EFFECT OF PETROLEUM OIL SPRAY ON PHOTOSYNTHESIS AND RESPIRATION IN CITRUS LEAVES[1]

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(WITH FOUR FIGURES)

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Introduction

Various formulations of petroleum fractions in the light lubricating oil range provide efficient general insecticides for control of insect pests of citrus and have been used for this purpose for many years. The relatively low cost and high insecticidal efficiency of these materials make it probable that their widespread use in citrus culture will continue for some time. Many types of injury to citrus trees have been reported, however, as resulting from such sprays.

The injurious effects of oil sprays on citrus trees have been summarized in reviews by EBELING (3) and RIEHL (12). Conclusive evidence has been presented to show that the application of oil sprays to citrus trees results in a reduction of the total percentage of soluble solids in the juice of citrus fruits. This decrease in the solids content of the juice was first noted by YOTHERS and MCBRIDE in 1929 (16). SINCLAIR *et al.* (14), STOFBERG and ANDERSSEN (15), and BARTHOLOMEW *et al.* (2) have extended the results and have shown that, when fruits from oil-sprayed trees and from non-oil-sprayed trees are compared, the use of oil spray in the usual commercial dosage causes a decrease of as much as 15% in the total soluble solids in the juice.

The relatively large portion of the citrus crop marketed at present in the form of a frozen, concentrated juice having a uniform solids content makes this lowering of the solids in the juice, resulting from oil sprays, of more importance than formerly. Since the primary constituents of the soluble solids of citrus juice are sugars and citric acid, the reduction caused by oil sprays is probably due to an interference with the net production of photosynthate by the tree, or to a decrease in the translocation of elaborated foods from the leaves to the fruit, or to a combination of these factors.

The present study was undertaken for the purpose of elucidating the effect of petroleum oils on photosynthesis and respiration of citrus leaves in the hope that knowledge concerning the mechanism of the toxic effect of oils would be useful in developing means of overcoming these effects in field practice.

KNIGHT *et al.* (8) measured the effect of petroleum oils on the photosynthesis of citrus leaves by painting oil on both surfaces of the leaves of

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plants which had been starved in darkness for 15 days. Sections were cut from these leaves and tested for starch daily. It was found that when an oil having a viscosity measurement of 106 seconds was applied, the starch content of the treated leaves had not returned to normal at the end of 40 days. With an oil having a viscosity measurement of 50 seconds, the starch returned to normal on the fourth day. These same workers, using a carbon dioxide absorption-titration method, found a very marked stimulation of the respiration of citrus cuttings as a result of oil painted on the leaves. The same oil applied as a spray also stimulated respiration, but not to the same degree. The data given indicate that although the plants were illuminated, the oil application stimulated respiration so much that it was considerably in excess of photosynthesis.

GREEN and JOHNSON (**6**) found that oils containing less than 84% unsulphonated residue (U.R.) caused an increase in the respiration of bean leaves, whereas oils containing more than 84% U.R. brought about a decrease in the rates of respiration. GREEN (**5**), working with both poorly refined oil (84% U.R. or less) and highly refined oil (94% U.R. or more) on bean plants, apple leaves and twigs, and barley seedlings, found that there was, in general, an increase in the rate of respiration in response to oils supplied by atomization, but that the increase caused by the low U.R. oils was more than three times as much as that caused by the high U.R. oils. There was considerable variation within individual samples, however, and in some cases respiration was inhibited by high U.R. oil. He also found that small amounts of oil produced the same changes in the respiration of barley seedlings as large amounts of oil.

SCHROEDER (**13**), using summer spray oils applied to apple leaves, found that 1% of an oil of 80-second viscosity, 85% U.R., reduced carbon dioxide absorption 17% one to three days after oil application. The use of 2% of the same oil gave a reduction of 38.4% three to four days after application. In general, he found that inhibition of photosynthesis increased with higher viscosity and greater amount of oil applied.

