Vol. 4 No. 3

PLANT PHYSIOLOGY

JULY, 1929

ON SOME LIMITING FACTORS IN THE USE OF SATURATED PETROLEUM OILS AS INSECTICIDES[1]

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(WITH TWO FIGURES)

Introduction

Owing to the increased use of saturated petroleum oils (*i.e.*, petroleum oils from which all, or nearly all *unsaturated hydrocarbons* have been removed, usually 98 per cent. or more) as insecticides when applied to foliage-bearing fruit-trees, it has become important to study the effects thus produced on the host plant.

The insecticidal efficacy has already been established by DE ONG and the two senior authors of this paper (**2**). It was noted that these oils apparently produce some adverse physiological effects on citrus trees, but this phase of the problem was not especially investigated.

The general nature of the deleterious effects accompanying the use of *heavy*, white-oil sprays has recently been described by WOGLUM, LA FOLLETTE and LANDON (**6**) as follows:

"The bad effects which have been noted on oranges in some degree since highly refined heavy oil sprays have been used are numerous. They include retarding of blossoming; reduction of blossoming; reduction of crop; retarding fruit coloration; interfering with normal sweat room coloring; drop of immature fruit; drop of mature fruit; roughened texture; mummifying of fruit; increasing crystallization; producing insipid flavor;

[1] The investigation reported as Part I of this paper was conducted by KNIGHT and SAMUELS, Part II by KNIGHT, and Part III by CHAMBERLIN. The work was done while the authors were in the employ of the California Spray-Chemical Company, of Watsonville, California. The studies reported on in Part III were made independently.

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decrease in acidity, soluble solids and sugars; increase of dead wood; occurrence of fruit burn and leaf drop; difficulty in cleaning fruit, and "gumming-up" packing house conveyors. . . . Some of these troubles were costly to the grower. For instance extreme application of the heaviest oil sprays on Valencias during October and November, in some cases reduced the succeeding crop as much as 50 per cent., and affected the quality of the fruit then on the trees."

Not all of these effects have been fully verified. Some of them are established in the discussion which follows. It should be emphasized that these effects result primarily from the application of *heavy oils* (*i.e.*, saturated lubricating oils of high viscosity).

The quick-breaking white oil sprays were developed particularly with reference to California citricultural conditions and it is there that they have been most extensively used. As insecticides they are markedly successful and because of the decreasing efficacy of HCN fumigation in California due to the development over wide and increasing areas of HCN resistant scale (**5**), heavy oil sprays have rapidly come into use. The practical need of a substitute for present methods of HCN fumigation is not likely to lessen, but the results herein presented indicate that this substitute should be something other than a *heavy* petroleum oil spray. Our data emphasize the extreme importance of a knowledge of the metabolic and pathological results following the use of oil sprays.

Part I

Some notes on the physiological effects of saturated, white petroleum oils on Citrus

In order to ascertain something of the metabolic changes induced in the plant by the application of petroleum oil as insecticides, the following experiments were made. The problem was attacked from three angles, namely transpiration, photosynthesis and respiration. The plants used were "seed-bed stock" of sour orange (*Citrus aurantium* Linn.).

Transpiration.—The plants for the transpiration tests were taken from two nurseries, one in Riverside, California, and one in Glendora, California, and were about 25 centimeters in height. Preliminary tests showed that the source of the plants is important. Those from the Riverside nursery taken in August had an average weekly transpiration rate of 40 grams of water per square decimeter of leaf surface, while plants, apparently just as thrifty, taken from Glendora, where cultural conditions were not the same, gave a weekly average of 21 grams per same unit area. Plants from the Riverside nursery taken in February showed a transpiration rate of 12.5 gm. per week. This indicates that the transpiration rate varies greatly with differences of environment—cultural, climatic and seasonal.

Two sets of experiments were run, the first between August and October, 1926; the second between February and May, 1927. The data are presented in tables I and II. The experimental conditions, aside from season, were identical in the two cases. The plants were grown in sealed jars containing HOAGLAND'S nutrient solution. The weekly transpiration losses were replaced with distilled water. The leaf surface of each plant was measured and the loss of solution determined by daily weighings. The average transpiration loss for the two weeks prior to the application of the oil was taken as a norm for purposes of comparison. All results are expressed in two forms: first, in grams of water transpired per week per square decimeter of leaf surface; and secondly, as percentage of the "normal" rate as determined prior to treatment. Individual variations between plant and plant are considerable and differences of less than fifteen to twenty per cent. can not be considered significant. The data presented in any one vertical column are comparable, but those in different vertical columns are not, owing primarily to fluctuations in humidity.

Both humidity and temperature records were kept for the first test (table I). They are not included for the reason that no significant correlation could be found. Within the normal range of variation temperature alone is not significant, for, if the ratio of relative humidity to temperature remains constant, there is no significant change in transpiration. When the relative humidity drops, however, there is an immediate increase in transpiration. But since the change in transpiration caused by weather conditions was constant for all lots included in the tests, it may be disregarded in the present connection.

The application of oil causes a sharp and abnormal drop in the transpiration rate, the extent of the disturbance varying directly with the viscosity of the oil. In the case of kerosenes and light lubricating oils, recovery is effected in from one to three weeks. With saturated oils of high viscosity (100–110 seconds SAYBOLDT) recovery does not begin under six or seven weeks and may be delayed much longer than that. The length of time required for recovery is also correlated with the amount of oil applied. It is more rapid where the oil has been applied in small amounts (*i.e.* when applied as an emulsion), than where it is applied in large amounts (*i.e.* painted on in pure form). As indicated by experiment no. 12, table I, the presence of even a small percentage of unsaturates in an oil is sufficient to cause a sharp drop in the transpiration rate and to postpone recovery materially.

