EFFECT OF PETROLEUM OILS ON THE RESPIRATION OF BEAN LEAVES

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(WITH FIVE FIGURES)

The injurious effect of petroleum oils on fruit trees is a serious objection to their use as insecticides. The damage appears in a great variety of ways (**10**). Trees sprayed in dormant stage show injury during the following spring as a wrinkling or withering of the bark and retardation of leaf formation. The damage to sprayed leaves generally appears as oil soaked spots or a more acute drying or burning of the leaf.

The exact physiological actions accompanying injury from oil are not well known. The problem appears to be divided into two major possibilities; plugging of stomata and poisoning of cells. In either case respiration should be affected. It has been the purpose of this work to determine the change in rate of respiration of plants which have been sprayed with oil.

Mechanical injury has been shown by RICHARDS (**9**) and others (**3**) to increase the rate of respiration. Toxic and anesthetic materials at certain concentrations are also expected to increase the respiration of plants (**2, 8**).

KNIGHT, CHAMBERLIN and SAMUELS (**6**) have determined the effect of a viscous, highly refined oil in the rate of respiration of citrus trees. Their results show that respiration is greatly increased and remains abnormally high for a long period of time after treatment with this type of oil.

Believing that respiration is generally increased when a plant is injured, an attempt has been made in this study to show the injurious effect on the respiration of bean leaves by the different types of oil used as sprays.

Methods

PLANTS

Dandelion and potato leaves were first used, but later bean leaves were found to give more uniform results. Three- to six-gram samples of bean leaves were taken and the stems placed in water as soon as they were cut. Four samples were used for checks and four were sprayed with oil.

SPRAYING

The spraying was done with an atomizer operated with compressed air (figure 1). It was calibrated before each determination to deliver a definite amount of oil. This was done by spraying weighed filter papers and then weighing after spraying to determine the amount of oil delivered. The leaves were then sprayed with the same nozzle adjustment, temperature and air pressure. The time of spraying the leaves was adjusted to apply 10 milligrams of oil on a circular area 7 centimeters in diameter, which was the size of the filter-paper used for calibration.

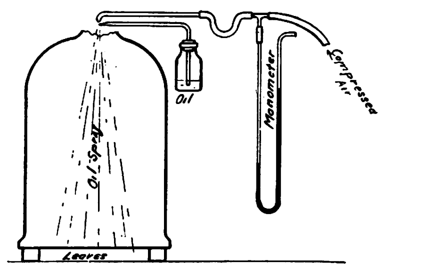

FIG. 1. Apparatus for spraying leaves.

Considerable preliminary work was done on the effect of the amount of oil within the range between very light spraying and dipping the leaves in oils. The amount finally selected gave a visible coating of oil on the leaves and, when calculated, is 0.26 milligram of oil per square centimeter.

OILS

Petroleum oils that are used for spraying are usually of the lubricating type. Nine kinds of oils were selected for experimental use. They had widely varying characteristics, as is shown in table I. The oils were ap-

TABLE I

OILS USED IN RESPIRATION EXPERIMENTS

No.	MANUFACTURER	MANUFACTURER'S NO.	TRADE NAME	SULPHONATABLE RESIDUE	VISCOSITY
				per cent.	*deg.*
1	Standard	13604R	100 Pale	15	100–110
2	Standard	13605R	Calol red engine	44	220
3	Standard	13606R	Oronite technical	5	100
4	Standard	13607R	Mineral seal	10	50
5	Standard	14510R		35	110
10	Standard	13240R		39.7	140
13	Shell	R–L–99		18.3	72
21	Shell	106		14.4*	53
24	Sonneborn & Sons		Amelie	none	67

* SO_2 treated.

plied in the pure state. Emulsions were not used because they would have introduced too many variable factors for this problem.

Method of measuring respiration

A simple method for measuring respiration was devised, following the plan used by Lund (7). After the leaves had been sprayed they were fastened, by means of a rubber band, to a stopper on the tube *a*, figure 2, and were placed in a one-half gallon Mason jar made of white glass. The tube *a* extended downwards about one-half the depth of the jar, and had another stopper on the lower end to keep the leaves apart.

