bug, potato psyllid on tomatoes, lygus on peaches.
Oklahoma A & M College, for control of squash bug, cucumber beetle and harlequin bug.
California Experiment Station, for control of bean thrips on beans, pears, peas.
Idaho Experiment Station, for control of squash bug.
Texas Experiment Station, for control of Hawaiian beet webworm on beets.
Virginia Experiment Station, for control of beet webworm on spinach.
Ohio Experiment Station, for control of strawberry leaf roller.
New York Experiment Station, for control of pentatomids on tomatoes.
New Jersey Experiment Station, for control of blueberry fruit worm.

Also recommended and used by thousands of commercial growers for control of sugar-beet webworm, bean leafhopper, grape leafhopper, cucumber beetle, squash bug, harlequin bug, aphids, cabbage worms, Mexican bean beetle, potato flea beetle, potato leafhopper, pea weevil, cranberry leafhopper, garden flea hopper on tomatoes, and many other insects attacking food crops.

MULTICIDE

Insecticide specially made for commercial growers.

A Pyrethrum insecticide containing spreader, ready to spray, on diluting with water. Used for control of celery leaf-tiers, cranberry fire worm, cabbage worm, and many other insects attacking truck crops.

EVER GREEN

Largest selling pyrethrum home-garden spray.

A ready-to-use Pyrethrum insecticide especially made for the home-gardener. An ideal insecticide for protecting "Food for Freedom" gardens. Kills aphids, cucumber beetles, leafhoppers, asparagus beetles, Mexican bean beetles, tarnished plant bugs, cabbage worms and many other insects.

We can thank our British Allies in Kenya Colony, East Africa, that Pyrethrum, the most versatile insecticide raw material, is still available and that the price is stabilized at a reasonable level. If you wish further information on any of these uses of Pyrethrum, write to . . .

McLAUGHLIN GORMLEY KING COMPANY · MINNEAPOLIS, MINNESOTA

This advertisement appears in the February, 1942 issue of SOAP AND SANITARY CHEMICALS

Army in India became interested in pyrethrum for malaria control *(90, 201, 815)* and very considerable quantities of concentrated pyrethrum extract were sold by an American manufacturer to the British authorities for that purpose, from 1935 until exports were prohibited by the United States Government in 1942.

McLaughlin Gormley King Co. licensed and designed a pyrethrum extraction plant which was established in South Africa in 1939 and assisted in establishing a smaller plant in India in 1941. Both of these plants were destined to play a part in supplying pyrethrum extract to the British army for malaria control during the war. An extraction plant of 500 tons annual capacity was put into operation in Kenya by the British Ministry of Supply in 1945.

The successful use of pyrethrum in the eradication of *Anopheles gambiae* from Brazil in 1939-40 established its value for mosquito control beyond all doubt. During the early years of the war, the demand for pyrethrum from Government sources was so small that American inporters were much concerned as to whether or not the manufacture of pyrethrum insecticides would be considered an essential business by the Government agencies which controlled man-power, raw materials and supplies.

Domestic sales of pyrethrum insecticides proceeded normally in 1939, 1940 and 1941, but with the entry of the United States into the war, the civilian demand increased enormously because of the fear of shortages caused by war conditions. In June 1942 pyrethrum was put under allocation by the War Production Board and in August 1943, all pyrethrum was allocated to the aerosol bomb program.

From 1938 to 1941 the quantity of pyrethrum flowers imported annually into the United States declined from 14,537,000 to 11,020,000 pounds. If, however, the pyrethrin content of these flowers (page 416) is calculated on the basis of 1.3 per cent for British East Africa and Belgian Congo flowers and 0.9 per cent for flowers from other sources, it will be found that the quantity of pyrethrins imported was remarkably constant, as shown in Table CIX.

TABLE CIX. IMPORTATION OF PYRETHRINS INTO THE UNITED STATES.

Pounds	1938	1939	1940	1941	1942	1943	1944
Pyrethrum flowers	14,537,000	13,569,000	12,591,000	11,020,000	9,452,000	6,796,000	10,658,000
Pyrethrins	142,300	143,700	154,900	140,200	121,300	85,900	129,700

In 1942 there was a marked decline in the quantity of both flowers and pyrethrins imported, followed by an even greater decline in 1943, which proved to be the low point, as the 1942 level was regained in 1944 and even larger quantities are anticipated in 1945.

There were several reasons for the decline in imports, among which were: increased use of pyrethrum by the British armed forces for malaria control, decreased production in Kenya because other crops were more profitable and loss of shipments in transit to the United States by enemy submarine action.

These figures show why it was necessary to place pyrethrum under allocation in 1942 and why all pyrethrum was allocated to the aerosol bomb program in 1943. They also emphasize the fact that pyrethrum would have been employed by the armed forces for many other purposes had supplies been available. Lacking pyrethrum, substitutes were used. It is generally admitted that the use of some pyrethrum in the formula would have improved the DDT louse powder, which was so dramatically employed by the army to prevent a typhus epidemic in Naples in 1944.

In 1938 Burdette *(1253)* published an interesting report on "air-floated" oil particles and the relation of their size to the toxicity of contact oil sprays to insects. Oil was atomized with a pressure sprayer and the resulting fog was collected on microscope slides covered with liquid soap, on which the oil particles retained their shape; their size was determined by microscopic examination. Nearly half of the fog delivered consisted of droplets of ultramicroscopic sizes and possibly partly of gas; the remainder consisted of droplets 1 to 79 microns in diameter. Droplets larger than 10 microns settled out of the air suspension in about 4 minutes. Toxicity of the fog to bees was due to the portion consisting of droplets less than 10 microns in diameter.

Ginsburg *(1502)* has reviewed the literature of insecticidal aerosols and has mentioned his own use of aerosols of oil-pyrethrum for the control of adult mosquitoes. The use of the Phantomyst sprayer for the production of pyrethrum aerosols has been mentioned (page 562).

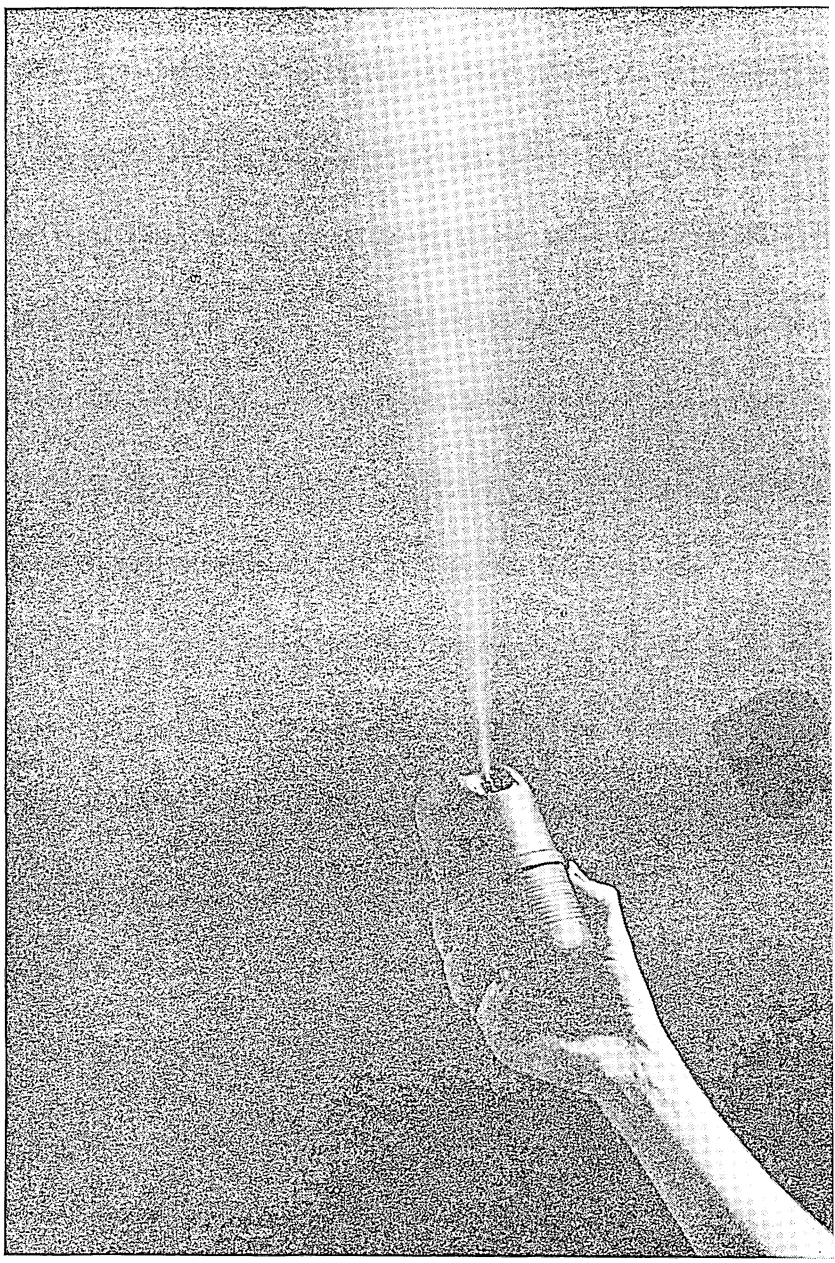
Goodhue and Sullivan *(1524)* produced pyrethrum aerosols by burning a mixture of pyrethrum, ground cornstalks and sodium nitrate. The toxicity to flies was poor and they decided that pyrethrum cannot be effectively dispersed by this method, thus confirming the conclusions of earlier investigators (page 275).