HOFFMAN (**7**) applied several summer spray oils to apple leaves and found that, on the day following an application of a 1% emulsion, photosynthesis was reduced to about 20% of the rate before treatment, and that there was a gradual recovery of photosynthetic capacity. Different oils of the same type had different effects on photosynthesis. Under the conditions of his experiments, respiration occasionally exceeded photosynthesis. OBERLE *et al.* (**10**) found that, in comparison with the controls, dormant spray oils reduced the respiration of treated tissues. These workers also found a direct relation between the amount of oil applied and the inhibition of respiration.

Materials and methods

Plants of Washington Navel orange and of Eureka lemon, two important commercial species of citrus grown in California, were used in these experiments. The plants treated were rooted cuttings 18 months of age and 24 to

30 inches in height, growing in a greenhouse at a temperature of 80° F and at 50% relative humidity.

Oil treatments were made in the laboratory. The navel orange cuttings were sprayed with a laboratory sprayer operated with compressed air at a pressure of 30 pounds per square inch. The lemon cuttings were sprayed with a small-scale replica of a field spray rig operating at a pressure of 500 pounds per square inch. All the plants were given a drenching spray with full coverage of both upper and lower surfaces of the leaves. Immediately upon completion of the spray applications, the plants were returned to their former location in the greenhouse, where they remained throughout the experiment.

Plants of both citrus species were treated with an emulsion containing 2% of a California medium-grade (**4, 9**) emulsive-type spray oil. Inspection indicated that the oil deposit on the leaves was comparable in amount to that obtained under field conditions. In the experiment with navel orange three plants were sprayed with the oil mixture, and three similar plants were sprayed with water alone, to serve as controls. In the experiment with lemon six plants were sprayed with the oil mixture, and six similar plants were sprayed with water.

Determinations of photosynthesis and respiration were made with the use of a Warburg respirometer having a rectangular tank and modified to provide illumination from below the tank by means of a horizontal bank of 10 thirty-watt fluorescent lamps spaced with one eighth inch clearance between the tubes and placed 2.5 inches from the bottom of the flasks. Color quality of the light was balanced to more closely approximate sunlight by alternating Daylight (blue-white) and Warm-white (pink-white) lamps. These lamps provided an illumination of approximately 1300 fc at the bottom of the flasks.

The flasks used were of 16 ml. capacity, with two side arms. A constant carbon dioxide tension of approximately 1.5% was maintained in the system by means of diethanolamine-carbonate buffer (**11**) contained in the well and side arms. The flasks were shaken at a rate of 100 cycles per minute.

For each sampling, three leaves were harvested from the oil-treated and three from the control plants. In the experiment with orange plants, one leaf from each plant was collected at each sampling date. In the experiment with lemon plants one leaf was collected from each plant on alternate days of sampling. In both experiments, the first samples were collected 24 hours after the application of the oil spray, and sampling continued periodically until no leaves which had been oil sprayed remained on the plants.

After harvesting, the leaves were kept with their petioles in a tube of water until disk samples had been obtained. These disks were made by means of a sharpened cork borer having an inside diameter of 1 cm. Six disks were taken from each leaf, three from each side of the midrib. These disks were distributed six to a flask, so that each of the replicate flasks contained punches from all the leaves used for the treatment represented. The leaf disks were placed in the flask with the upper surfaces next to the bot-

tom of the flask, thus preventing obstruction of the stomata which occur primarily on the lower surface of citrus leaves (1). Movement of the disks during shaking was prevented by moistening the bottom surface of the flask with three drops of distilled water. By this means, overlapping of the disks and a consequent reduction in illumination was prevented.

Respiration determinations (O_2 uptake) were carried out in darkness for a total of one hour. At the end of this time the lights were turned on and the manometers were opened. After an equilibration period of 10 minutes, the system was closed, and apparent photosynthesis, as represented by the O_2 evolved, was measured for one hour. This procedure was followed by a second determination of respiration for one hour. Readings were made at 10-minute intervals, but the final data are expressed as the means of half-hourly readings for all flasks. True photosynthesis was approximated by adding the mean of pre-illumination and post-illumination determinations to the values obtained during the period of illumination. This mean of the two respiration determinations is also used as the value for respiration of the leaf disks.