PHOTOSYNTHESIS AND RESPIRATION.—To determine the extent to which photosynthesis was affected by an application of oil, a thrifty potted sour orange plant somewhat more than 60 centimeters in height was kept in a dark chamber for fifteen days, when the leaves gave no further starch

TABLE I

THE EFFECTS OF PETROLEUM OIL ON THE TRANSPIRATION OF SOUR ORANGE NURSERY STOCK (AUGUST TO OCTOBER, 1926)

No. of experiment	General specifications of oil[1]	Mode of application	Transpiration rate at start of test[2]	Transpiration rate per week after application of oil. Weeks numbered 1–8[3]							
				1	2	3	4	5	6	7	8
1	Saturated; white petroleum; viscosity 106	Both sides of leaf painted with pure oil	38.9 gm. per week	15.3 gm.	15.7 gm.	18.4 gm.	14.3 gm.	12.3 gm.	12.6 gm.	18.1 gm.	22.3 gm.
			100 per cent.	39 per cent.	40 per cent.	47 per cent.	37 per cent.	32 per cent.	32 per cent.	47 per cent.	57 per cent.
13	Same as above	Same as above	46.7 gm. per week	12.2 gm.	28.0 gm.	25.7 gm.	30.8 gm.	31.3 gm.	38.0 gm.	45.3 gm.	.....
			100 per cent.	26 per cent.	60 per cent.	55 per cent.	66 per cent.	69 per cent.	81 per cent.	97 per cent.	.....
11	Saturated; white petroleum; viscosity 50	Same as above	34.4 gm. per week	27.7 gm.	27.2 gm.	31.2 gm.	27.7 gm.	.....	.....	.....	.....
			100 per cent.	81 per cent.	79 per cent.	91 per cent.	81 per cent.	.....	.....	.....	.....
6	Same as above	2 per cent. quick-breaking emulsion	30.1 gm. per week	16.1 gm.	35.7 gm.	.....	.....	.....	.....	.....	.....
			100 per cent.	54 per cent.	119 per cent.	.....	.....	.....	.....	.....	.....
9	Highly refined nearly saturated kerosene	Both sides of leaf painted with pure oil	43.5 gm. per week	29.8 gm.	51.3 gm.	44.0 gm.	.....	.....	.....	.....	.....
			100 per cent.	68 per cent.	118 per cent.	101 per cent.	.....	.....	.....	.....	.....
12	80 per cent. saturated; lubricating petroleum; viscosity 45	Same as above	31.5 gm. per week	12.3 gm.	18.1 gm.	18.8 gm.	11.0 gm.	15.6 gm.	11.4 gm.	9.8 gm.	8.1 gm.
			100 per cent.	39 per cent.	57 per cent.	60 per cent.	35 per cent.	50 per cent.	36 per cent.	31 per cent.	25 per cent.
10[4]	None	Check; no treatment	34.2 gm. per week	.....	.....	39.2 gm.	49.0 gm.	40.2 gm.	29.4 gm.	37.6 gm.	21.7 gm.
			100 per cent.	.....	.....	114 per cent.	143 per cent.	117 per cent.	86 per cent.	109 per cent.	63 per cent.

[1] Degrees of saturation and viscosities are fairly close approximations. Viscosities are given in seconds SAYBOLT at 100° F.
[2] Average rate as determined by two weeks' observation, in grams of water per square decimeter of leaf surface per week.
[3] Percentages to nearest whole per cent. only.
[4] Owing to an accident in setting up, there are no readings for the check for the first two weeks following the oil treatments.

TABLE II

THE EFFECTS OF PETROLEUM OIL ON THE TRANSPORTATION RATE OF CITRUS (SOUR ORANGE) NURSERY STOCK (FEBRUARY TO MAY, 1927)[1]

NO. OF EXPERIMENT	GENERAL SPECIFICATIONS OF OIL	MODE OF APPLICATION	TRANSPIRATION RATE AT START OF TEST	TRANSPIRATION RATE PER WEEK AFTER APPLICATION OF OIL. WEEKS NUMBERED 1–8							
				1[2]	2	3	4	5	6	7	8
21	Saturated; white petroleum; viscosity 106	2 per cent. quick-breaking emulsion	10.4 gm.	2.0 gm.	2.4 gm.	4.5 gm.	9.1 gm.	6.1 gm.	7.0 gm.	15.8 gm.	.....
			100 per cent.	19 per cent.	23 per cent.	42 per cent.	87 per cent.	59 per cent.	62 per cent.	152 per cent.	.....
26	Same as above	Same as above	16.7 gm.	5.9 gm.	4.2 gm.	5.6 gm.	9.8 gm.	7.0 gm.	8.4 gm.	21.0 gm.	.....
			100 per cent.	35 per cent.	25 per cent.	33 per cent.	59 per cent.	42 per cent.	50 per cent.	125 per cent.	.....
24	None	Check; no treatment	11.8 gm.	7.3 gm.	14.0 gm.	13.3 gm.	11.7 gm.	10.5 gm.	12.6 gm.	9.8 gm.	13.6 gm.
			100 per cent.	62 per cent.	118 per cent.	112 per cent.	99 per cent.	89 per cent.	107 per cent.	83 per cent.	115 per cent.
37	None	Check; no treatment	15.7 gm.	8.1 gm.	16.3 gm.	16.6 gm.	14.6 gm.	11.7 gm.	15.9 gm.	11.1 gm.	15.3 gm.
			100 per cent.	52 per cent.	104 per cent.	106 per cent.	93 per cent.	74 per cent.	101 per cent.	71 per cent.	97 per cent.

[1] See footnotes 1, 2, 3, table I, which likewise apply to the data in this table.
[2] Initial low transpiration during the first week following treatment due to humid weather. Note figures for checks.

reaction to the iodine test. In the evening of the fifteenth day the plant was placed in very subdued light and was treated as follows:

Several leaves were painted on both surfaces with (1), a saturated white oil of 106 seconds viscosity; (2), with a saturated white oil of 50 seconds viscosity; and, (3) with a nearly saturated, highly refined kerosene oil. The treated leaves were then tagged and the plant returned to the dark chamber till the following evening when it was put outside, hence 36 hours lapsed before exposure to sunlight.

Sections were cut from the treated leaves daily and tested for starch. It was not until the fourteenth day that those of lot 1 gave a faint reaction to starch, and they *had not returned to normal on the fortieth day when the test was terminated.* Lot 2 showed a faint starch reaction on the first day and had returned to normal by the fourth. Lot 3 gave starch reaction the first day. Checks gave a normal starch reaction the first day.