Sullivan, Goodhue and Fales *(2194, 1525)* produced pyrethrum aerosols by dissolving pyrethrum oleoresin in safrol and spraying the solution with an atomizer on an electric hot plate at 375° C. Dense clouds of white smoke containing the insecticide were formed. The safrol solution contained 0.5 per cent pyrethrins and 10 cc. of solution were sprayed per 1100 cu. ft. Exposure of flies to the aerosol for 1 hour caused mortalities of about 72 per cent after 48 hours. There was little or no toxicity to the American cockroach. Aerosols produced in the same way with alcoholic extract of pyrethrum were toxic to mosquitoes.

The use of compressed air or carbon dioxide to propel an insecticide from a closed container is well known. The employment of liquid carbon dioxide in the Sparklet bulb by Mackie and Crabtree to spray airplanes has been referred to (page 563). It should be noted that in these instances the insecticide is not dissolved in the propellant. Henning *(1631)* used methyl bromide as a propellant for fire extinguishing liquids such as carbon tetrachloride. Rotheim *(2080)* patented the method of spraying coating compositions by dissolving them in dimethyl ether in a pressure flask and releasing the solution through a suitable valve. He points out that minute atomization of the coating composition is obtained by the evaporation of the dimethyl ether on contact with the air and states that the particle size can be controlled by varying the percentage of dimethyl ether in the solution. Bichowsky *(1209)* patented the use of chloro-fluoro derivatives of hydrocarbons for propelling non-self propelling substances from containers. This patent, which covers the use of dichlorodifluoromethane (Freon) as a propellant, has 16 claims and is owned by the manufacturer of Freon. Iddings *(1684)* has patented a method of making a liquid self-propelling. He refers particularly to insecticides and discloses the use of 19 propellants, among them dichlorodifluoromethane and methyl chloride. There are eleven claims; claim ten is:

> "A method of making a liquid self-propellant consisting in cooling by boiling methyl chloride to its boiling point, making a solution of light petroleum oils and extract of pyrethrum, cooling a container and the solution to substantially the same temperature of the methyl chloride, adding and stirring the methyl chloride into the solution in the cooled container and finally sealing the container."

The other propellants mentioned are ethylene, ethane, acetylene, carbon dioxide, methyl fluoride, ketene, carbon oxysulfide,



THE PYRETHRUM AEROSOL BOMB IN ACTION.
(COURTESY WESTINGHOUSE ELECTRIC CORPORATION)

normal propane, ammonia, dichlorodifluoromethane, dimethyl-ether, methyl chloride, vinyl chloride, sulfur dioxide, methyl amine, trimethylamine, ethyl chloride, ethyl amine and 1,2 butadiene.

Goodhue and Sullivan filed an application for a patent on July 29, 1941. The original claims relating to pyrethrum were:

1. A composition of matter comprising a liquefied gas and a parasiticide dissolved therein.

2. A composition of matter for the production of parasiticidal aerosol comprising dichlorodifluoromethane and pyrethrins.

3. A composition of matter comprising dichlorodifluoromethane, pyrethrins and sesame oil as a mixture spontaneously convertible into a parasiticidal aerosol.

5. A composition of matter comprising a liquefied gaseous halogenated hydrocarbon and a parasiticide spontaneously convertible to a parasiticidal aerosol.

Claims 4 and 6 referred to rotenone and nicotine, respectively.

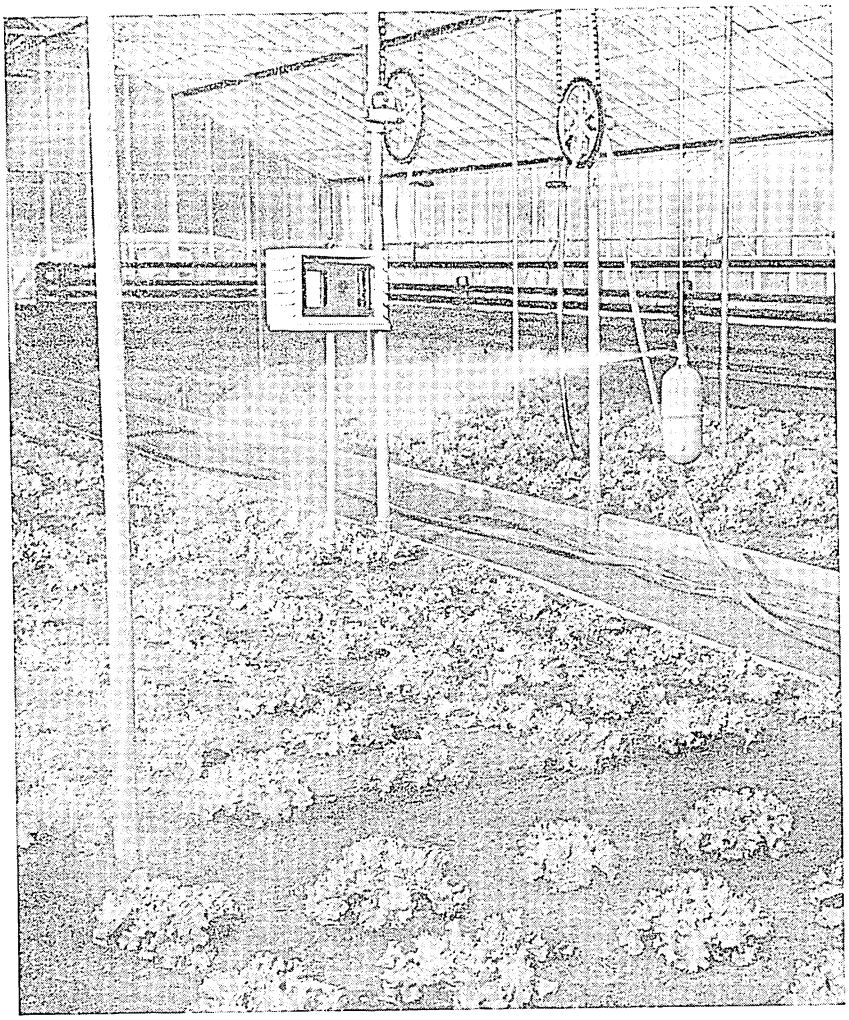
A patent was finally granted *(1528)* June 8, 1943, with six claims, each of which limited the proportion of volatile solvent or propellant to not less than 90 parts to 10 parts of parasiticidal material. Claim one is as follows:

"The method of producing an aerosol comprising releasing into the atmosphere in the form of finely divided droplets a solution under pressure of not more than 10 parts of the material to be dispersed as an aerosol in not less than 90 parts of a solvent, the vapor pressure being such that the solvent will boil violently under atmospheric conditions, whereby the violent boiling of the solvent will cause the droplets to be further subdivided, so that when all of the solvent has evaporated, the solute material will remain colloidally suspended in the atmosphere." The first public announcement of this invention was made on November 11, 1941.