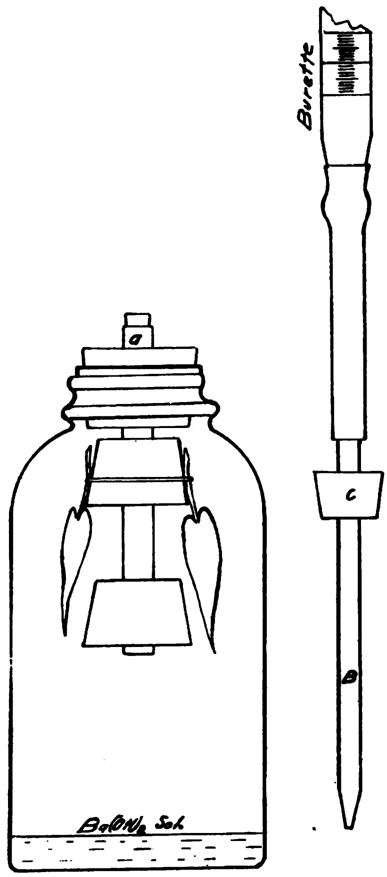

Fig. 2. Respiration jar and burette for measuring and titrating $Ba(OH)_2$ solution.

A titration set was prepared with long tips *B* that would reach down through the tube *a*. One burette was used for adding the $Ba(OH)_2$ solution which absorbed the CO_2 from the plants, and another for titrating the excess $Ba(OH)_2$ with HCl at regular intervals. The tips were selected so as to fit the tube *a* closely, which was kept stoppered except during titrations. The tip had a rubber stopper *c* near the top which was held down tightly against the tube *a* while titrations were being made to exclude as much outside air as possible.

When the leaves were in place, 25 to 50 cc. of approximately 0.010 N $Ba(OH)_2$ were measured into the jar. Two blank jars without leaves were prepared in exactly the same way, and all were placed on a shaker in an asbestos-lined, constant temperature box maintained at 28° C., figure 3. The shaker was operated with a small motor with a fan on its shaft for circulating the air in the constant temperature box. A very slight shaking motion was sufficient to rock the solution and to circulate the gases in the respiration jars, thus continually exposing a new surface of $Ba(OH)_2$ to the CO_2.

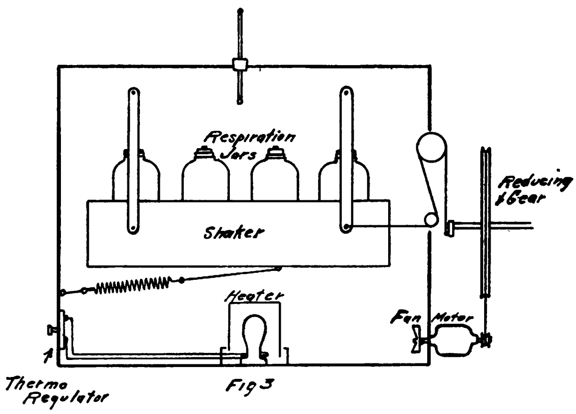

FIG. 3. Respiration jars on shaker in constant temperature box.

Titrations were made at two-hour intervals with exactly 0.005 N HCl. Phenolphthalein was used as the indicator. In the preliminary work the indicator was added in alcoholic solution in the ordinary way, but, to avoid the effect of the alcoholic vapors on the leaves, the phenolphthalein was later placed directly in the standard $Ba(OH)_2$ solution. When the $Ba(OH)_2$ solution was prepared a standard aqueous solution of the phenolphthalein was added until the desired color was obtained. The effect of a small amount of alcoholic vapor in the closed system of the respiration jars was not known, but the probabilities are that it is considerable.

In calculating the results the titrations of the blank jars were subtracted from those of the samples, the differences representing the CO_2 given off from the leaves. One cc. of 0.005 N HCl equals very nearly 0.11 mg. of CO_2.

