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Honey-Dew Smut and Photosynthesis.

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Most homopterous sap-feeding insects excrete a considerable quantity of honey-dew which spreads over the foliage and stems and supports the growth of certain epiphytic fungi. The mycelium of these fungi is black and usually forms a crust adhering to the surface of leaves, stems and fruit.

Sugar cane leaves are commonly blackened by this "smut" where the *Perkinsiella* leaf-hopper occurs in quantity. Avocado foliage is frequently badly crusted by the presence of mealy-bugs *(Pseudococcus nipae)*. Orange leaves and fruits as well are commonly blackened by the mold. In California this is due primarily to the black scale *(Saissetia oleae)* and is considered as a very serious detriment to successful citriculture. In Florida a similar smutting on citrus leaves is caused by Aleyrodidae.

Plants which become encrusted with honey-dew smut are more or less dwarfed or stunted or otherwise injured. Sugar cane plants are commonly stunted and citrus foliage when smutted is usually dwarfed. Fruit trees often fail to set fruit when the foliage is badly covered with smut.

Very obviously a part, at least, of this injury is due to the removal of sap by the insects, a state of starvation resulting. It is commonly believed, however, that the presence of a black crust on the leaves is an additional injury, cutting off part or all of the light energy which is needed for photosynthesis, and thus reducing the rate of food manufacture in the leaves and bringing about a condition of starvation.

This appears to be a very logical conclusion, for plant physiology teaches that luminous energy is one of the indispensable factors in photosynthesis, and when a screen is interposed between a green leaf surface and the light source in such a way as to exclude all light, photosynthesis ceases. The honey-dew smut is black and often thick enough to exclude

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much or all of the light falling on a leaf surface, hence photosynthesis theoretically must cease and starvation of plant tissues result.

However, we know that for many years fruit growers have sprayed their orchard trees with insecticides and fungicides containing lime, and that the coating left on the foliage sometimes for weeks appears to work no injury whatever. Instead, the foliage seems more healthy and vigorous. This lime coating is not black and opaque, but it is semi-opaque and excludes a considerable part of the illumination falling upon it.

The question naturally arises, then, as to the actual effect upon photosynthesis of a leaf coating of any sort or color. Does a coating or crust of any material on a leaf surface function to reduce or retard photosynthesis as does an opaque screen when interposed between leaf and light source?

When a green plant is surrounded by a screen which excludes all light but admits air, the reserve starch in the leaves is used up and none is elaborated to replace it. An examination after several hours shows an absence of starch.

During the night most plants use up the excess starch stored in the leaves during the day, so that an examination at dawn will normally show an absence of that carbohydrate. Such is the case with orange leaves.

Now, if a leaf coating of honey-dew smut or of carbon-black or of any other substance acts like an opaque screen to reduce or inhibit photosynthesis, we should expect to find a shortage or absence of starch in the leaf beneath the coating.

There are two means of determining the presence or absence of carbohydrates in green leaves. One of these, known as Sach's Method, is comparing dry weights of equal areas of leaf tissues. The other is the familiar iodine test for starch. The first tests for all carbohydrates, the second for starch only.

In connection with protracted investigation carried on by the writer in California upon the subject of alleged injury to orange trees by deposition of cement dust on the foliage, some important data is available on the larger subject which forms the basis of this paper.

The Effect on Photosynthesis of Various Leaf Coatings.

The luminous portion of radiant energy appears to be indispensable in the photosynthetic work of green leaves, and it is a prevailing idea that this energy must fall upon the leaf surface as illumination to be effective in carbon assimilation. A screen reduces the rate of carbon fixation in proportion to the amount of illumination intercepted, seeming therefore to bear out these statements. It is not at all clear in what manner the illumination falling upon a leaf cell transfers its energy to the chlorophyll to enable it to carry on its photosynthetic function.

Temperature is recognized as a very important factor in photosynthesis, perhaps even more important than illumination, for these are chemical reactions and subject to the Van t'Hoff-Arrhenious law of increasing rate of reactions in a rising temperature, even though the coefficient of increase may differ in the several chemical processes which comprise photosynthesis. Much of the radiant energy falling upon and absorbed by a leaf surface appears to be utilized by the cells as heat, only about 0.5 to 3.0 per cent being used as *light* energy, according to the views of many physiologists.