In describing experiments with the pyrethrum-aerosol bomb, Sullivan, Goodhue and Fales *(2195)* stated: "The nontoxic nature of this insecticide to man and animals, its noninflammability, its ease of application with no power requirements, and its nonstaining properties appear to make it satisfactory for the control of mosquitoes on airplanes."

The preparation of pyrethrum aerosols with other gases and the design of apparatus for their experimental use were described by Goodhue and Sullivan *(1526)*. The use of aerosols in greenhouses was suggested.

Goodhue *(1518)* suggested a formula composed of 5 grams of pyrethrum concentrate containing 20 per cent pyrethrins, 2



USE OF AEROSOL BOMB FOR CONTROL OF GREENHOUSE INSECTS.
(U. S. D. A. PHOTOGRAPH BY KNELL)

grams of refined sesame oil, as synergist, and 93 grams of dichlorodifluoromethane (Freon-12). This mixture was very toxic to mosquitoes when as little as 5 mg. of pyrethrins per 1000 cu. ft. were used. It was also toxic to houseflies and stable flies when used at higher concentrations and to Sciaridae and Phoridae on mushrooms. Goodhue also pointed out that certain pyrethrum extracts are quite irritating, a quality that is especially objectionable in aerosol sprays when they are deeply inhaled. The settling rate of aerosol bomb sprays was studied and compared

with the settling rates of pyrethrum-oil sprays and aerosols produced by spraying solutions on hot plates. The aerosol bomb sprays were atomized at about 80 pounds pressure and the pyrethrum-oil sprays at 12½ pounds. The former remained in the air considerably longer, which might be partly explained by the difference in the pressures at which the two materials were applied.

Pyrethrum-aerosol sprays were toxic to cockroaches and bedbugs *(2195)* and to adult cheese mites, *Piophila casei (1210)*. Monro and others *(1903)* reported them toxic to mushroom flies, blackflies, cockroaches and sawflies.

Goodhue *(1519)* considered dichlorodifluoromethane the best propellant for the aerosol bomb. Methyl chloride also worked satisfactorily but it is toxic to man and hence is not as safe as Freon, which is nontoxic. Methyl chloride is a better solvent for pyrethrum than Freon, but it is also somewhat flammable and Freon is not. "Pyrethrum is invaluable in the aerosol and no substitute has been found for it." The particle size of the aerosol can be controlled by the amount of nonvolatile material included and, when Freon is used, the optimum particle size appears to be that produced by 15 per cent of nonvolatile material. This would correspond to a concentration of not more than 85 per cent Freon in the aerosol solution. This confirms the work of McGovran, Fales and Goodhue *(1871)* who found that an aerosol solution containing 16 per cent of nonvolatile material was more toxic to flies than solutions containing 8, 4 or 2 per cent nonvolatile matter, although all four solutions were sprayed in the Peet-Grady chamber in such amounts that 8 mg. of pyrethrins and 40 mg. of sesame oil were present in each test. The nonvolatile material consists of the pyrethrum extract, sesame oil, and materials other than the propellant.

Goodhue, Fales and McGovran *(1521)* summarizing their work on dispersants for aerosols state: "As a propellant for aerosols used in the presence of man, dichlorodifluoromethane (Freon-12) appears to be the most satisfactory. Other liquefied gases were studied as substitutes or diluents for Freon-12. These include propane, butane, dimethyl ether, methyl chloride, chlorodifluoromethane, chlorofluoromethane, carbon dioxide and nitrous oxide. As a diluent for Freon-12 and a solvent for insecticides, methylene chloride appears to be most practical. This diluent is practically nontoxic and nonflammable. It is an excellent solvent for insecticides. It is liquid at room temperature, but even with

its low volatility it can be substituted for Freon-12 up to one-third of its weight without reducing the effectiveness of the aerosol." About 20 per cent of Freon-12 can be replaced with a mixture of equal weights of propane and butane without forming a flammable material. Dimethyl ether can be substituted for Freon-12 to the extent of about 25 per cent. This mixture is non-flammable. Methyl chloride can be used alone or with Freon-12, but it is toxic to man; it is cheaper than Freon-12. Other members of the Freon series were not as satisfactory as Freon-12. Sixteen per cent of carbon dioxide in acetone gave a pressure of 300 lbs. and produced a wet, flammable spray. Because of its low cost, carbon dioxide is worth further investigation.

Pyrethrum Aerosol Spray

The first pyrethrum aerosol bombs contained *(1518)*:

Pyrethrum extract, 20% pyrethrins	5%
Sesame oil	2%
Freon-12	93%
	100%

Pyrethrin content 1.0%

This was soon changed, in 1943, to 4 per cent pyrethrum extract, equivalent to 0.8 per cent pyrethrins, 6 per cent of sesame oil and 90 per cent of Freon. Later in 1943, a formula containing 2 per cent of pyrethrum extract, 8 per cent of sesame oil and 90 per cent of Freon was used, giving a pyrethrin content of 0.4 per cent *(2032)*. This reduction in pyrethrin content was made because of the shortage of pyrethrum.

In 1945, after long preliminary tests, 3 per cent of DDT was added to the aerosol bombs, but the pyrethrin content was not reduced below 0.4 per cent. However, the sesame oil was replaced with 5 per cent of lubricating oil, and 5 per cent of cyclohexanone was used to dissolve the DDT.

Other solvents have been used to replace cyclohexanone because of its objectionable odor and the tendency of DDT to decompose when dissolved in it, with resulting corrosion of the container. Propylene oxide and xylene have been so used.

Lindquist and associates *(1806)* conducted tests of pyrethrum and DDT aerosols against mosquitoes and houseflies. In order to obtain the necessary concentration of DDT in Freon-12, cyclohexanone was used as an auxiliary solvent. The most satisfactory results were obtained with combinations of pyrethrins

and DDT. The knockdown obtained with DDT alone was relatively poor. It was found that other oils could be substituted for sesame oil. The addition of motor oil to the bomb made it possible to reduce the concentration of DDT and cyclohexanone without lowering the effectiveness.