Discussion

In the closed system for respiration one of the first questions that arises is the effect of the change in concentration of gases; however, the change is very small. The oxygen in a one-half gallon jar can be decreased only very slightly by 3 to 6 grams of leaves in 10 hours. According to KOSTYCHEV (**5**) the concentration of oxygen has very little effect on the respiratory process as long as some oxygen is present. It is stated that a reduction to one-half the normal concentration of oxygen does not change the rate of respiration.

The concentration of CO_2 is kept constant in the closed system, as the moving $Ba(OH)_2$ absorbs it as fast as it is formed. The concentration of water vapor may be important. KELLEY (**4**) states that a high humidity favors oil damage, while on the other hand YOUNG (**10**) finds that in field work the greatest damage from oil occurs when trees are suffering from drought. In the method used, the leaves were in a saturated atmosphere and seemed to go through the ten-hour tests without any apparent injury.

The effects of light and temperature were reduced to a minimum. The constant temperature box was heated with an electric light bulb, automatically controlled. This was covered to eliminate the effect of light in the box. The leaves were in total darkness except for the 3 to 5 minutes required to make titrations at the two-hour periods. Any effect of light and temperature changes was very nearly the same on all the samples.

The age and vitality of the leaves were found to have a great influence on their respiration. Young leaves respire at a more rapid rate than older ones, per gram of leaf. For this reason much care was used in selecting the samples. The curves show that in some determinations the average rate of respiration was much higher than in others even though samples were selected from plants of very nearly the same age.

Because of the variability of samples, several leaves were used in each test and four checks were always run against four treated samples. At the conclusion of the work it was found that even this number was not sufficient. A calculation of the variability of two check samples against two other checks of the same series, showed that the average variability for a group of 17 determinations was 7.5 per cent. It is apparent that the variability of four checks compared with four others, as in the case of the experiments, would be less, probably of the order of one-half to one-third less, making the actual error of the work 4 to 6 per cent.

The variation of the treated samples was much greater than the normals. The average variation of the treated samples, when computed in the same way, was 11.5 per cent., which indicates that respiration was greatly affected by oil.

In the beginning, increasing amounts of oil were expected to gradually accelerate respiration until a maximum should be reached, after which more oil would reduce the rate of respiration.

A series of experiments, using kerosene, was performed to show this effect, because kerosene was expected to be more injurious than lubricating oils. Four experiments were conducted in which the amount of kerosene was doubled each time and finally one set of samples was dipped in kerosene. The results are shown in table II and in figure 5, curves nos. 18, 19, 20, 21 and 22. The respiration rate increased quite regularly with increasing amounts of oil until a maximum was reached after which more oil caused decreased respiration. When the leaves were dipped in oil their respiration dropped to 52.2 per cent. of the normal rate.

TABLE II

RESPIRATION OF NORMAL BEAN LEAVES AND BEAN LEAVES SPRAYED OR DIPPED IN KEROSENE

CURVE NO.	TREATMENT	CO_2 GIVEN OFF, MEASURED AT 2-HOUR INTERVALS					TOTAL CO_2 FOR 8 HOURS	DIFFERENCE	GAIN	LOSS
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent.*	*per cent.*
18	Sprayed 1 min.	1.34	1.36	1.57	1.43	1.40	7.10			
18	Normal	0.69	1.13	1.24	1.05	1.02	5.13	1.97	38.4	
19	Sprayed 2 min.	1.23	1.37	1.58	1.47	1.51	7.16			
19	Normal	1.01	1.11	1.22	1.08	1.04	5.46	1.70	31.1	
20	Sprayed 4 min.	1.28	1.66	1.85	1.60	1.54	7.93			
20	Normal	1.14	1.33	1.28	1.04	0.91	5.70	2.23	39.1	
21	Sprayed 8 min.	1.34	1.65	1.63	1.29	1.34	7.25			
21	Normal	1.14	1.45	1.17	1.05	1.04	5.85	1.40	23.9	
22	Dipped in kerosene	1.15	0.62	0.38	0.47	0.46	3.08			
22	Normal	1.73	1.59	1.25	1.32	1.28	7.17	4.09		57.0

The lubricating oils used may be divided into two classes: light colored, and dark colored. The dark colored oils were usually more viscous and contained greater amounts of sulphonatable residue than light colored oils.