Citrus, especially sweet orange, leaves are very favorable for these studies because there are no stomata on the upper surface, and also because during the night practically all the starch stored during the day is used up. Microtome sections of leaves removed from the tree just before dawn and stained in iodine show that only a few chloroplastids here and there in the mesophyll cells have any starch remaining in them.

Structure of an Orange Leaf.

An orange leaf has a rather thick epidermis, especially on the side exposed to the sunlight directly. Beneath this there are two layers of palisade cells, and a partial third layer, very closely crowded together. Leaves which grow near the trunk or heart of the tree and are thereby shaded by the outer foliage are very much thinner than the outer leaves, from one-half to four-fifths as thick. The palisade cells, moreover, are in only one layer with sometimes a partial second layer. The mesophyll cells are very spongy, with large cavities between them. The stomata are all on the under surface of the leaf.

METHODS.

Several methods were used to determine the effects of the surface coatings on leaves. Two methods were found especially satisfactory. One of these was essentially Sach's method for determining by weight the increase of dry matter in a leaf during the daytime. Many leaves were prepared on the tree with one-half of the upper surface coated with a given substance and the other half not coated and the lower surface remaining normal. Usually the midrib was taken as the dividing line between the two halves, but in some leaves the half near the petiole or in others the apical half was coated. In all cases a sufficiently large number of leaves was employed to secure more accuracy. Several days were allowed to elapse between the time of coating the leaves and using them further in the experiment so that the leaf might accommodate itself to the changed conditions. In the morning, before daybreak, one circular disc of one sq. cm. area was cut from each half of a hundred or more leaves very carefully, and quickly killed in an oven after having been carefully cleaned in water by rubbing with the fingers. In cutting these discs the larger veins were avoided, although it may be said that orange leaves do not have as prominent veins as many other plants often used in such experiments as this. In the middle afternoon the same leaves were cut from the tree, cleaned in the laboratory thoroughly and as quickly as possible, and then a number of discs were cut, with the same instrument as used in the morning, from each half. These four lots of discs were dried at 100 degrees C. for about two days, or until thoroughly dried, and then weighed to .001 of a gram. The relative weights per square meter area were computed, the greater weight of the discs cut in the afternoon representing accumulated dry matter. No attempt was made to determine what this dry matter consisted of, but presumably it was mostly starch and sugars.

Some objections have been raised by certain physiologists with the thought that this method, of cutting out discs and weighing them dried, is not a correct or adequate means of determining the amount of carbon assimilation during the day. There is more or less truth in these assertions, for it is difficult to determine the rate of translocation, and hence it is only a test of the accumulation of products. It is possible that it is a slight shock to the leaf to have a couple of round discs of one sq. cm. area cut from it, and perhaps there is a slight temporary effect on the metabolic activities of the remaining portion of the leaf. But, nevertheless, for the purposes of these studies this method is wholly satisfactory and adequate. It is safe to assume that the shock to one-half the leaf is not greater nor less than to the other half, and hence the value of the comparison is not impaired whether there is a slight shock to the leaf, or not. Again, since light seems to have somewhat of an inhibitory effect on the diastase action in digestion and removal of starch, and since translocation of sugars appears to be somewhat increased in rate when the temperature rises and it appears, further, the presence of a dust coat or surface coating of any sort tends to slightly increase the temperature and, therefore, slightly accelerate translocation of sugars, we may conclude safely that in the coated half of the leaf the rate of translocation is at least not reduced, and when we consider all the facts it appears that probably it is substantially unchanged. We may, therefore, conclude that the weight of accumulated dry matter in each half of a hundred or more leaves is, at least, a good comparative test when we are seeking to determine the ratio of photosynthetic activity in two parts of the same leaf. Whether or not this method is adequate for testing the total assimilation of carbon during a given period of time is a question with which we are in this study not in the least concerned.