Schroeder and associates *(2099)* compared the pyrethrum-sesame oil aerosol with aerosols containing DDT and pyrethrins to determine the residual effect against houseflies. Best results were obtained with the following formula:

Pyrethrum extract (20% pyrethrins)	1.0%
DDT	3.0%
Cyclohexanone	5.0%
Lubricating oil	5.0%
Freon	86.0%

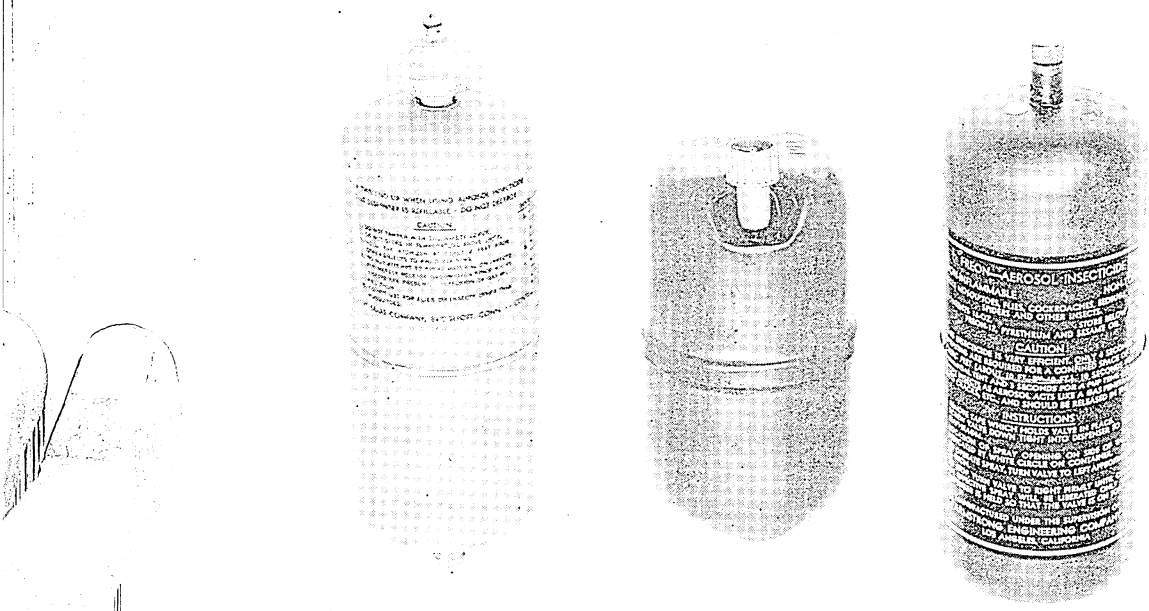
McGovran and Goodhue *(1873)* have patented an aerosol containing an insecticide, such as pyrethrum, rotenone or nicotine and a germicide, e.g., carbolic acid, hexylresorcinol or propylene glycol in a liquefied gas such as Freon-12 or methyl chloride. Other materials, including a conditioner or carrier may be added.

Specifications for the pyrethrum concentrate used in the aerosol bomb at first called for nonirritating pyrethrum extract containing 20 per cent pyrethrins. Members of the industry had great difficulty in finding out exactly what was required. The first written specifications, issued December 15, 1942, provided for a pyrethrin I content of 9½ per cent, by the Seil method, "which will assure 20% total pyrethrin content," and further provided that not more than 10 per cent of the concentrate should be insoluble in Freon, when tested according to the procedure described. It was soon found that 10 per cent of Freon-insoluble material caused blocking of the discharge tubes of the bombs, especially when they became chilled in shipping. The specifications were accordingly changed to require 20 per cent (± 0.5%) pyrethrins and not more than 4.0 per cent Freon-insoluble matter, or 0.05 per cent moisture. Odorless refined kerosene base oil was specified as a solvent and no addition of other solvents, perfumes, synergists or antioxidants was permitted. The pyrethrin content was determined by the Seil method and Freon-insoluble matter was determined by the method suggested by Goodhue and Sullivan *(1526)* as modified by Westinghouse Electric corporation *(1109, 1122)*.

After the development of the highly purified pyrethrum concentrate by Gnadinger and Clark in 1943 (page 518), the maximum for Freon-insoluble material was lowered to 1½ per cent *(1120)*. The method for determining Freon-insoluble solids suggested by Wachs, Morriello and Mages *(2263)* has been used in some Government specifications *(1120)*.

The Bomb

The containers for aerosol sprays, generally referred to as bombs, are built to withstand high pressures. They are equipped with rupture plugs or disks which release the contents at comparatively low pressures if the bombs are overheated. The bomb loaded with Freon aerosol spray is under a pressure of about 80 pounds per square inch at 68° F. The bomb that has been produced in largest quantity is about 2⅞ inches in diameter and 5¼ inches high. This bomb weighs about 11 ounces and the contents weigh 18 ounces. Some bombs containing 2, 5 or 10 pounds of solution have also been packed. The spray is discharged through a capillary tube which varies in diameter in the



Pyrethrum aerosol bombs. Left, Bridgeport Brass Co. Center, Westinghouse Electric Corp. Right, Armstrong Engineering Co.

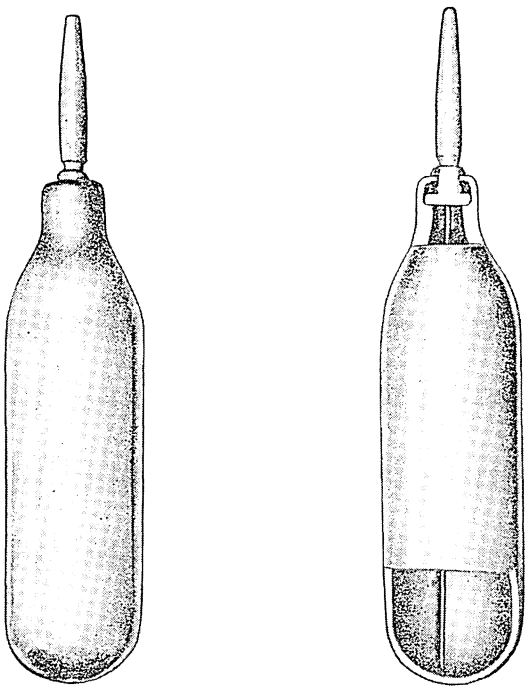
different types of bombs. One bomb has a capillary with a diameter of 0.017 inch; another discharges through an orifice of 0.008 inch diameter. The first bombs were equipped with screw caps, but all are now fitted with valves. About 15 minutes are required to discharge an 18 ounce bomb, and the resulting spray will treat approximately 200,000 cu. ft. of space for mosquito control. The dosage ordinarily recommended for practical applications is about 2 to 3 grams of aerosol per 1000 cu. ft. Inability to control the dosage accurately results in waste of material and this is one of the principal disadvantages of the aerosol bomb.

Some bombs are refillable, but others are intended to be discharged and then discarded. Nusbaum has patented a bomb, without a capillary tube, which discharges through an orifice shaped like the frustum of a cone, decreasing in diameter toward the discharge end *(1954)*. More than 25,000,000 bombs, from the several manufacturers, have been supplied to the armed forces up to July, 1945. Most of these bombs have been used in combat areas; a few have been used for insect control on aircraft entering the United States. Dunnahoo *(1389)* has described the use of pyrethrum-Freon aerosols in aircraft.

A small compact aerosol bomb has been developed by the British Central Scientific Board and Sparklet Limited, London, using the standard Sparklet carbon dioxide cylinder as a container. This bomb is ¾ inch in diameter and 3½ inches long and weighs slightly more than 1 ounce when filled. To discharge the bomb, it is held upright and the tip is broken off with the fingers; there are no valves or caps and the entire contents, about ⅓ ounce, are discharged in 8 to 10 seconds. One bomb is said to be sufficient for a room 12 x 12 feet. The Sparklet bomb can be used with Freon, methyl chloride or carbon dioxide. Sparklet bombs are also manufactured in the United States where the bomb is filled with the following mixture:

Pyrethrum extract (20% pyrethrins)	1%
Sesame oil	5%
Cyclohexanone	5%
Odorless kerosene	5%
DDT	5%
Freon-12	79%

One advantage of this small bomb is that it automatically delivers a definite amount of aerosol and the discharge need not be timed to prevent waste.