The term sulphonatable residue designates that portion of an oil that can be extracted with strong sulphuric acid under strictly analytical specifications. It consists largely of unsaturated hydrocarbons, sulphur, nitrogen and aromatic compounds. There is evidence that some of these sulphonatable compounds are toxic to plants. GRAY and DE ONG (**1**) have shown from field tests that injury increases very closely with the percentage of sulphonatable residue. Perhaps the unsaturated hydrocarbons are responsible for the trouble, but many of the compounds in the sulphonatable residue are unstable and may have deleterious effects on living plants.

A comparison of the effect of oils of low sulphonatable residue, figures 4, 5, oils nos. 3, 4 and 24, with those of high sulphonatable residue,

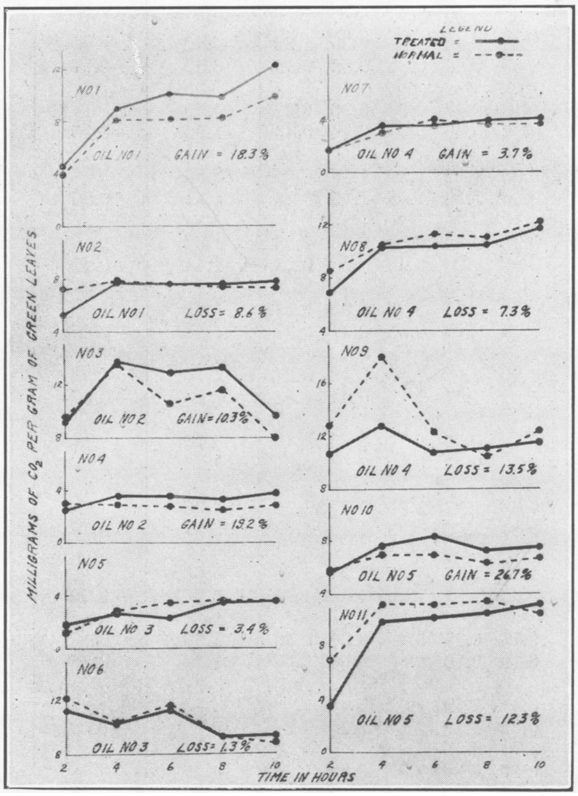

FIG. 4. Curves showing the effect of oil on the respiration of bean leaves.

nos. 2, 5 and 13, show that the former group caused an average loss of 5.8 per cent. in rate of respiration in 7 determinations, while the latter caused an average gain of 8.1 per cent. in 6 determinations. By arranging all of the light colored oils of less than 16 per cent. sulphonatable residue in one group and the darker colored oils of more than 16 per cent. sulphonatable residue in another group, a similar difference is shown. In 8 determinations the light colored oils caused an average loss of 5.0 per cent. in rate of respiration, while, in 9 determinations, the dark oils caused an average gain of 7.5 per cent. The analytical results are shown in table III and are graphically represented in figures 4 and 5, curves 1 to 17.