The other of the two principal methods used was the examination microscopically of microtome sections of leaves stained in iodine. This, of course, is a check only on the starch and not on all carbohydrates. Leaves were treated as outlined above, a portion of the upper surface being coated and the remainder left clean. Narrow strips were cut from these leaves in the afternoon and after fixation in chrome-acetic solution and infiltration with paraffine, they were sectioned with a microtome. Sections were in all cases made 10 micro-mm. thick. They were stained in iodine dissolved in xylol, mounted in balsam, and as soon as possible a typical spot was photographed. Study of photographing of these sections must be done quickly, for the iodine is soluble in balsam and the color soon fades out. By this method one can gain a good idea of the comparative amounts of starch in sections, but by no means is it a quantitative test. Unless the sections are of uniform thickness the comparison is valueless, for it is obvious that the mass color effect of the stained starch in two layers of cells would be much greater than that of one layer of cells. A great many leaves were sectioned and photographed in this manner.

Increased Dry Matter.

In determining the effect of a cement dust coating on orange leaves the studies were made in two localities—in the region about the Riverside Portland Cement Company's plant where the leaves were coated by dust blown into the atmosphere, and also in Claremont. In the latter place leaves were partially covered with a paste made from "treator dust," which is the same as that blown into the atmosphere at the cement plant. In the Riverside region the procedure was as follows: Many of the most heavily coated leaves were very carefully cleaned with a dampened cloth on one-half of the surface. Usually the midrib was taken as the dividing line, but in some series the basal or apical half was cleaned. Thus cleaned, the leaves were allowed to remain on the tree for several days, and then discs were cut out before daybreak and again in the afternoon, as above set forth. Records were kept of the average temperatures during the experiments and of the conditions of the sky, weather, etc., whether bright or cloudy. These experiments were repeated on several days throughout the late summer, fall, winter and spring.

Another means of comparing the quantity of carbon fixation in coated and uncoated leaves was tried. One-half of the surface of many leaves was cleaned, as before, and the leaves allowed to remain on the tree for several days. At about the middle of the afternoon these leaves were cut from the tree, quickly brought into the laboratory and carefully cleaned, and then a number of discs were cut from each half. These were dried thoroughly and their dry weights compared. As a check on this method, I first cut a number of ordinary orange leaves, cleaned them carefully, and from each side of the midrib of each leaf cut an equal number of discs. The two lots of discs taken from opposite halves of each leaf were then dried and weighed. The dry weights of these two lots of discs were almost identical, showing that when a considerable number of leaves are used the dry weight is practically the same on each side of the midrib, per unit area. A number of tests were then made to determine any difference which might exist between the clean and coated halves of leaves. This does not determine the quantity of carbohydrates made nor does it do more than simply give a comparison between the two. If unit areas on each side of the leaf normally weigh the same, but when one side is coated with a dust film it should be found that the dry weight per unit area diminishes, it might then be assumed that less carbon was being fixed beneath the coating on the surface. If, however, the dry weight of the coated side remains practically the same as that of the other side, then we may conclude that it corroborates the data of the tests just preceding. This is the case, as the following tables show. The differences in dry weights of the two halves are practically identical, the slight differences being probably only experimental error. This method, alone, of determining carbon assimilation is wholly inadequate, but as a corroborative test with that in which the hourly rate of fixation was made, it is quite worth while.

COMPARISON OF DRY WEIGHT PER SQUARE METER OF LEAF TISSUE OF CLEAN AND COATED LEAVES.