Results

The effect of oil-spray treatment on the true photosynthesis of navel orange leaves is shown in figure 1. Here the rate of photosynthesis, in μl. O_2/hour/flask, is expressed as a percentage of that of the control plants. The least significant difference at the 5% level is shown by the vertical line at each point. On the first day after spray application the photosynthesis of the treated plants was slightly though not significantly higher than that of the controls. On the second day the rate of photosynthesis of the treated plants had decreased to 31% of that of the controls. With some fluctuations

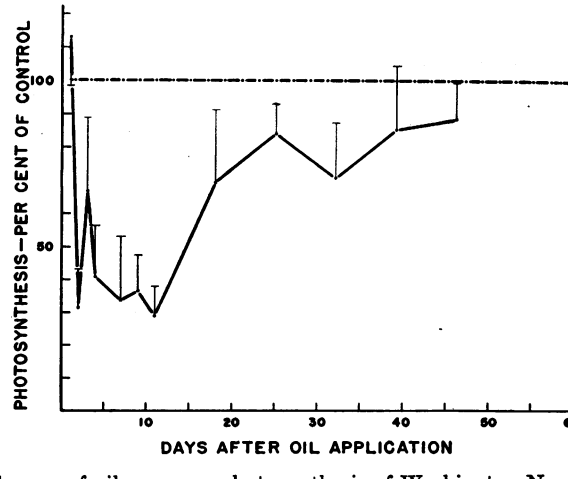

FIG. 1. Influence of oil spray on photosynthesis of Washington Navel orange leaves. Each point represents the mean of six determinations for the treated plants and is expressed as percentage of the mean of six determinations for the control plants, made at the same time. Vertical lines represent least significant differences at the 5% level.

in the relative rates, the photosynthesis of the oil-treated plants remained at a low level for at least 11 days. On the nineteenth day, the leaves had begun to recover their photosynthetic abilities. This recovery continued until the experiment was terminated at the end of 46 days, when the difference between the oxygen evolution of the two sets of plants was only 12%, or barely significant.

The rate of respiration of the same leaf samples (fig. 2) was consistently lower in the oil-treated leaves than in the controls from the second day until the nineteenth day. The next two samplings did not show statistically significant differences. In general, respiration of the leaves seems to be affected by oil in the same way as photosynthesis, but to a lesser extent. For example, it will be noted that the increased photosynthesis on the third day

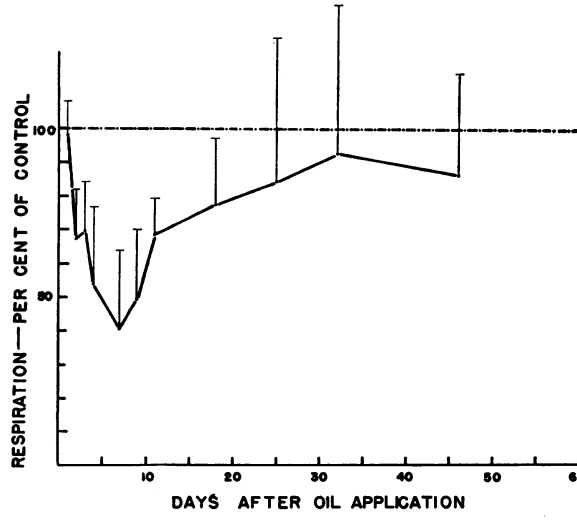

FIG. 2. Influence of oil spray on respiration of Washington Navel orange leaves. Each point represents the mean of six determinations for the treated plants and is expressed as percentage of the mean of six determinations for the control plants, made at the same time. Vertical lines represent least significant differences at the 5% level.

is reflected in an increased respiration rate for that day. This same rather close relationship between the effects of petroleum oil on photosynthesis and on respiration has been noted in other experiments in this laboratory. At no time have we found a stimulation of oxygen uptake, as was reported by KNIGHT *et al.* (8), GREEN and JOHNSON (6), GREEN (5) and others.