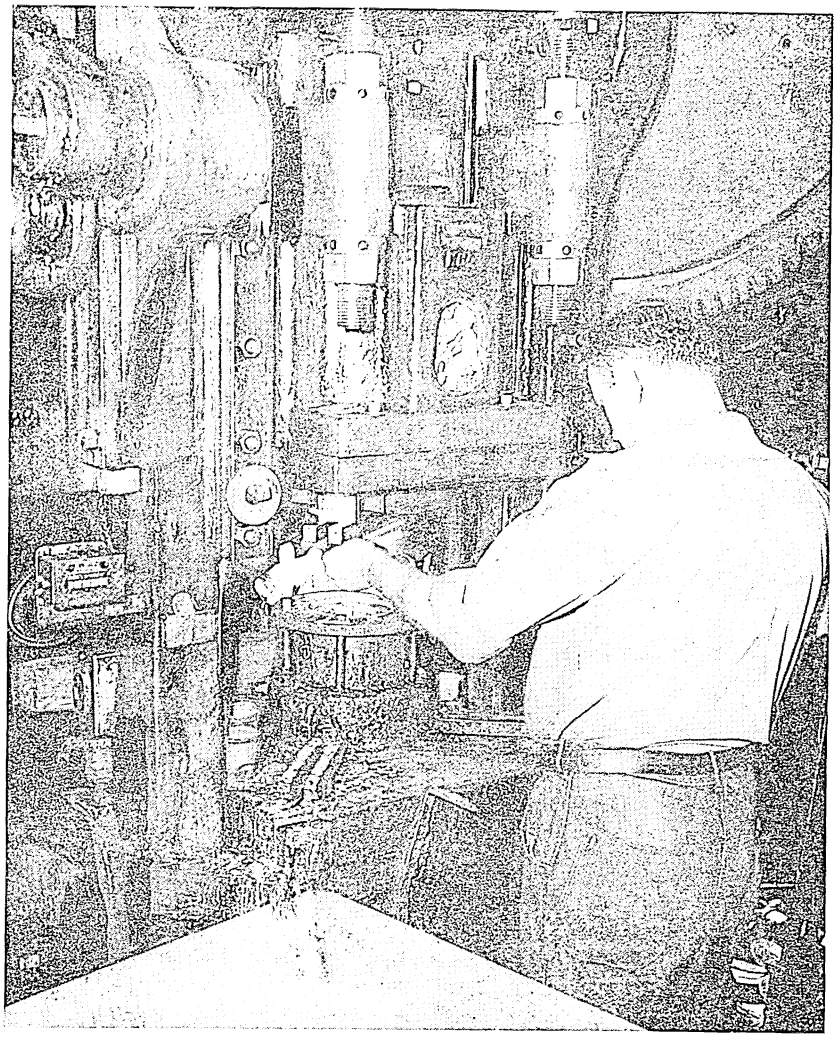


SPARKLET AEROSOL BOMB, ACTUAL SIZE. RIGHT, CUT AWAY TO SHOW CONSTRUCTION (COURTESY SPARKLET DEVICES, INC., DIVISION OF KNAPP-MONARCH CO.).

THE PROPELLANT

Rhodes *(2032)* defines a liquefied gas aerosol as "an air colloid produced as the result of a change from the liquid phase to the gaseous phase of a compressed and liquefied gas, having a gauge pressure exceeding 25 pounds per square inch at 70° F. in which has been dissolved a chemical or chemicals capable of assuming colloidal particle size when propelled into the air and freed of their solvent because of its change of state." Rhodes continues, "In this definition I have incorporated the definition of a compressed and liquefied gas which is found in Paragraph 300 of H. A. Campbell's Freight Tariff No. 4. The first official mention of these aerosols is in Supplement 8 of the above Freight Tariff where they are listed as 'Insecticide, liquefied gas.' On September 14 [1944] the specification for the small aerosol container issued as an Order of the Interstate Commerce Commission as ICC No. 9.

"The Aerosol Committee of the Compressed Gas Manufacturers' Association have through its Sub-committee spent a great deal of time in the consideration of test methods and specifica-



SHAPING AEROSOL BOMBS FROM FLAT BLANKS OR "COOKIES."
(COURTESY WESTINGHOUSE ELECTRIC CORPORATION)

tions for containers which will hold up to three pounds of aerosol. Above that amount it is felt that the ICC 4B 300 container will be suitable. Every one of the war contractors was represented on the committee and all of them displayed an eagerness to have a container which would be perfectly safe in the hands of the public. The adoption of this specification prevents entrepreneurs from selling aerosol to the public in tin cans or other dangerous type dispensing devices, and in the United States it will be impos-

sible to transport by any means liquefied gas aerosol which is not contained in a cylinder covered by ICC specifications.

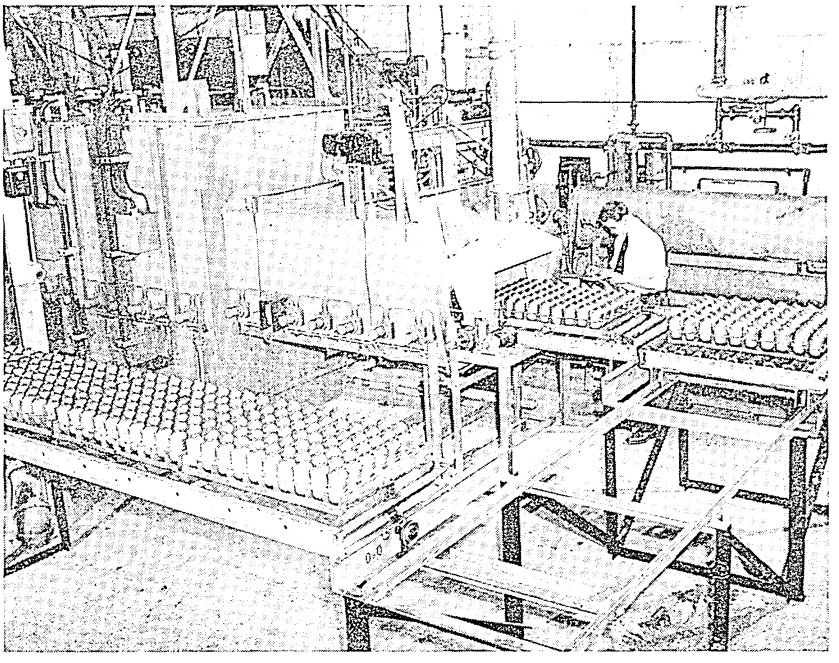
"When an aerosol, containing 'Freon-12' as the propellant, changes from the liquid to the gaseous phase, it expands at 70° F., two hundred and sixty times the volume it occupied in the liquid phase. This action may be likened to an explosion because it occurs in an infinitesimal period of time."

Rhodes *(2031)* has discussed the postwar use of aerosol bombs, especially in regard to design of the bombs, safety of spray ingredients and the necessity of protecting the public from injury.

Smith and Goodhue *(2147)* have observed that "At 80° F. the density of saturated Freon-12 vapor is 0.0377 gram per cc. As liquid is withdrawn from an aerosol container during use, appreciable quantities of Freon evaporate from the solution remaining in the container to maintain this high vapor concentration in the increasing space not occupied by the liquid. As a result the insecticide concentration of the remaining solution gradually increases as the container empties. While this change is less serious than if the solution progressively weakened, still the need for conservation of insecticide suggests that some consideration be given to the matter, especially in connection with packaging the solution." For a solution of 5 per cent sesame oil in Freon-12, a quantity of liquid equal to 22 per cent cent of the original liquid content must be withdrawn before the concentration of insecticide in the remaining liquid rises 1 per cent; removal of 80 per cent causes a 5 per cent increase and delivery of 92.5 per cent increases the concentration 9 per cent. This is important when filling small containers from a large one, as in filling aerosol bombs. Some bomb manufacturers add sufficient pure Freon-12 to the reservoir at intervals to maintain approximately the original concentration.