Time of cutting discs	Number of discs	Weight, per sq. meter clean	coated	Difference
7 a. m.	143	118.321	118.955	0.634 grams
2 p. m.	64	110.938	111.250	0.212 "
2 p. m.	100	105.408	105.287	0.121 "
3 p. m.	138	103.607	103.110	0.497 "
1 p. m.	218	106.835	106.718	0.117 "
1:30 p. m.	276	106.70	107.370	0.670 "
	Average:—	108.635	108.781	0.146 "

Check:—114 discs cut from each side of midrib of 40 normal leaves:—Lot 1, 101.316 grams
Lot 2, 101.623 "
Difference—0.307 grams

It appears very certain from these data that the presence of a coating of cement dust is not diminishing in the least the rate of carbon assimilation. The differences in weights of coated and clean parts of the leaves by both methods of testing, above outlined, are very small and to be accounted for simply as experimental error. These same tests were repeated in Claremont, using leaves which were heavily coated on one-half of the upper surface with a paste made of cement dust. This coating was much thicker than that found on the leaves in the dustfall zone about the cement plant. By weighing discs cut in the early morning and in the afternoon, as before, it was found that even this thick coating did not reduce the amount of carbon assimilation in the least. The average of several tests made in Claremont shows a difference in favor of the clean side of the leaves of 0.047 grams.

It seemed evident from the latter tests that not much illumination could enter the leaf through such a thick coating, and this led to making similar tests with orange leaves coated on half of the upper surface with lampblack paste. Obviously this would intercept practically all the luminous rays of sunlight. Proceeding as before, it was determined by the weighing of many discs that fixation of carbon progressed very normally beneath this heavy coating of lampblack, the gain in dry matter being quite equal to that of the unblackened half of the leaves.

At the same time similar tests were made to determine the effect of smut growth on scale-infested trees, and it was found that here again the black covering was not reducing in the least the amount of carbon assimilation. It appears from this latter that the real injury to the orange tree is not from the smut (except as it affects the salability of the fruit), but from the sucking out of sap by the scale insects.

As a check on the foregoing tests a great many microscopic examinations of leaf sections have been made, as previously explained. Leaves in the Riverside district coated by the falling cement dust were partially cleaned and left on the trees for several days. Then sections were cut from the coated and the clean portions of the leaf and stained with iodine. In no case, among the many hundred sections made, was there any indications of there being substantially more starch on either of the two sides or portions of the leaf. Many leaves at different seasons of the year and on different kinds of days, cloudy and sunny, have been examined and no differences have been discovered. Peirce * asserts that leaves partially cleaned, as I have explained above, and sectioned and stained with iodine, show four or five times as much starch in the cells of the clean side as of the coated side. Certainly nothing in the tests reported above would bear Peirce out in this statement even to a slight degree. Peirce used hand sections decolorized and stained in iodine, and it is quite probable that there is a source of error, for it is obvious that a slightly thicker section would appear to have more starch in it than a thinner one; and furthermore, in a freehand section the chloroplastids are quite apt to fall out and leave the cells empty, and thus increase the effect of starch shortage.

In the same way a great many sections have been made of leaves partially coated with lampblack, thickly. These examinations confirm the conclusions derived from the comparison of dry weights of discs cut from blackened and clean parts of leaves, for in no case did there appear to be any less starch in the coated portion than in the clean. In fact, not a few

* Peirce, C. J., Plant World, Vol. 13, p. 286, 1910.

sections showed more accumulated starch in the palisade cells under the black coat than where the coat was absent.

Since such a coating of lampblack as was used in these tests absorbs and intercepts practically all the luminous rays of sunlight which fall upon the upper surface of the leaf, and since it was found that carbon assimilation progressed normally in spite of the black coat, only two possible explanations were apparent. Either enough diffuse light entered the leaf from beneath, for this surface was not coated, or else the light rays falling upon the black surface were absorbed, transformed and transmitted as another form of energy into the leaf cells beneath where this energy became effective in photosynthesis.

Upon the under surface of some orange leaves, which several days before had been coated heavily with lampblack on the upper surface, were attached some lightproof boxes. Some of these were Ganong's partial leaf form light screen, and others were a somewhat different home-made type which were even more effective than the Ganong type for excluding light while admitting the passage of air to the stomata on the lower leaf surface. These light screens were attached to the partially coated leaves at evening time and allowed to remain until the next afternoon. The leaves were then removed from the tree and narrow strips cut from three areas, as follows: (1) From an area which had been uncoated above and not screened beneath, (2) from an area which had been coated with lampblack above and not screened beneath, and (3) from an area which had been coated with lampblack above and protected from the entrance of light to the lower surface by a light screen. In some instances the portion clean above but screened beneath was also cut for sectioning. These strips were then prepared for microtome sectioning, sectioned ten microns thick and stained with iodine and photographed.