In order to determine the extent to which photosynthesis was interfered with under orchard conditions, analyses of the starch content of leaves taken from a lemon grove which had been sprayed with a white, saturated petroleum oil emulsion (viscosity 106 seconds SAYBOLDT) at 2 per cent. concentration, were made at approximately regular intervals. The results are set forth in table III. These lemon trees were sprayed on December 15, 1926. Surrounding lemon groves were fumigated. Leaf samples were taken at random from both the sprayed and fumigated trees, those from the fumigated trees being taken at the beginning and at the end of the experimental period. These latter may be considered as a check. The

TABLE III

STARCH ACCUMULATION IN LEMON LEAVES SPRAYED WITH A SATURATED WHITE PETROLEUM OIL SPRAY
(VISCOSITY 106)
(FIELD CONDITIONS)

NATURE AND DATE OF TREATMENT	GRAMS OF STARCH IN 5 GRAMS OF LEAVES	PER CENT. OF STARCH TO DRY WEIGHT OF TREATED LEAVES	DATE SAMPLED
	gm.	*per cent.*	
Fumigated with hydrocyanic acid gas, December 15, 1926	0.0986	1.97	Dec. 29, 1926
	0.0960	1.92	Feb. 25, 1927
Sprayed with white oil emulsion at 2 per cent. concentration, December 15, 1926	0.0910	1.92	Dec. 29, 1926
	0.0744	1.49	Jan. 12, 1927
	0.0566	1.13	Jan. 21, 1927
	0.0604	1.21	Feb. 8, 1927
	0.1160	2.32	Feb. 25, 1927

results show that the starch content of the unsprayed trees remained constant within the limits of normal variability and experimental error. In the sprayed block the starch content dropped off markedly the first five weeks, and then rose rapidly until at the termination of the test it was far in excess of that of the check trees. Results similar to the above, showing increased starch content in the leaves two or more months after spraying have been noted in several other groves.

An attempt was also made to determine the effect on respiration, by the application of a heavy white oil as follows:

The above-ground portion of a potted Citrus plant was sealed under a large bell-jar. A small opening was provided at the bottom for the inflow of air, and a second small opening at the top to permit samples to be drawn off. The air taken out at the top was run through a gas train which removed the moisture and then the carbon dioxide was trapped in soda-lime and weighed. The amount of air passing through the system was controlled by means of an aspirator connected to the train. In this way the volume of water flowing from the aspirator was equal to the volume of air entering the system. Thirty-six liters of air were drawn through the apparatus for each determination. Several determinations were made with the empty apparatus to determine the amount of CO_2 normally present in the air. This was found to be 0.0166 grams per 36 liters. It is evident that when the same amount of air is drawn through the apparatus with the plant enclosed and more than this amount of CO_2 is found, that the plant is giving off CO_2, or, in other words is respiring in excess of photosynthesis. The point at which these two quantities are equivalent is indicated by line "A" in fig. 1, where the results of this test are shown in graphic form. The distance of the plotted curve above or below this line therefore shows the extent to which the one or the other of these processes predominates.

Two thrifty untreated Citrus trees about six decimeters in height were placed under bell jars as before indicated and the CO_2 value was determined for each on four consecutive days. The curve (fig. 1) is well below line "A" and shows that photosynthesis was therefore in excess of respiration, the plants fixing an average of ten milligrams of CO_2 each day during the period covered by the test. Each run lasted approximately five and one-half hours. The four-day average is indicated in fig. 1 by the broken line designated "Norm." This is to be considered an average of photosynthetic activity for the plants in question, only for this season of the year; it would probably be greatly exceeded during the growing season.

The plants were then treated as follows: The entire leaf surface of one was painted with saturated oil of 106 seconds viscosity. The other was sprayed with a 2 per cent. emulsion of the same oil.

The graph, fig. 1, is self-explanatory. Respiration was enormously increased, the plants evidently oxidizing great quantities of reserve food.

Neither plant had returned to normal at the expiration of a month after the application of the oil. The reaction of the two plants differed only in degree, as was to be expected.

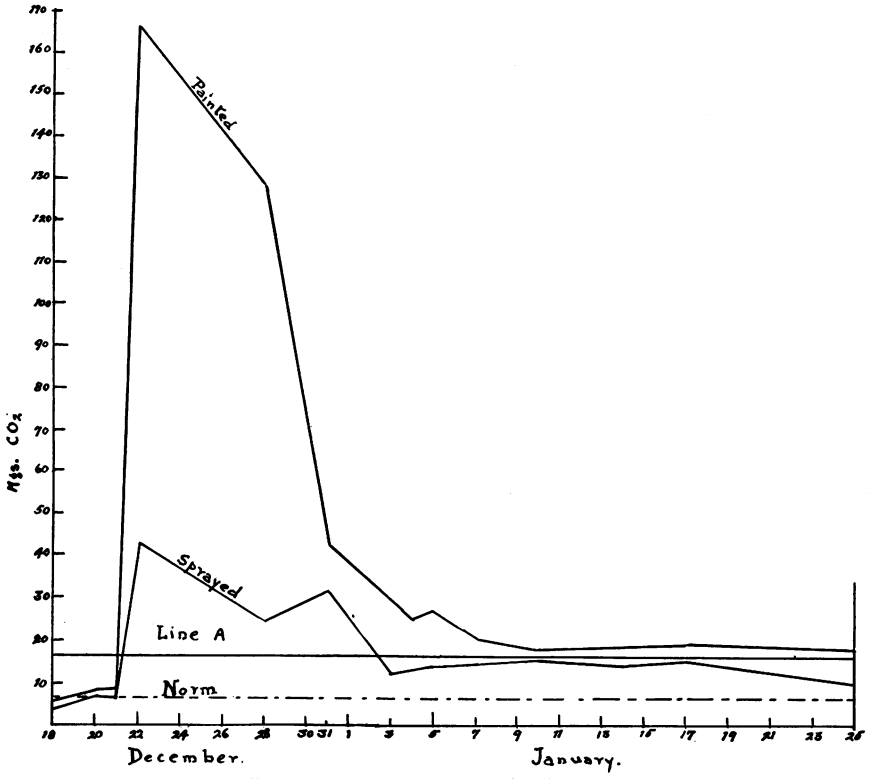

FIG. 1. Effect of oil sprays on respiration of Citrus trees.

It is recognized that the data presented are not sufficient to furnish a basis from which to draw definite conclusions. They indicate, however, that the application of a viscous, saturated petroleum oil induces profound metabolic changes in Citrus trees which persist for an extraordinary length of time.

It seems desirable to emphasize the fact that mere lack of "corrosive" qualities or the possession of "neutrality" or "chemical inertness" on the part of an oil does not imply that it is without deleterious effects when applied to living plants.

Part II

THE TRANSLOCATION OF PETROLEUM OIL IN THE LIVING PLANT (CITRUS)

Part I of this paper presented in a general way some of the physiological effects produced on the metabolism of *Citrus* by an application of a highly

refined, saturated petroleum oil, and indicated that the inhibitory effects are of surprisingly long duration.