Aerosols vs. Oil Sprays

Fales and Goodhue *(1432)* compared the toxicity of pyrethrum aerosols and oil-pyrethrum spray to houseflies. The tests on the aerosol were made in a Peet-Grady chamber, as described by McGovran (page 475), and the spray was tested by the Peet-Grady method. Both aerosol and spray were so used that 20 mg. of pyrethrins were introduced into the Peet-Grady chamber in each test. The aerosol was applied at 80 pounds pressure and the spray at 12½ pounds. Fales and Goodhue give the following



AEROSOL BOMBS READY FOR THE COPPER BRAZING FURNACE.
(COURTESY BRIDGEPORT BRASS CO.)

summary of their results: "The comparative effectiveness against houseflies of sprays produced with a standard Peet-Grady atomizer and aerosols produced by liquid Freon has been determined. The aerosol is superior, when sprayed directly on the insects, being slightly better even with exposure periods as short as 1 minute. When the insects are not exposed until some time after the insecticide has been distributed, the aerosol is more effective after 20 minutes than the spray is after 5 minutes. The 10-minute knockdown effect was about 7 per cent greater with the spray, but the knockdown with the aerosol was still 74 per cent after a delay of 20 minutes before exposing the flies."

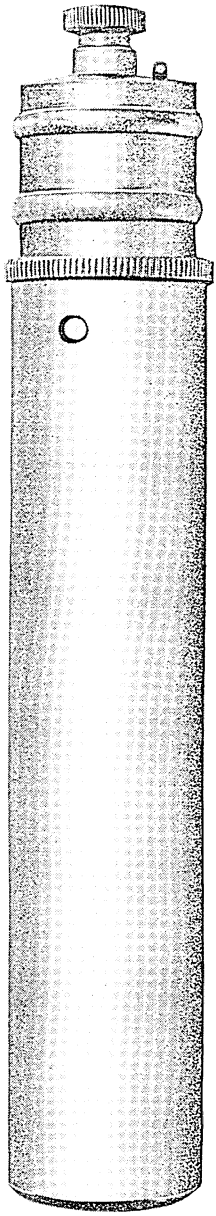
Gothard *(1531)* does not agree with the conclusion of Fales and Goodhue. Gothard made practical tests of pyrethrum aerosol and pyrethrum spray in a room of 2500 cu. ft. capacity, using 1000 to 1200 flies for each test. Using the aerosol bomb, 175 mg. of pyrethrins were introduced into the room. The oil spray was applied with a conventional sprayer so as to disperse 118 mg. of pyrethrins in the room. Roaches were exposed to the aerosol fog by placing them in Petri dishes on a table in the center of the room. Tests were also made in a Peet-Grady chamber introduc-

ing 19.2 mg. of pyrethrins for each test with aerosol or spray. Gothard concluded: "We find that the current aerosol bomb, as at present used, and which is claimed to be effective against flies and roaches, is not as effective as the conventional sprayer. In a full scale room test, using 48 per cent more pyrethrins and with the benefit of a synergist, the bomb gave a knockdown 15 per cent lower and a kill 11 per cent lower. In order to equal the knockdown and kill obtained by the conventional sprayer, it seems evident that an excessive amount of pyrethrum must be used in the bomb. We find further that the knockdown with the aerosol bomb is excessively slow, as well as ineffective, and would not be satisfactory to the average user."

Stage *(2179)* states: "Lindquist, Schroeder and Knipling reported studies on particle size and pointed out that, when finely atomized, pyrethrum sprays are equally as effective as the liquefied gas aerosols. These workers later demonstrated that the amount of active ingredient applied was the governing factor in effectiveness of the spray and that the amount of diluent, within limits, was of little importance." For example, 1 ml. of 20 per cent spray was as effective as 20 ml. of 1 per cent spray. Lindquist and his associates developed and applied for a patent on a small pocket sprayer in which a concentrated extract is used. This sprayer delivers a very fine fog.

Lindquist, Schroeder and Knipling *(1805)* demonstrated by biological tests "that a finely divided spray will remain suspended in the air almost as long as a liquefied-gas aerosol. Preliminary studies of particles deposited on slides indicate that there is very little, if any, difference in size or abundance of particles between the spray and the aerosol." Comparisons were made between aerosol of standard formula and spray containing the same ingredients, without Freon, applied with the pocket-size capillary tube sprayer. "The aerosol was applied at the rate of 3.3 g. and the spray at 1 ml. per 1000 cu. ft. There was practically no difference in the rate of knockdown or mortality between the two types of sprayers." The tests were made against free-flying houseflies and mosquitoes in rooms. An ordinary household sprayer, rebuilt by substituting a capillary tube of 0.017 inch inside diameter for the customary large siphon tube, produced a spray having a surprisingly low settling rate.

Knipling *(1739)* says: "With a small sprayer filled with the concentrated solution, having a total weight of about 8 ounces, as much space can be treated as with 1 gallon of O.T.I. fly spray applied with an ordinary hand sprayer. The pocket-size sprayer



U. S. D. A. POCKET SPRAYER, ACTUAL SIZE.

contains the equivalent of one aerosol bomb weighing 26 ounces. Tests with finely atomized sprays have shown that for practical purposes they are as efficient as liquefied gas aerosols." See Table CX.

TABLE CX. EFFECTIVENESS AGAINST FLIES AND MOSQUITOES OF DDT PLUS PYRETHRUM WHEN APPLIED AS AN ATOMIZED SPRAY AND AS AN AEROSOL (KNIPLING).

Dosage DDT-Pyrethrins		Method of Application	Mortality in 24 Hours	
			Anopheles quadrimaculatus	*Musca domestica*
1000 cu. ft.	Mg. per		%	%
2	20	Aerosol	83	64
		Atomized spray	73	60
4	40	Aerosol	94	86
		Atomized spray	90	89

Rhodes *(2031)* and Cowin *(1321)* have also discussed the postwar possibilities of aerosols and sprays. The latter has called attention to the danger of spraying halogenated hydrocarbons, such as dichlorodifluoromethane in rooms where flames are exposed, because volatile decomposition products are formed which are exceedingly irritating and possibly injurious.

The aerosol bomb is certain to bring about the development of more efficient sprayers.

MOSQUITO REPELLENTS

Granett *(1553)* has described a method of testing mosquito repellents and has given the results of tests of nearly a thousand substances as repellents. The most satisfactory material was a mixture of diethylene glycol monobutylether acetate and diethylene glycol monoethylether (Sta-Way). Oil of citronella, citronellol and pine oil were about 60 per cent as efficient and pyrethrum mixtures about 40 per cent as effective as Sta-Way. The latter was effective against blackflies, sand flies, stable flies, deer flies and chiggers when frequently applied. Granett's tests were made against salt marsh mosquitoes, mainly *Aedes sollicitans* and *Aedes cantator*.

MacNay *(1825)* tested the repellency of numerous compounds against the mosquitoes found in the wooded areas of Ontario, *Aedes hirsuteron*, *A. stimulans*, *A. vexans*, and *A. trichurus*. The most promising material was a mixture of oil of thyme, 0.5 fl. oz., pyrethrum-oil extract (containing 3 g. pyrethrins per 100 cc.), 1 fl. oz. and castor oil, 3 fl. ozs. This formula is said to have been supplied to Indian troops in Malaya and to have kept these soldiers almost entirely free from malaria *(1080)*. Protection against mosquitoes, blackflies and other blood sucking insects lasted from 3 to 5 hours.

Adams *(5)* has patented the use of pyrethrum with the repellent alkyl phthalates.

ONE METHOD OF USING THE PYRETHRUM AEROSOL BOMB FOR MOSQUITO CONTROL. (U. S. D. A. PHOTOGRAPH BY KNELL)

Knipling and Dove *(1740)* have described the mosquito repellents used by the armed forces. Those recommended are dimethyl phthalate *(1906)*, 2-ethyl-1,3-hexanediol (Formula 612) and butylmesityl oxide oxalate (Indalone) *(1729)*. Dimethyl phthalate is most effective against *Anopheles quadrimaculatus*, Formula 612 is most effective against *Aedes* and Indalone is the most effective against the stablefly, *Stomoxys calcitrans*. Repellents are more effective when applied to the clothing than when applied to the skin. A patent has been granted Travis and Jones *(2241)* on a mixture of these three materials as a mosquito repellent. Some of these synthetic compounds exert a solvent action on clothing made of rayon and on certain plastics, paints and varnishes.