TABLE III

RESPIRATION OF OIL SPRAYED AND NORMAL BEAN LEAVES

CURVE NO.	OIL NO.	CO_2 GIVEN OFF, MEASURED AT 2-HOUR INTERVALS					TOTAL CO_2 FOR 8 HOURS	DIFFERENCE	GAIN	LOSS
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent.*	*per cent.*
1	1 Sprayed	0.46	0.90	1.01	0.99	1.23	4.59			
1	Normal	0.39	0.81	0.81	0.82	0.99	3.82	0.77	18.3	
2	1 Sprayed	0.13	0.37	0.36	0.36	0.38	1.60			
2	Normal	0.33	0.39	0.36	0.34	0.33	1.75	− 0.15		8.6
3	2 Sprayed	0.90	1.38	1.29	1.33	0.96	5.86			
3	Normal	0.95	1.35	1.05	1.16	0.80	5.31	0.55	10.3	
4	2 Sprayed	0.25	0.36	0.36	0.33	0.38	1.68			
4	Normal	0.30	0.29	0.28	0.25	0.29	1.41	0.27	19.2	
5	3 Sprayed	0.20	0.27	0.23	0.35	0.36	1.41			
5	Normal	0.10	0.28	0.35	0.37	0.36	1.46	− 0.05		3.4
6	3 Sprayed	1.15	1.02	1.13	0.92	0.94	5.16			
6	Normal	1.22	1.04	1.17	0.92	0.88	5.23	− 0.07		1.3
7	4 Sprayed	0.16	0.36	0.36	0.40	0.42	1.70			
7	Normal	0.16	0.30	0.42	0.37	0.39	1.64	0.06	3.7	
8	4 Sprayed	0.68	1.03	1.04	1.05	1.18	4.98			
8	Normal	0.84	1.05	1.13	1.12	1.23	5.37	− 0.39		7.3
9	4 Sprayed	1.07	1.28	1.07	1.11	1.15	5.68			
9	Normal	1.23	1.81	1.23	1.05	1.25	6.57	− 0.89		13.5
10	5 Sprayed	0.16	0.37	0.44	0.33	0.36	1.66			
10	Normal	0.19	0.30	0.30	0.24	0.28	1.31	0.35	26.7	
11	5 Sprayed	0.31	0.99	1.02	1.06	1.12	4.50			
11	Normal	0.68	1.12	1.12	1.15	1.06	5.13	− 0.63		12.3
12	5 Sprayed	0.70	0.86	1.02	0.89	0.88	4.35			
12	Normal	0.70	0.90	1.00	0.90	0.89	4.39	− 0.04		0.9
13	10 Sprayed	0.48	0.72	0.78	0.69	0.66	3.33			
13	Normal	0.46	0.67	0.69	0.61	0.62	3.05	0.28	9.2	
14	13 Sprayed	0.29	0.90	1.08	0.97	0.97	4.21			
14	Normal	0.30	0.91	1.05	0.90	0.83	3.99	0.22	5.5	
15	21 Sprayed	0.72	1.05	1.00	0.93	0.79	4.49			
15	Normal	0.69	1.02	0.99	0.94	0.83	4.47	0.02	0.4	
16	24 Sprayed	0.72	0.96	0.82	0.86	0.71	4.07			
16	Normal	0.71	1.04	0.88	1.02	0.78	4.43	− 0.36		8.1
17	24 Sprayed	0.56	0.81	0.91	0.73	0.84	3.85			
17	Normal	0.64	0.96	1.04	0.80	0.88	4.32	− 0.47		10.9
		Total							93.3	66.3

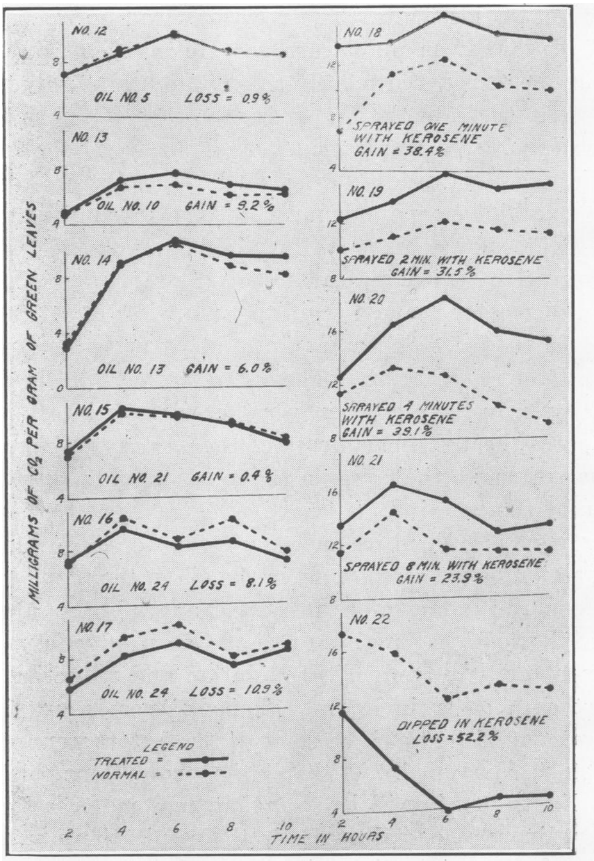

FIG. 5. Left, effect of lubricating oil on the respiration of bean leaves. Right, effect of varying amounts of kerosene on the respiration of bean leaves.