Field observations also indicate that the heavier oils are absorbed by the leaves and remain therein for a very long period of time. The experiments already presented show that the functional activity in the leaf is partially resumed within about sixty days. In order to ascertain, if possible, how the oil was finally disposed of by the plant a histological study of oil penetration into the leaf was undertaken. To accomplish this successfully it was necessary to devise a special technique for staining the oil within the leaf tissue. It was recognized at the outset that none of the solvents in general use could be employed, as they are all oil solvents; and the problem was to fix or clear the leaf without dissolving the oil. Consequently the use of alcohol, xylol, or any of the essential oils as well as canada balsam was precluded, as was also the paraffin method of preparing sections.

Two kinds of preparations were desirable, (1) a flat gross preparation in which the distribution of oil in the leaf could be observed over a relatively large area, and (2), cross-sections in which the individual cells could be observed in their relation to the absorbed oil thus securing some insight into the penetration of oil into the cells themselves.

For the gross preparations it was necessary to "clear" the leaf, that is, to dissolve the chlorophyll and render the leaf transparent without dissolving or disturbing the oil; and for cross-sections, to fix the tissues for staining and sectioning also without disturbing the oil.

A short note in "Stain Technology" (**4**) gave a suggestion which materially helped to solve both phases of the problem. This note called attention to the use of pyridine in aqueous solution as a carrier for a fat stain. Pyridine is soluble in water as well as oil. Both Sudan III and IV, and Oil Soluble Red O are soluble in pyridine. When an oil-treated leaf is immersed in a saturated solution of Oil Soluble Red O dissolved in an aqueous pyridine solution, the stain, having a greater affinity for the oil than for the aqueous pyridine solution, is taken up by the oil. The solution of pyridine can then be removed by washing. In addition it was found that an aqueous solution of pyridine is an excellent solvent for chlorophyll, thus permitting "the killing of two birds with one stone" for it thereby became possible to clear the leaf and stain it at the same time without disturbing the oil.

The technique developed is substantially as follows:

For flat gross preparations.—Immerse in sixty per cent. aqueous solution of pyridine. Heat over water-bath. When discolored pour off and refill with fresh solution. Repeat (usually twice is sufficient) till solution remains clear and specimen becomes transparent.

Immerse for twenty-four hours in saturated solution of Oil Red O dissolved in 70 per cent. aqueous solution of pyridine.

Differentiate in 50 per cent. pyridine.

Wash in running water.

Pass through (1) glycerine-water (equal parts), followed by (2) pure glycerine.

Clear in carbol-glycerine (1 part carbolic acid to 2 parts glycerine). Heat gently and watch carefully under dissecting microscope till clear. Specimen should be turned under-side up, when oil droplets can be seen distinctly.

Pass through glycerine again and mount in glycerine-jelly.

Allow to harden and seal with clear "Duco."

For cross-sections.—Fix in chrome-acetic acid for 48 hours.

Wash in running water.

Immerse in 5 per cent. formalin for 30 minutes.

Wash again in running water.

Immerse in 50 per cent. pyridine (aqueous solution) for 10 minutes.

Stain for twenty-four hours in saturated solution of Oil Red O dissolved in 70 per cent. aqueous solution of pyridine.

Differentiate in 50 per cent. aqueous solution of pyridine until color ceases to stream (watch carefully).

Wash in running water.

Section. See below.

Pass through (1) glycerine-water, followed by (2) glycerine.

Mount in glycerine-jelly and seal with clear "Duco."

Inasmuch as imbedding in paraffin is precluded, cross-sections were made by means of pith or cork. The freezing method could no doubt be used, but the writer has not had the opportunity of trying it. The operations of staining, washing, clearing and so on, are carried on with the aid of watch-glasses or small shell vials. If watch-glasses are used for staining they should be placed in closed petri dishes to prevent excessive evaporation. Watch-glasses are preferable for use with cross-sections. Staining may be done either before or after sectioning. If done before, shell vials will be found preferable.

For purposes of comparison the following oils were used:

(1) Heavy, saturated, white petroleum oil of 100–110 seconds viscosity SAYBOLDT at 100° F.

(2) A medium lubricating oil consisting of an equal mixture of 1 and 3. Saturation about 97 per cent.; viscosity about 67.

(3) Light, lubricating petroleum oil of about 50 seconds viscosity and 96 per cent. saturation.

(4) Light, lubricating oil of 44 seconds viscosity and 67 per cent. saturation; an unsaturated oil probably blended with a distillate.

It is regrettable that accurate data regarding other physical constants of these different oils are not available, such as vapor pressure at ordinary temperature, acidity, rate of oxidation, etc. Until such information is available much of the value of this type of study is lost.

Experimental data

Four potted orange plants were selected, of uniform size (about 6 decimeters in height) and having one or two lateral branches. Several of the leaves, from the tip to center of one limb, were carefully treated with oil applied by means of a small artist's brush, the oil being applied to both surfaces of the blade as far back as the petiole. The limb was tagged at the base of the lowest treated leaf. After treatment the plants were set away in the lath house.

Oil number 1 was taken as an index for the initial penetration tests, samples being cut from the leaves at intervals of 1, 2, 4, 8 and 24 hours. Owing to the great amount of labor involved, it was not possible to take samples from all four treated plants for this initial test. This is unimportant, the only possible difference being one of rapidity of penetration, the lighter oils penetrating more rapidly. Oil number 3, for example, disappeared from the surface in approximately three days, while oil number 1 remained a week or more. (See also the data on penetration in part III of this paper.)

At the expiration of the first twenty-four hours and at certain intervals afterwards, samples were taken from all four plants simultaneously and were treated together, each sample being cut to a distinctive pattern to facilitate its recognition.

After the first 24 hours, samples were taken every three days until it became evident that the withdrawal or disappearance of oil from the tissue of the leaf, even in the case of the lighter oils, was a very lengthy process. The time between samplings was then lengthened to one week.

The disappearance of oil, either from the surface or from within the leaf, was not caused by evaporation, as is generally supposed, except perhaps in a very minor degree. As a matter of fact, the evaporation of oils of high boiling points and low vapor pressures (lubricating oils) is probably a negligible factor at ordinary atmospheric temperatures, especially when enclosed within the intercellular spaces of the leaf tissue. Only those oils with boiling points within or near atmospheric temperature ranges evaporate quickly, *i.e.*, benzine, gasoline, kerosene, etc.

Each set of samples was prepared and mounted both in flat gross mounts and cross-sections, carefully studied under the microscope and compared with preceding lots.