Roy and Ghosh *(2081)* state that pyrethrum mixtures were effective as mosquito repellents for much longer periods than any other preparations tested. The following formula, suggested by MacNay, is said to protect for five hours:

Pyrethrum extract, 3 g. pyrethrins per 100 cc.....	0.5 fl. oz.
Castor oil.....	4.0 fl. oz.
Citronella oil.....	5 drops

Other Uses

Angevine *(1017, 1018)* employed a pyrethrum ointment for the control of lice and scabies. This ointment was made from a jelly-like by-product of the manufacture of pyrethrum extract, petrolatum and dimethyl methylene ether of allyltetrahydroxybenzene. The latter was added to prevent the entrance of allergic substances into the lymphatic system. The pyrethrin content was 2 per cent. No cases of dermatitis were observed in 279 cases of scabies treated.

Another formula, recommended for head lice and scabies by the U. S. Department of Agriculture *(1102)*, contains:

Benzyl benzoate	10.0 grams
Dinitroanisole	2.0 grams
Pyrethrins (in 20% extract)	0.2 gram
Isobutylundecylenamide	0.5 gram
Ethyl alcohol to make	100 cc.

This liquid was also recommended as a delousing spray, applying it directly to the body. The use of these ingredients (SYLN) for louse control and treatment of scabies has been described by Eddy *(1406)*.

The insecticide powder developed by Knipling and Dove *(1740)* for the armed forces for the control of body crawling insects had the following composition:

Pyrethrins (in 20% extract)	0.20%
Isobutylundecylenamide (synergist)	2.00%
2,4-Dinitroanisole (ovicide)	2.00%
Isopropyl and diisopropyl cresols (antioxidants)	0.25%
Pyrophyllite (diluent)	95.55%

This powder was recommended for the destruction of crab lice, body lice and head lice and for the prevention of chigger and tick bites. Application was made to clothing and bedding. The development and use of this formula (MYL) has also been described by Bushland and his associates *(1255)*.

In a study of the use of repellents against fleas, Lindquist, Madden and Watts *(1804)* obtained the longest average protection time with a mixture of 1 per cent of pyrethrins and 2 per cent of isobutylundecylenamide in mineral oil of 342 seconds viscosity at 100° F., Saybolt.

Bushland, Eddy and Knipling *(1254)* obtained excellent control of the body louse with pyrethrins alone and with combina-

tions of pyrethrins and synergists, of which isobutylundecylenamide proved to be the most effective.

Madden, Lindquist and Knipling *(1826)* demonstrated that pyrethrum dusts are effective repellents for chiggers. Treatment of the clothing with liquid repellents gave the best protection. Liquid pyrethrum preparations were apparently not tried. Stone and Haseman *(2186)* report that applications of pyrethrum to socks, trousers and feet gives protection against chiggers.

Roy and Ghosh *(2084)* recommend pyrethrum in odorless mineral oil for the control of head and pubic lice. One treatment is said to be sufficient.

Simmons and Dove *(2136)* investigated various materials for the control of the dog fly, *Stomoxys calcitrans,* breeding in beach deposits of marine grasses. Surface applications of fuel oil containing pyrethrum extract killed newly emerged flies as they forced themselves upward to the surface of the infested material.

Glasgow *(1506)* has used an emulsion of mineral oil containing pyrethrum, similar to Ginsburg's formula (page 274) to control blackfly larvae in a stream, without harm to fish or aquatic birds.

Smith and Gouck *(2149)* found pyrethrum extract, emulsified in water with soap, effective agains the ticks, *Ixodes scapularis* and *Ambylomma americanum.* Isobutylundecylenamide was used as an activator. There was no appreciable damage to vegetation. Robinson *(2067)* concluded that pyrethrum I is more toxic than rotenone to the argasid tick, *Ornithodorus moubata.*

A new liquid material, Lousex, for use against body vermin is reported by Zumpt *(2364)* to consist of a chlorinated hydrocarbon mixture containing pyrethrum. It has high wetting power, is not irritating and is very effective.

Jones, *et al. (1713)* studied the use of pyrethrum extract for impregnating underwear to control lice attacking man. Dipping or spraying suits of underwear with a mixture of pyrethrum extract and the synergist isobutylundecylenamide controlled lice introduced 6 weeks after treatment. Suits were still effective when worn after being stored for ten months. The supply of pyrethrum was too critical to permit its use in this way during the war.

Deonier and Lindquist *(1364, 1365)* found pyrethrins in a mixture of mineral-oil and carbon tetrachloride effective against larvae of the Clear Lake gnat, *Chaoborus astictopus.* A solution of pyrethrins in kerosene was effective as an ovicide. The habits of the larvae and the difficulty of applying the ovicide effectively make both methods of control impracticable on Clear Lake. Com-

parisons of the toxicity of pyrethrins and DDT to larvae and pupae of *Chaoborus punctipennis*, by Lindquist and Bushland *(1802)*, indicated that the pyrethrins are more toxic and quicker acting.

Heavy mineral oil containing about 0.1 per cent pyrethrins applied to screens with a brush or rag will repel or kill salt-marsh sand flies (Culicoides). This was demonstrated by Hull and Shields *(1673)* in laboratory and field tests. Best results were obtained with:

Pyrethrum concentrate, 2 g. pyrethrins per 100 cc...	1 part
Lubricating oil, S.A.E. 5.....	20 parts
or	
Pyrethrum concentrate.....	1 part
Kerosene	6 parts
Lubricating oil, S.A.E. 10.....	12 parts

CHAPTER XXVII

POSSIBLE SOURCE OF PYRETHRUM FLOWERS IN THE UNITED STATES

During the past eight years little progress has been made in the development of a domestic source of supply of pyrethrum. Shortly after the United States entered the war in 1941, the Government suddenly became interested in growing pyrethrum, in order to insure supplies to the armed forces for malaria control. Tentative plans were made for plantings in the United States, but these were soon changed and interest shifted to pyrethrum cultivation in Mexico, Chile, Peru and Central America where the work is still in progress (page 418).

Probably the largest planting of pyrethrum in the United States is that owned by McLaughlin Gormley King Co. in southwestern Colorado. This planting has not produced on a commercially successful basis, but much information has been gained on methods of planting, cultivating and harvesting. One of the most difficult problems encountered is weed control. With the exception of some hand weeding between plants in the row, all operations can be conducted mechanically, including planting, cultivating, harvesting and drying.

The pyrethrin content of the 1944 crop, by the Seil method, was:

Pyrethrin I	0.95 per cent
Pyrethrin II	0.62 per cent
Total	1.57 per cent

This project is being continued, and a similar one, on a somewhat smaller scale, is being conducted by the same Company in western Oregon. Other small plantings are located in California and Tennessee.

Culbertson *(1334)* has made a detailed report on his work on the commercial production of pyrethrum, covering the period from 1927 to 1937. Culbertson first selected Virginia, North Carolina, South Carolina, Georgia, Alabama, Tennessee, Florida, California, Oregon and Washington as suitable localities. Initial plantings were made in Virginia, North Carolina, South Carolina, Georgia, Kentucky, Indiana and Pennsylvania. The plantings were later extended to 31 other states. Pyrethrum behaved as an annual in the extreme south, as a biennial a little farther

north and as a perennial lasting at least 8 years in favorable growing areas between 37 and 50 degrees north latitude. Culbertson classified the states in three groups:

1. Suitable for commercial production: Pennsylvania, New York, New Hampshire, Vermont, Connecticut, Michigan, Minnesota, California, Idaho, Indiana, Illinois, Iowa, Ohio, South Dakota, Oregon, Washington, Wisconsin, Montana and West Virginia.
2. Production possible, but hazardous: Colorado, Kentucky, Maryland, New Jersey, North Carolina, South Carolina, Tennessee, Virginia, Missouri and Nebraska.
3. Production a doubtful undertaking: Alabama, Arizona, Florida, Georgia, Kansas, Mississippi, Louisiana, New Mexico and Texas.