Oil applied to Eureka lemon plants has an effect on photosynthesis similar to that reported for applications to plants of Washington Navel orange. The photosynthesis of leaves of oil-treated plants plotted as percentage of the rate of that of leaves of control plants is given in figure 3. Comparison of these results with those obtained with orange leaves shows that the depression of photosynthesis by oil was effective more rapidly on lemon leaves, since the rate was 41% of the control on the first day after oil application.

The lowest point in the time-course of photosynthesis was reached on the second day, and on the third day the process had recovered to 68% of the control rate. From this point there was a gradual recovery of photosynthesis; but at the end of 59 days after treatment, the rate was still lower than the control, although the difference was not significant. These data, together with other similar determinations made on lemon leaves, seem to indicate that the effect of oil on photosynthesis in lemon leaves differs from its effect on photosynthesis in orange leaves in that there is a more rapid initial inhibition of the process in the former.

The respiration of lemon leaves, plotted in the same manner as the data previously given, is shown in figure 4. The effect of oil on this process in lemon leaves differs considerably from its effect in orange leaves, the initial response being slight, and even this slight effect persisting for only about 10

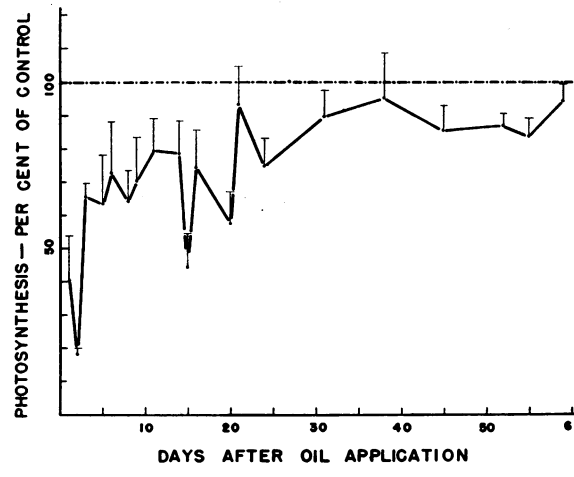

FIG. 3. Influence of oil spray on photosynthesis of Eureka lemon leaves. Each point represents the mean of six determinations for the treated plants and is expressed as percentage of the mean of six determinations for the control plants, made at the same time. Vertical lines represent least significant differences at the 5% level.

days. After 20 days there was a second downward trend which continued for approximately 20 days. After 45 days there was no longer any significant difference between the rates for the two groups of plants. This delayed inhibition of respiration has also been observed in other experiments with lemon leaves, and seems to constitute a real difference in the response of lemon and orange leaves to oil sprays.

A summary of the actual rates of true photosynthesis and respiration of the control leaves of both the Navel orange and Eureka lemon plants is given in table I. It can be seen that there was considerable variability in the photosynthesis and respiration of the leaves from day to day. Since the temperature and relative humidity of the greenhouse were controlled to approximately $\pm 5\%$, it is thought that the variation reflects differences in the light intensity to which the plants were exposed and differences in the

physiological age of the leaves selected on any one day. These fluctuations in the photosynthesis and respiration of the control plants contribute to the variability indicated by the least significant differences in figures 1 to 4. However, even though in some cases other factors were partially limiting the rates of the two processes, the presence of oil on the leaf caused an even greater depression of the rate, indicating that the relative inhibition of photosynthesis and respiration due to oil was of considerable magnitude. In fact, the inhibition was probably even greater than is indicated by the data obtained under the conditions of this experiment, where light intensity during the measurements was almost certainly a partial limiting factor.