Manner of penetration

Samples taken at the expiration of one hour after treatment showed that the cutinized outer integument covering the epidermal cells absorbs oil rapidly. On the upper surface of the leaf the oil accumulates in the depressions between the cells as shown in fig. 2 A. On the lower epidermis it pours through the stomata and runs along the cell walls, frequently coalescing into droplets between the outer walls of the guard cells (fig. 2 B, C). Such droplets disengage themselves and pass into the intercellular spaces of the spongy chlorenchyma. When drops are not formed at this point the oil may run along the outer surface of the epidermal cell wall and gradually spread out between the cells of the spongy chlorenchyma.

At the end of the second hour this process shows further developments. A considerable amount of oil now shows along the outer surface of the

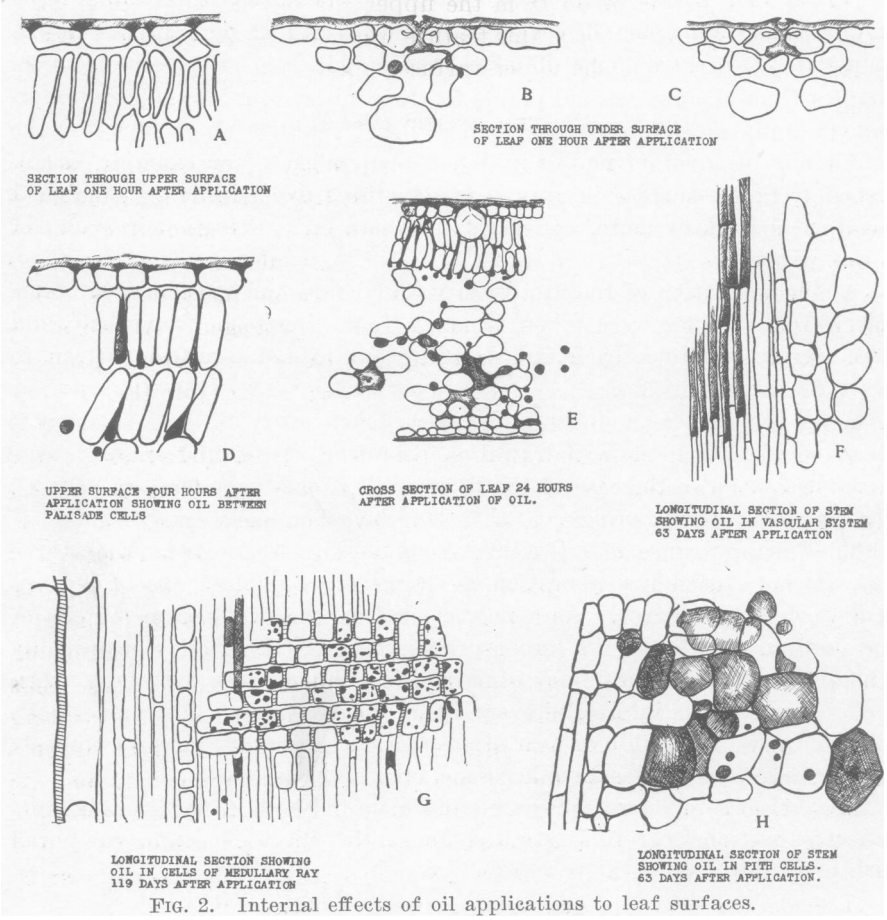

FIG. 2. Internal effects of oil applications to leaf surfaces.

epidermal cells and there are a number of small droplets scattered here and there in the intercellular spaces. Little difference is to be observed on the upper surface except that the oil has penetrated deeper into the depressions between the epidermal cells. By the fourth hour, however, oil begins to appear in the palisade parenchyma (fig. 2 D), apparently having been absorbed by the cell walls and carried thence through the epidermal layer. It is first seen as a minute "thread" squeezed between the long cells, gradually expanding as it descends and finally coalescing in drops at the bottom of the palisade cells and passing into the intercellular spaces of the spongy chlorenchyma. At the end of twenty-four hours oil is entering the leaf from both upper and under surface (fig. 2 E). Quantitatively, however, there is little doubt that the greatest penetration occurs beneath. See part III.

As the penetration of oil from the upper surface had not, apparently, been demonstrated previously, this feature was checked with positive results by treating leaves upon the upper surfaces only.

General observations

The oil in the intercellular spaces apparently extracts or dissolves materials from the adjacent cells. In unstained specimens the oil becomes green in color, having evidently dissolved chlorophyll. In stained specimens minute black specks begin to appear in the oil globules at about the third day. These bodies grow larger and coalesce, forming black masses which pass to the periphery of the droplet and become disengaged. At this stage oil can be observed within the cell itself in the form of very minute droplets. It gradually accumulates along the larger veins and passes into the vascular system.

When it became apparent that oil was being translocated steps were taken to ascertain what became of it. Sections were cut from the lateral branch below the treated leaves and also from the main trunk below the point of union with the lateral. Longitudinal and transverse sections of both of these showed large quantities of oil in the phloem (fig. 2 F), the medullary rays (fig. 2 G), and finally in the large storage cells of the pith and old wood fiber of the xylem (fig. 2 H). Some cells became full of oil, others were apparently in process of being filled. Even in those cells already packed with starch grains, oil can distinctly be seen penetrating between them. No oil has been observed in wood of the current season's growth; it is carried across and deposited in the pith parenchyma and the *old* wood fibers. How far down the limb this process extends is not known, but it is probable that most of the oil is deposited in the smaller twigs and limbs and this may account for the great increase in deadwood noted by observers in the field following an application of heavy oil.

Summarizing, the several distinct phases in the elimination of oil from the leaves are seen to be:

(1) Penetration into the intercellular spaces from the leaf surface.
(2) Entrance of the oil into the cell.
(3) Entrance of the oil into the vascular system and translocation down the leaf trace into the stem.
(4) Transfer from the phloem across the medullary rays.
(5) Deposit of the oil in the large storage cells of the pith and in the old wood fibers of the xylem.

Observations on particular oils

Oil number 4.—This was the first one to disappear from the leaves. It is less viscous than oil number 3 although of about the same degree of volatility. It is an unsaturated oil and caused some injury to the leaves, destroying the cells in spots, and causing some of the leaves to drop. The older leaves lost their dark green color and became yellowish, but they regained their color later when the oil disappeared. The last trace of oil was found in the leaf on the 65th day after application. One hundred days after treatment, sections were cut from the limbs below the treated leaves. Both longitudinal and transverse sections showed much oil in the phloem, the medullary rays and the pith and old wood fibers of the xylem.