The pyrethrin content was little influenced by type of soil, fertilizer treatment, pH, latitude or elevation. There was no significant difference in the pyrethrin content of flowers from 17 seed sources.

In seed treatment and germination tests the most practical results were secured by soaking the seed in hot water at 50° C. for 20 minutes and then in a 1½ per cent solution of copper sulfate for 1 to 3 hours. Seed was injured by temperatures above 52° C. Soaking in Semesan, 1 teaspoonful to 1½ quarts of water, for 1½ to 3 hours was also effective. Pyrethrum seed properly stored retained a high percentage of viability for 5 years. Field seeding was impracticable under the humid conditions of the east. Plantings established by division of old crowns showed reduced yields, higher mortality and higher costs than plantings established from seed. One ounce of seed was sufficient for 60 sq. ft. of seed bed or ¼ acre of planting having 3250 plants. Germination required 4 to 10 days, and plants were ready for transplantings in 8 to 10 weeks. Mechanical transplanting was successful. The most favorable spacing of plants was 30 inches between rows and 15 inches between plants in the row. Three to five cultivations were necessary each season, taking care not to cover the crowns. Yields of seed were 180 to 490 pounds per acre; the weight per bushel was 21 to 24 pounds. There was little difference in the pyrethrin content of flowers dried in the shade, in the sun or artificially at 105° to 110° F. Flowers lost about 75 per cent of their weight in drying. It was advisable to mow the stems after harvest. An application of 500 pounds of 4-12-4 fertilizer was necessary to maintain production. Fall

bloom had no effect on the succeeding crop. Replacements amounted to 3 per cent annually.

Culbertson's costs for two flowering seasons were $62.01 per acre the first year and $53.99 the second year. Mean yields were 756 pounds and 703 pounds, making the cost per pound 8.2 cents and 7.6 cents. The maximum acreage in Pennsylvania was 250 acres divided among 150 farmers. There was no correlation between yield of flowers and pyrethrin content. The pyrethrin content did not diminish as the age of the plants increased.

Leaf diseases were caused by *Alternaria* spp., *Cercospora* sp. and *Septoria chrysanthemella*. Crown and root diseases were due to *Fusarium* spp., *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Sclerotinia trifoliorum*, *Sclerotium delphinii* and *Sclerotium rolfsii*. The nematode *Heterodera radicicola* also caused root gall. A systemic virus caused aster yellows, and other diseases were due to low temperatures. The most destructive disease was due to *Sclerotinia*.

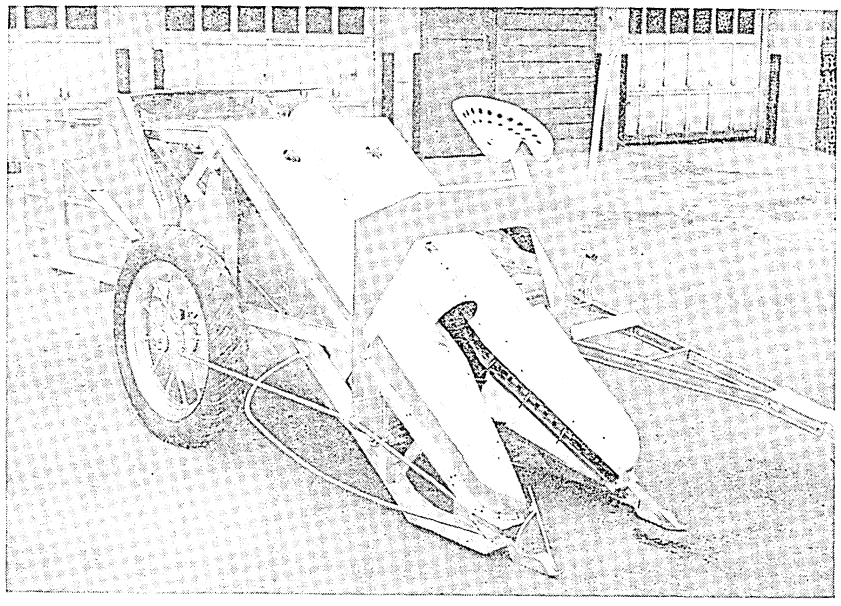
Culbertson found the somatic chromosome number in pyrethrum to be 18. The chromosomes are rod-shaped and vary in length from 4 to 14 microns. Seed formation appears to result from self fertilization. There was little difference in the pyrethrin content of flowers from mother plants and from progeny clones and open- and self-fertilized lines.

Culbertson's work did not result in the development of a domestic source of supply.

Harvesting of pyrethrum in the principal producing countries is done by hand picking. In Kenya mature flowers and immature buds occur on the plants at the same time, so that mechanical harvesting is impossible. In the United States the cost of hand picking would be prohibitive, hence the development of an efficient mechanical harvester is one of the problems that must be solved before pyrethrum can be commercially produced in this country.

Probably the most satisfactory harvester so far developed is that described by Sievers, Lowman and Hurst *(2129)*. This machine has been tried on small plantings with fairly good results. The principle of this harvester appears to be sound, but some improvements in design are still necessary before a multiple-row machine, capable of efficiently handling a large acreage, can be produced.

Acree, Schaffer and Haller *(997)* have investigated the pyrethrin content of fresh pyrethrum flowers. Ripert *(744)* had



PYRETHRUM HARVESTER, IMPROVED MODEL OF U. S. DEPARTMENT OF AGRICULTURE MACHINE. (COURTESY GULF OIL CORPORATION)

previously reported that the pyrethrins occur in fresh flowers. Gnadinger, Evans and Corl *(355)* had also shown that the pyrethrins occur in the freshly picked flowers and that different methods of drying do not greatly affect the pyrethrin content of the flowers. These conclusions were confirmed by Acree, Schaffer and Haller, whose more comprehensive investigations also showed no appreciable difference in the pyrethrin content of fresh flowers and the same flowers after drying, calculating the pyrethrin content to the moisture free basis. They concluded that enzymes and moisture have little effect on the synthesis or decomposition of the pyrethrins in the process of drying the flowers. Moore* has recently confirmed these conclusions.

Drain *(1384)* obtained good results in Tennessee with 4-8-4 nitrogen-phosphorus-potassium fertilizer, using about 400 pounds per acre. High nitrogen applications increased losses from disease and reduced blossom yields. Best yields were obtained with plants spaced 12 inches apart in the row, with 30 inches between rows; this spacing required 17,424 plants to the acre.

Jary *(1703)* found that flowers harvested two years after the seeds were planted contained 0.84 per cent pyrethrin I and 1.00

*Unpublished.

per cent pyrethrin II. With succeeding crops the pyrethrin content decreased until at the sixth harvest the flowers contained only 0.47 and 0.40 per cent of pyrethrins I and II respectively.

A small field experiment on the fertilizer requirements of pyrethrum was conducted by Martin, Mann and Tattersfield *(1852)*. The fertilizers used were lime, fish scrap and complete artificial fertilizer composed of ammonium sulfate, superphosphate and potassium chloride. Lime, applied the first year only, at the rate of 4 tons of calcium carbonate per acre, was beneficial, causing a very slight increase in yield each year for four years; there was also a very slight increase in pyrethrin content each year, and a decrease in plant losses in the fourth and fifth years. Yearly application of moderate dressings of the other fertilizers were accompanied by very slight increases in yields in the second and fifth years and very slight increases in the pyrethrin I content in the fourth and fifth years.

Yip *(2355)* experimented with pyrethrum grown in culture solution. Pyrethrum was found to require small amounts of boron, copper and zinc and if any one of these was withheld, the plants showed pronounced deficiency symptoms. When raised in culture media, the plants produced flowers the first year and the pyrethrin content was very high, varying from 2.03 to 5.16 per cent. The yields of flowers, however, were very low.

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