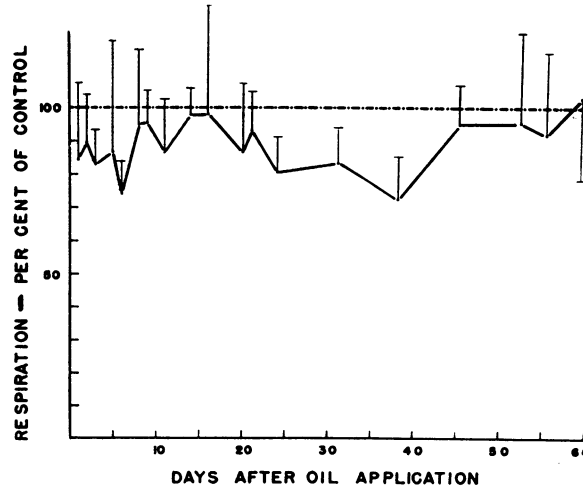

FIG. 4. Influence of oil spray on respiration of Eureka lemon leaves. Each point represents the mean of six determinations for the treated plants and is expressed as percentage of the mean of six determinations for the control plants, made at the same time. Vertical lines represent least significant differences at the 5% level.

Discussion

The data presented here, which are in general agreement with several other experiments of the same type, show that petroleum oils of the kind used as insecticides on citrus in California cause inhibition of the processes of photosynthesis and respiration in citrus leaves. The duration and magnitude of these inhibitions seem to be affected by species differences as well as by a number of environmental factors which are not clearly understood. The present experiments, which were carried out under controlled conditions in the greenhouse and laboratory, using small rooted cuttings, agree with similar determinations made of photosynthesis and respiration of trees growing in commercial groves to which petroleum oils have been applied by the usual methods. These field experiments, which are to be reported completely elsewhere, showed the same trend as was found in the work reported here, but differed in that there was more variation between the relative rates of control and oil-treated plants at different sampling rates. This

TABLE I

RATES OF TRUE PHOTOSYNTHESIS AND RESPIRATION OF UNTREATED NAVEL ORANGE AND EUREKA LEMON LEAVES.

Days	Navel orange		Eureka lemon	
	True photosynthesis	Respiration	True photosynthesis	Respiration
	$\mu l.\ O_2/cm.^2/hr.$	$\mu l.\ O_2/cm.^2/hr.$	$\mu l.\ O_2/cm.^2/hr.$	$\mu l.\ O_2/cm.^2/hr.$
1	61.6	9.6	46.1	7.7
2	62.6	11.3	57.7	8.7
3	48.2	12.0	58.1	8.7
4	76.5	12.9	....	....
5	....	....	47.0	9.5
6	....	....	59.0	8.3
7	38.5	8.0	....	....
8	50.0	7.2	....	....
9	58.0	8.6	35.8	8.0
11	47.6	8.0	37.0	8.1
14	....	....	21.4	7.2
15	....	....	28.5	4.7
16	....	....	31.2	4.7
18	35.6	4.9	....	....
20	....	....	38.3	5.5
21	....	....	40.3	8.1
24	....	....	46.4	8.2
25	44.2	5.0	....	....
31	....	....	44.7	6.9
32	59.0	6.4	....	....
38	....	....	37.9	5.8
39	61.2	7.2	....	....
45	....	....	33.2	7.6
46	44.2	8.0	....	....
52	....	....	66.1	5.9
55	....	....	68.2	6.7
59	....	....	29.3	6.1

fluctuation in the relative inhibition of both photosynthesis and respiration in the field is thought to be due to differences in environmental conditions. In experiments which are now under way, an attempt is being made to correlate these differences with various environmental factors.

The mechanism by which oil sprays affect photosynthesis and respiration in citrus remains to be determined. On the basis of present evidence, there seem to be at least two alternatives. First, there may be a direct interference with both photosynthesis and respiration, possibly due to an altered permeability of the plasma and/or other cell membranes to the passage of one or more of the gases involved. Second, there may be an indirect effect on photosynthesis caused by an accumulation of the end products of the process brought about by an inhibition of outward translocation from the leaf.

Data collected by the authors on dry weight and carbohydrate content of leaves indicate that there is an inhibition of outward transport due to the presence of oil. This type of inhibition of photosynthesis would require a

direct inhibition of respiration to prevent the stimulation of this process which would otherwise be expected from an accumulation of substrate due to inhibition of translocation. There are other possible explanations, and it seems quite likely that several factors are involved.