Oil number 3.—The last trace of this oil was found in the leaf on the 107th day after treatment, 42 days after oil number 4 had disappeared. Oil was still present in the veins of the leaf at this time. One hundred and nineteen days after application and twelve days after the oil had disappeared from the leaf, sections were taken from the stem about seven to eight centimeters below the lowest treated leaf (ten normal untreated leaves occupied this interval). A large quantity of oil was found in the phloem, in the medullary rays and in the pith and old wood fibers of the xylem.

Oil number 2.—The last trace of this oil was found in the leaf 121 days after application. There was still much oil in the veins, and sections from the stem made later showed the same condition as that described above for number 3.

Oil number 1.—This was the most viscous oil tested (106–110 seconds Saybolt). *A large quantity of this oil was still in the leaf 257 days after application.* The translocation of this particular oil is extremely slow, in correlation with its high viscosity. It may, either partially or completely choke certain elements of the vascular system of the leaf for long periods of time. Inasmuch as oil has been found in the leaves of specimens taken in a commercially sprayed grove no less than sixteen months (480 days) after application, it is not improbable that some oil remains in the leaf during most, if not all, of its life period. It was therefore decided to

terminate the test. The stem was sectioned below the treated leaves. As in the previous instances a large quantity of oil was found in the phloem and some in the cells of the pith.

Checks from untreated plots prepared by the same technique were frequently made, both from leaves and stems. Fatty substances, lipoids, resins, essential oils and cutin associated with the leaves and cells were stained but could easily be distinguished from the petroleum by their much darker color.

From the data presented it may be concluded that viscosity is the single factor of greatest importance in determining the mobility of an absorbed oil, and the length of time the oil remains in the leaf and vascular system. The period of persistence is apparently independent of unsaturation provided the tissues are not actually killed. Volatility, as a factor influencing the disappearance of an oil of high boiling point from the intercellular spaces of the leaf, may be disregarded in practise, inasmuch as this study demonstrates that the bulk of this disappearance is due to translocation to other parts of the plant. The lighter oils, being the easiest to move, are the first to disappear.

It appears quite evident that the *accumulation of starch in the leaves, noted in part I of this paper, is due, not to any real stimulation of the plant, but simply to the fact that as the leaf begins to function again, it manufactures carbohydrates which it cannot effectively translocate due to the overloading of the conducting vessels with oil.*

If this condition persists over a protracted period of time it must eventually react unfavorably on the root system, thus weakening the entire tree.

The foregoing data suggest an adequate explanation for the many adverse physiological effects observed in the field following the application of heavy, saturated, white petroleum oil sprays.

Part III

On some factors in the problem of the absorption and translocation of saturated petroleum oil in the living plant

The initial penetration of oil into the leaf.—It is generally assumed that penetration of oil into the leaf is by way of the stomata. On the whole, this belief seems to be sound. The length of time a surface film remains on a treated leaf is found to vary with the type of oil and the character of the cuticle. Thus a light oil (kerosene for example) applied to a succulent, herbaceous leaf which is supplied with large numbers of stomata disappears from the surface rapidly, the time varying from a few minutes to an hour or so. On leaves of the California Live Oak no free oil (of the kerosene type) persists longer than twenty-four hours. No doubt some of

this disappearance is due to volatility. But with a lubricating oil of high viscosity (100–110) the case is different. Oil applied to a *Malva parviflora* leaf did not disappear entirely until the end of the third day. In the case of the leathery evergreen leaves of the California Live Oak (*Quercus agrifolia*) and California Bay (*Umbellularia californica*), the time is very greatly increased. On the Bay leaves there were traces of free oil sixteen days after application, and oil did not disappear from the Live Oak leaves till some time later. The presence of oil was still visible *inside* the leaves of both Bay and Live Oak seven months after application although in apparently reduced amount. Leaves of these two plants were also treated on their upper surfaces only. The period of persistence of free oil was greatly lengthened, and in both leaves when penetration did occur it took place primarily along the margins and tip of the *underside* where the oil had drained from the upper surface. Some dorsal penetration was observed to occur in the Live Oak leaf where it could not be explained by "underrun" but this did not take place to an appreciable extent until the March following the application—a period of three months.

In order to learn more concerning the rate of this initial penetration, leaves were removed from different plants and immersed in pure oil. The time necessary for complete penetration was recorded. The entrance of oil into the intercellular spaces causes the leaf to become translucent. Complete translucence was taken as the criterion of complete penetration. A representative sample of the data obtained is given in the following table (table IV).

The great difference in susceptibility to oil-penetration by different leaves is clearly illustrated in the table mentioned. Even those leaves most resistant to complete penetration (*e.g.*, Live Oak) show considerable and almost immediate partial penetration. Evergreen leaves are, on the whole, more resistant to penetration than herbaceous leaves, and leaves adapted to xerophytic conditions are most resistant of all.

The effect which viscosity plays in penetration is evident in the tests which follow. The leaves of Cranesbill (*Erodium* sp.) immersed in kerosene show complete penetration in 40 to 60 minutes. In saturated petroleum oil of 110 seconds viscosity, it required 300 to 360 minutes.

The resistance of xerophytic leaves of either the succulent or leathery type (*e.g.*, Stonecrop and Live Oak) is due to imperviousness of the epidermis. Penetration in such leaves is very rapid if an artificial opening is made through the epidermis. The succulent tissues of the Stonecrop absorb oil as so much blotting paper if the epidermis be first stripped off. They seem to resist penetration indefinitely if it be left intact. The variation of the method of penetration into different leaves is shown by the following experiment. A lemon leaf and a Live Oak leaf were taken and the tips sliced

TABLE IV

PENETRATION OF KEROSENE OIL INTO LEAVES OF VARIOUS KINDS. (ISOLATED LEAVES IN PANS OF PURE KEROSENE AT ROOM TEMPERATURE)

SPECIES OF PLANT	TYPE OF LEAF	NATURE AND DEGREE OF PENETRATION AT END OF 15 MINUTES[1]	TYPE OF PENETRATION	TIME OBSERVED FOR COMPLETE PENETRATION TO OCCUR
Montia sp. (Miner's Lettuce)	Succulent, moisture-loving mesophyte	100 per cent.	Uniform	A few seconds; absorbs oil like "blotting paper"
Sambucus sp. (Elder)	Soft; mesophytic. Veins non-sclerenchymatous[3]	About 50 per cent.	Grease-spot[2]	1 hour
Coprosma sp. (New Zealand Looking Glass Plant)	Strongly cutinized arid mesophytic. Midrib and large veins sclerenchymatous	25–30 per cent.	Large checkered[4]	3–4 hours
Rubus sp. (Blackberry)	Soft, hairy, mesophytic. Middle and large veins sclerenchymatous	A few large polygons show partial penetration	Large checkered	10–12 hours
Citrus sp. (Lemon)	Oily, leathery. Non-sclerenchymatously veined	Indefinite blotches show faint partial penetration	Grease-spot	
Umbellularia californica (California Bay tree)	Oily, leathery. With fine network of heavily sclerotic veins	Scattered polygons show faint penetration	Small checkered	About 6 days (140–150 hours)
Quercus agrifolia (California Live Oak)	Leathery, non-oily xerophytic. With fine network of heavily sclerotic veins	Scattered polygons show faint penetration	Small checkered	40 per cent. complete at end end of eighth day (192 hours)
Sedum sp. (Stonecrop)	Xerophytic, succulent (of "cactus type")	None	None	At end of eighth hour there was no indication that penetration had so much as begun. (Not observed further)

[1] Degree of penetration, eye estimates only.