Careful examination of hundreds of these sections has convinced me that as much carbon was fixed in that portion blackened above and screened beneath as in the portion entirely clean and not screened beneath. This, then, leads us to the conclusion that the energy which effects the carbon assimilation is entering through the surface coating, no matter what that material may be nor what its color or opacity may be.

Discussion of Data.

It is very obvious from the foregoing tests that a surface coating does not affect leaf functions in the same way that a screen does, for in the latter case most or all of the heat energy of the light is excluded at the same time that the illumination is reduced. It must appear from the foregoing data that the presence of a dust coat or even of a black coating, as lampblack or a smut growth, is not injurious to the leaf in so far as the function of carbon assimilation is concerned.

Orange leaves coated for two or three years are just as bright and green when the dust is removed as a normally clean leaf of the same age is. Chlorophyll solutions of equal area of tissue of cleaned and of coated leaves have been repeatedly compared and no diminution of chlorophyll has been detected by the writer in leaves coated with dust. Similar tests of leaves coated with lampblack for four or five months have been made with the same results. These latter leaves appeared as normal and thrifty and green on the black-coated half as on the other, although the coat had been applied for four months.

This has an important bearing on several problems in horticultural science. First, spraying trees does not impair the function of food manufacture in the leaves, provided the spray is not caustic. Second, dust coats on leaves, so long as the stomata are not clogged, do not impair the food-making functions, assertions to the contrary notwithstanding. Third, fungus smut on scale-infested trees does not reduce carbon assimilation. Two injuries may be discovered in such trees. The scale insects themselves are sucking out a very large amount of sap and starving the trees; and, second, the black coat may increase transpiration to a dangerous degree.

It appears from certain of these tests that more radiant energy is absorbed by a coated leaf, and hence, the internal temperature would be increased. No attempt has been made in these experiments to determine the leaf temperature, but it seems probable that any increased temperature would be equalized by the increase of transpiration, so that the actual temperature would be about the same as in an uncoated leaf, but the number of heat units in the leaf would be much greater.

Martin * states that increase in transpiration in a spray-coated leaf probably is not to be explained by the increased temperature. If, however, a leaf normally reflects from its surface a large percentage, perhaps 50 per cent, of the incident radiant energy, but a coated leaf perhaps not more than 20 per cent, the increase in heat units within the coated leaf would be considerable and might well explain the increased transpiration.

In most of the stained sections of coated and uncoated leaves it was found that more starch accumulated in the palisade cells beneath a surface coating, whether the coating were opaque or not, than accumulated in the palisade cells of a clean leaf. This might indicate that some element or quality of direct sunlight inhibits somewhat the photosynthetic activity in the outer palisade cells, and that this inhibitive element or quality is removed by the presence of a surface coating. Perhaps it is merely the illumination itself which is the inhibitive factor, and that the normal function of the outer densely chlorophyllous palisade cells is to *screen out* the illumination and transform its energy to another form useful to the leaf cells. In an uncoated orange leaf the greatest amount of starch, by the iodine-stained section test, is accumulated in the mesophyll cells, but in a coated leaf as great an amount seems to be accumulated in the palisade cells.

It is interesting to note, too, that in those leaves which were coated above with lampblack and darkened beneath with a light screen the mesophyll cells next to the lower epidermis showed as much starch accumulation as did the corresponding cells in a normally illuminated leaf.

CONCLUSION.

From the foregoing experimental data we may draw at least one conclusion. While photosynthesis depends on illumination for its energy, that illumination need not fall actually upon the leaf surface. A more or less opaque surface coating on the leaf does not exclude the energy of illumination falling upon it, but transfers that energy in some form through to the leaf cells.

* Martin, W. H., Journal of Agr. Res., Washington, 1916: 529-547. See also, Duggar, The Effect of Surface Films on the Rate of Transpiration, Ann. Missouri Bot. Garden.