The question of direct respiratory inhibition has been studied by the use of disks punched from the albino portions of the leaves of variegated ivy which had been sprayed with petroleum oil emulsions. In these experiments no effect on respiration was found, but this observation is not conclusive, since substrate may have been limiting the process in the chlorotic regions of these leaves, and, in addition, the effect of oil on the respiration of this plant may be very different from its effect on citrus.

Summary

The photosynthesis and respiration of disk samples from leaves of Washington Navel orange and Eureka lemon have been studied by the use of a Warburg respirometer modified to provide illumination from a bank of fluorescent lamps placed below the flasks.

The application of petroleum oil emulsions in amounts approximating those used as insecticides on citrus was found to depress the rate of photosynthesis in leaves of both species. Some inhibition of the process persisted as long as determinations were made (59 days).

A corresponding but smaller decrease in respiration was found in these oil-treated leaves.

The data suggest that at least part of the decrease in percentages of soluble solids in the juice of the citrus fruits accompanying oil-spray applications may be due to inhibition of photosynthesis.

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LITERATURE CITED

1. Bartholomew, E. T. and Reed, H. S. General morphology, histology, and physiology. In: The Citrus Industry, Vol. I. Edited by H. J. Webber and L. D. Batchelor, pp. 669–717. University of California Press, Berkeley and Los Angeles. 1943.
2. Bartholomew, E. T., Stewart, W. S., and Carman, G. E. Some physiological effects of insecticides on citrus fruits and leaves. Bot. Gaz. **112**: 501–510. 1951.
3. Ebeling, W. Subtropical Entomology. Lithotype Process Co., San Francisco. 1950.
4. Erb, L. W. California Dept. Agr. Spec. Pub., Div. Chem., California State Printing Office, Sacramento. June, 1932.
5. Green, J. R. Effect of petroleum oils on the respiration of bean plants, apple twigs and leaves, and barley seedlings. Plant Physiol. **11**: 101–113. 1936.

6. GREEN, J. R. and JOHNSON, A. H. Effect of petroleum oils on the respiration of bean leaves. Plant Physiol. **6**: 149–159. 1931.
7. HOFFMAN, M. B. The effects of several summer oils on the carbon dioxide assimilation by apple leaves. Proc. Amer. Soc. Hort. Sci. **32**: 104–106. 1935.
8. KNIGHT, H., CHAMBERLIN, J. C., and SAMUELS, C. D. On some limiting factors in the use of saturated petroleum oils as insecticides. Plant Physiol. **4**: 229–321. 1929.
9. MARSHALL, W. G. Economic poison decisions. In: Economic Poisons. California Dept. Agr. Spec. Pub. **116**: 7–8. 1932.
10. OBERLE, G. D., PEARCE, G. W., CHAPMAN, P. J., and AVENS, A. W. Some physiological responses of deciduous fruit trees to petroleum oil sprays. Proc. Amer. Soc. Hort. Sci. **45**: 119–130. 1944.
11. PARDEE, A. B. Measurement of oxygen uptake under controlled pressures of carbon dioxide. Jour. Biol. Chem. **179**: 1085–1091. 1949.
12. RIEHL, L. A. The use of petroleum oil fractions as insecticides for citrus in California. Third World Petroleum Congress Proc., Section V. (In press)
13. SCHROEDER, R. A. The effect of some summer oil sprays upon the carbon dioxide absorption of apple leaves. Proc. Amer. Soc. Hort. Sci. **33**: 170–172. 1936.
14. SINCLAIR, W. B., BARTHOLOMEW, E. T., and EBELING, W. Comparative effects of oil spray and hydrocyanic acid fumigation on the composition of orange fruits. Jour. Econ. Ent. **34**: 821–829. 1941.
15. STOFBERG, F. J. and ANDERSSEN, E. E. Effects of oil sprays on the yield and quality of navel and valencia oranges. Union So. Africa Dept. Agr. Sci. Bull. 296. 1949.
16. YOTHERS, W. W. and MCBRIDE, O. C. The effects of oil sprays on the maturity of citrus fruits. Florida State Hort. Soc. Proc. **42**: 193–218. 1929.