[2] Grease-spot penetration occurs in leaves in which the veins do not form more than a temporary barrier to the lateral diffusion of oil, and in which spread is from a more or less definite center, so that it appears as a circular grease-spot.

[3] The term *sclerenchymatous* is here used in the sense that such tissues are sufficiently abundant around the veins to completely or very nearly stop lateral oil diffusion across the veins.

[4] Checkered penetration occurs where there is a network of sclerenchymatous veins which inhibit lateral diffusion so that each enclosed polygon of the veinous network must be penetrated independently.

off with a razor. The cut leaves were then immersed in oil. The oil rose by capillarity in the lemon leaf and ultimately completely filled the intercellular spaces up to the point of union of the blade with the petiole. In the oak leaf, however, only the mesophyllic sclerenchyma-enclosed polygons which were opened by the razor cut filled with oil. This occurred almost immediately, but there penetration stopped indefinitely.

These observations suffice to illustrate the main features of the phenomena under consideration and to indicate that the factors surrounding oil penetration vary both with respect to the type of oil and the character of the leaf upon which it is applied. The main factor so far as oil itself is concerned is without doubt viscosity.

TISSUE KILLING BY SATURATED PETROLEUM OILS.—When white oils were first used as insecticides, verified evidence as to "burn," *i.e.*, actual tissue killing, was lacking. It has since been established that over-application of some of these oils does result in killing twigs and even branches of orchard trees. There are indications that true leaf-burn may also occur, but in all cases which have been personally observed this occurs only when the intercellular spaces are completely filled with oil and rendered translucent. This does not ordinarily occur in the field because the amount of oil applied is generally less than the capacity of the intercellular spaces.

Leaves of the California Live Oak and California Bay tree were painted with a saturated petroleum oil of 110 seconds viscosity on December 20, 1926. Five months later, of the 57 Bay leaves treated, 3 had dropped; 2 showed a ten per cent. tip burn, and nearly all the rest showed small marginal areas which had been killed by the oil and had eroded away leaving the leaf margins irregular. Of the 33 leaves observed as a check, one had dropped in that period; the rest were normal. The under surface of the Bay leaf normally possesses a distinct waxy bloom. This was destroyed and had not returned at the end of five months.

The results were similar but more severe in the case of the oak leaves. At the end of the five months observation period, of the 48 leaves treated, 36 had droppd; 2 showed 20 per cent. burn and ten appeared nearly normal. No drop or injury showed on the leaves kept under observation as a check. Leaves treated with ordinary kerosene behaved in all respects as the check lot. The bloom on the under surface of the Bay leaves although dissolved as completely by the kerosene as the heavy oil, had returned within six weeks after the application.

On succulent herbs the effect is still more pronounced. Of 100 "Cranesbill" (*Erodium* sp.) leaflets treated with the saturated viscous oil above noted, 33 were "normal" (still impregnated with oil); 27 burned or yellowed and 40 completely dead 15 days after treatment. At the end of one

month all were dead. On this plant kerosene kills or "burns out" large areas of leaf surface where penetration is greatest. This burning occurs within a day or two after the application. But those areas which are not immediately killed do not subsequently die or show other deleterious effects.

TRANSLOCATION OF OIL IN THE LIVING PLANT.—In order to verify the observations reported in part II concerning the translocation of oil from the leaves to the pith of Citrus plants, leaves of the English Laurel (*Laurocerasus laurocerasus*), a glossy leaved evergreen, were painted with saturated petroleum oil of 106–110 seconds viscosity. Six weeks later a section of the stem well below the treated leaves was removed, the bark shaved off, and the resulting woody cylinder carefully scraped to avoid any possible surface contamination; this was finely sliced with a sharp razor and extracted with CCl_4. A similar extraction was made of a sample from a normal branch. From the treated branch 11.2 mg. of CCl_4-soluble material per gram weight of tissue were recovered, while the check yielded just half as much or 5.6 mg. per gram of tissue.

Potato plants were grown and the leaves were treated with the same oil noted above, just prior to the time of tuber formation. After two weeks further growth, the plants were uprooted and the young tubers were washed, sliced and extracted with CCl_4. The tubers of the treated plant yielded 5.4 mg. of CCl_4-soluble material per gram while the check yielded 1.8 mg. The test was repeated, the treated plant yielding 18.6 mg. of CCl_4-soluble material per gram of tuber, the check yielding 7.8 mg. There is a large quantitative discrepancy here which may be explained by the fact that the second lot of material was more finely chopped and was extracted for a longer period of time, but inasmuch as each lot was treated in the same manner as its control, the general conclusion remains unchanged. In addition, the presence of oil exerted a profound effect on tuber formation, those of the treated plant were the size of peas at the time of testing, while those of the check were the size of large marbles, *i.e.*, four or five times as large.

The ability of the plant to absorb and translocate oil was then tested in a third way. Clean river sand was intimately mixed with the same oil noted above in the proportion of 6.8 cc. of oil to 340 grams of sand. Beans were germinated in this substratum and when the plants had reached a height of ten centimeters they were cut off just above the ground line, finely chopped and extracted with CCl_4. From one lot 8.2 mg. of CCl_4-soluble material per gram of tissue were obtained and from a second lot 10 mg. The check yielded 3.8 mg. under the same conditions. The test was repeated with substantially the same results; the plant grown in oiled sand yielded 15 mg. of CCl_4-soluble material per gram of tissue as compared with 5 mg. for the check.

The sand in the preceding experiment did not appear particularly oily and the plants grown therein were apparently normal.

These results demonstrate the reality of cellular penetration of oil and indicate that the plant is capable of translocating oil either from root to leaf or vice versa.