This evidence does not prove that the difference was due only to sulphonatable residue. There is some evidence that sulphonatable residues alone increase respiration. Oil no. 3 is a white oil with a sulphonatable residue of 5 per cent. and a viscosity of 100. Oil no. 5 is a yellow oil with 35 per cent. sulphonatable residue and a viscosity of 110. The difference of viscosity is small and the difference in sulphonatable residue is comparatively large. For 2 determinations oil no. 3 caused an average loss of 2.4 per cent. in CO_2 respired and for 3 determinations oil no. 5 caused an average gain of 4.3 per cent.

The indications are that the sulphonatable residue of an oil causes an increase in the rate of respiration of bean leaves and that the more highly refined oils cause a decrease in the rate. Satisfactory explanations for these two actions are difficult. The reactions leading up to the taking in or giving off of CO_2 from plants are very involved but in their simplest expression may be considered mainly as oxidation and reduction reactions.

An increase in the rate of CO_2 production over the normal is spoken of in this work as increased respiration. As it has been shown that the oils with high sulphonatable residues cause an increase in respiration it follows that the sulphonatable compounds must in some way favor oxidation in the plant. The light colored oils with low sulphonatable residue cause a decrease in respiration which indicates that these oils have favored carbon reduction or the greater use of CO_2 in the plant itself.

The exact means by which oxidation and reduction are promoted is as yet too difficult for explanation. These two actions are, no doubt, affected in a physical way by the oils whenever there is any interference with the exchange of gases through the stomata of the leaves. A change in the rate of flow of gases through their regular channels would cause changes in the concentration of the reacting material in the leaves at the points of action and may thus have a decided influence on the products formed. However, the chemical action of the compounds in the oil on the plant juices and cells is perhaps the more fundamental cause for changes in the rates of oxidation or reduction followed by changing rates of CO_2 production.

In any event the two tendencies, oxidation and reduction, are well balanced against each other in leaves. This balance is sustained by many factors. It was found that at least some of the factors were not even known and could not be controlled. With all the precautions taken to regulate temperature, light, etc., it will be seen that on some occasions either type of oil caused an increase or a decrease in the respiration rate.

In the case of oil no. 4 (a light oil of low sulphonatable residue) 3 determinations were made and 1 of these showed an increase, which is contrary to the rule. Also, with oil no. 5 (a dark colored oil of high sulphonatable residue) 3 determinations were made and 2 of these showed decreased respiration, which is decidedly different from the general trend of all the oils of this type. It is obvious that there are variables that are not yet under control and it is only by making a large number of determinations that any significance can be attributed to the results.

Both types of oil when used in high concentrations cause damage in the field (**10**). Oil no. 24 is highly refined and has no sulphonatable residue but when used in high concentrations it has caused injury in orchard tests. It may, however, be said that the oils with small amounts of sulphonatable residue cause less injury to foliage than those containing large amounts (**10**). Although this work was begun with the general impression that increased respiration was a sign of injury the question now arises regarding the relation of decreased respiration to injury. A positive answer can not now be given but the hypothesis will be advanced that any change in rates of respiration from the normal may be looked upon as the result of injury.

Summary

When bean leaves were sprayed with the dark petroleum oils containing more than 16 per cent. of sulphonatable residues, their rates of respiration were increased. In 9 determinations using five dark colored oils the average increase in CO_2 respired by the sprayed leaves over the normal was 7.5 per cent.

Light colored oils of less than 16 per cent. sulphonatable residue caused a decrease in the rate of respiration of bean leaves. In 8 determinations using four different light colored oils an average loss of 5.0 per cent. in CO_2 respired occurred.

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