Volatility as a factor in the disappearance of viscous petroleum oil

Volatility has been regarded as *one,* if not the *only,* important factor in oil disappearance from a sprayed plant. As late as August, 1928, de Ong (3) suggests volatility as the primary factor in oil disappearance. This is shown to be in error in the present paper. However, it is certain that even heavy lubricating oils when exposed to air do volatilize to a certain very limited extent. This was tested as follows: Oils were painted in thin films on clean glass plates. One set was exposed to sunlight for a period of eight months under conditions which admitted air but excluded dust. A second set was kept in the dark in the laboratory. Weighings were made from time to time and noted. The results are summarized in table V.

TABLE V

Volatility or weight changes in thin oil films on glass, in free air (November, 1927, to June, 1928)

Oil	Mg. oil to area of glass	Exposure	Per cent. change in weight after 2 months exposure	Per cent. change in weight after 8 months exposure
Saturated White Western Viscosity 106–110	10.8 mg. / 25.5 sq. cm.	Sunlight	Loss 43 per cent.	Loss 89 per cent.
	23.7 mg. / 51.6 sq. cm.	Dark	Loss 14 per cent.	Loss 27 per cent.
Saturated White Western Viscosity 75	16.6 mg. / 51.7 sq. cm.	Sunlight	Loss 87 per cent.	Loss 91 per cent.
	17.0 mg. / 51.7 sq. cm.	Dark	Loss 47 per cent.	Loss 82 per cent.
Saturated White Eastern Viscosity 75–85	15.8 mg. / 51.8 sq. cm.	Sunlight	Loss 12 per cent.	Loss 82 per cent.
	11.0 mg. / 51.2 sq. cm.	Dark	Gain 2 per cent.	Loss 4 per cent.

From these data it is evident that volatility even in the case of heavy oil does occur under the conditions of this experiment. It is important to keep in mind however that similar conditions do not obtain when oil is applied to the plant; in the latter case absorption takes place immediately and is

complete in a few days; once within the tissue, the oil is "bottled up." Optical observations in the latter case render it certain that volatility is negligible and does not nearly equal the figures given above. Furthermore, petroleum oils are not constant boiling point mixtures. Evaporation is therefore most rapid at first and involves only the lighter fractions leaving the heavier and less volatile residue behind. Once inside the leaf, oil disappearance is no doubt due primarily to translocation.

Summary and conclusions

1. Experience has shown that saturated petroleum oils, although not toxic to plants in the ordinary sense of the word, are nevertheless the cause of more or less profound and long continued metabolic disturbances in the plant which may ultimately become seriously deleterious.

2. These metabolic disturbances appear to be due to physical rather than chemical handicaps imposed by the intrusion of the oil into the plant tissue.

3. Oil applied as a free cuticular film, either by means of a brush or a quick-breaking emulsion, persists as such, for a period of a few minutes to fifteen or twenty days, depending primarily upon the morphology of the leaf and the viscosity of the oil.

4. The disappearance of the oil as a free surface film is due to its absorption by the leaf. A certain amount of oil is dissipated in gaseous form at this time. This amount is negligible except in the case of non-lubricating oils.

5. The leaves of plants which are adapted to xerophytic or arid conditions are much more resistant to this initial penetration than softer mesophytic or succulent leaves.

6. The resistance offered to this initial penetration is mostly epidermal, as shown by the fact that penetration in a xerophytic leaf may be extremely rapid once ingress to the interior is obtained. The same factors which protect such leaves from excessive water loss appear to be likewise effective as a protection against the ingress of oil. Penetration is always most rapid in areas rich in stomata but is not necessarily confined thereto. In citrus at least, penetration may occur by seepage between the epidermal cells of the upper (stomata-free) surface of the leaf.

7. Oil penetration is not usually uniform over an entire leaf; certain focal points are first penetrated and the oil tends to spread peripherally from those points. In certain leaves this early peripheral spread is checked more or less completely by the network of veins. Penetration in a leaf of this sort involves the independent absorption by each of the vein-bound polygons of mesophyll.

8. Beginning within a few days after entrance into the intercellular spaces of citrus and extending over a period of many months in the case of

a saturated petroleum oil of 106 seconds viscosity, the oil is taken into the vascular system of the plant and translocated to the storage tissues (pith and xylem parenchyma).

9. In Citrus plants the absorption of oil has been observed directly by microscopical means in the phloem of the leaf traces and stem; in the medullary rays of the stem, and in the pith and xylem parenchyma and sclerenchyma of the stem. No oil has been observed in xylem tissues of the current year's growth.

10. During the period of oil penetration and initial translocation, transpiration is sharply decreased and respiration enormously increased. Photosynthesis becomes temporarily inoperative. This may be due in part to the effects of the oil on the chloroplasts. Intercellular oil is shortly turned green by the extracted chlorophyll.

11. Recovery begins quickest in the case of light oils and is indicated by the return of transpiration and respiration rates towards normal and by the accumulation of carbohydrates in the leaves in abnormally large amounts. This latter phenomenon is correlated with the fact that the conducting vessels (phloem primarily) are still taxed to capacity with the oil and hence cannot adequately handle the carbohydrates now beginning to be produced in excess of the needs of the leaves.

12. The increase of carbohydrates in the leaves of Citrus trees recovering from an oil treatment is therefore in all probability a pathological phenomenon and not an indication of more vigorous synthetic metabolism or "stimulation" as has been maintained.

13. In addition to visual methods (microscopic) the fact of oil accumulation in pith and xylem has been verified by another means. Storage tissues of branches or plants whose leaves had been painted with oil were extracted by means of carbon tetrachloride and the oily materials in such tissues were found to be far in excess of the amounts in similar tissues of untreated plants.

14. It was also found that the plant is capable of transporting oils from the roots upward to the leaves. The leaves of young beans and peas grown in oil-treated sand show a much higher content of oily material than plants grown in normal soils.

15. The intracellular absorption of oils both from the intercellular spaces of the leaf and from the soil indicates the passage of oil molecules through the cell membrane into the cytoplasm of the cell.

16. Microscopic observation of all stages in the translocation of oil was rendered possible by devising a special staining technique which is described in the text.

17. Experiments with oil films on glass plates indicate that volatility is a real factor in the disappearance of even viscous oils in *free air*. In

practise however, due to the enclosure of the oil in the intercellular spaces, it is unquestionably negligible in comparison with translocation.

18. From the effects summarized in the preceding paragraphs the conclusion seems evident that *heavy* white oils (of a viscosity exceeding 60 seconds SAYBOLDT) must be used sparingly and with a great degree of caution, if, in the future, serious ultimate injury is to be avoided.

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