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NATIONAL BUREAU OF STANDARDS 1963-A

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EFFECT OF LEAD ARSENATE INSECTICIDES ON ORANGE TREES IN FLORIDA

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INTRODUCTION

One of the important methods used in the successful campaign to eradicate the Mediterranean fruit fly (*Ceratitis capitata* Wied.) from Florida was the application of a poison spray on which the adult flies fed. The most effective bait spray available in this emergency contained lead arsenate as the poison. Although it had been known for many years that arsenic hastened the maturity of citrus fruit by reducing the acid content and that its use had, in some instances, rendered the fruit unpalatable, the menace of this insect pest to the citrus industry of Florida and to fruit production elsewhere in the United States was so great that the authorization for the use of this poison was fully justified, and its use was approved by responsible State officials.

During the eradication campaign voluminous notes were accumulated as to the effect of the bait sprays on citrus trees and fruit. These notes were of a general nature, and the observations recorded were more or less conflicting. It seemed necessary, therefore, to conduct some carefully planned experiments to secure accurate information on the effect of arsenic on citrus trees. This bulletin reports the results of fundamental investigations carried on under funds providing for necessary research to eradicate this major pest from Florida.

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PREVIOUS WORK

Soon after arsenic was first used on citrus in Florida—in 1893, by Lyman Phelps—it was found that the fruit on arsenic-sprayed trees matured more quickly than other fruit (*5, p. 81*).[1] Since that time arsenical materials have been used considerably, sometimes to control insects but more often to hasten the legal maturity of the fruit. Certain insecticides that have been sold in Florida have been found to contain arsenic in considerable quantities. In one case, in 1915, as much as 2.93 per cent of arsenic trioxide (As_2O_3) was found in a commonly known material.[2]

In 1921 Gray and Ryan (*9*) published an account of some arsenical spraying in California. They found that when acid lead arsenate was used the acidity of oranges was reduced 50 per cent or more from the normal but that the sugars were not significantly changed. When basic, or neutral, lead arsenate was used there was little or no change in acidity. These workers believed the acid reduction to be due to the soluble arsenic liberated on the foliage. They stated that the effect on the tree probably was systemic, for all the fruits examined from the sprayed trees were of reduced acidity.

In 1925 Juritz (*11*) discussed the use of arsenic on citrus trees in South Africa. The treatment of the trees and the effect on the acid and sugar of the fruit are shown in Table 1, which is taken from his report.

TABLE 1.—*Analysis of sprayed and unsprayed oranges made by C. F. Juritz in South Africa, 1925*

Insecticide used per tree	Analyses of fruit	
	Acid	Sugar
	Per cent	*Per cent*
Lead arsenate mixture, 53 ounces	0.18	1.12
Lead arsenate mixture, 26½ ounces	.49	3.65
Check, unsprayed	1.12	4.14

Juritz stated that the effect remained more than a year and that the whole tree was systemically affected if only one-half was sprayed. He was the first worker to report that the soluble solids of the juice were seriously reduced.

In 1925 the State of Florida passed legislation restricting the sale of fruit that did not pass certain maturity tests of acid and soluble-solids requirements (*6*). When this was done and certain growers realized that the legal maturity of fruits could be hastened by arsenical spraying, many instances were found where the legal maturity was speeded up by spraying or dusting.

In 1926 Grossenbacher (*1*) suggested that there be some definite requirement regarding the ratio of solids to acid for a standard of legal maturity (i. e., a maximum as well as a minimum value for the ratio), thereby disqualifying arsenical-sprayed fruit. In 1927 the Florida Legislature passed a law forbidding the use of arsenic on citrus trees, but in 1929 amended it (*8*) to permit the use of arsenic when necessary in eradication measures such as those directed against the Mediter-

[1] Italic numbers in parentheses refer to Literature Cited, p. 20.
[2] Miscellaneous analyses 22611, made by C. H. Walker of the Bureau of Chemistry and Soils, U. S. Department of Agriculture.

ranean fruit fly, and again legislated against shipment of immature fruit (*7*).

Work done by Yothers and others in Florida in 1926 (*17*) established that only the part of the tree sprayed was affected. Examinations of sprayed trees showed that the fruit in the tops of the trees, which could not be reached by the spray, was normal, whereas that on the lower branches was considerably affected. This is shown in Table 2, which is condensed from a table in the article cited.

TABLE 2.—*Effect of lead arsenate on total soluble solids and ratio of acid to solids in oranges*

[Analyses of Nov. 10, 1926]

Source of fruit analyzed	Sugar	Acid	Ratio of acid to sugar
	Per cent	*Per cent*	
Top of tree	9.75	1.26	1 to 7.74
Lower limbs	9.00	.53	1 to 17.00
Top of tree	9.10	1.20	1 to 7.60
Lower limbs	8.85	.48	1 to 18.44

TABLE 3.—*Effect on oranges of arsenical applied as a dust*

[Analyses of Nov. 3, 1926]

Source of sample	Sugar	Acid	Ratio of acid to sugar
	Per cent	*Per cent*	
Dusted trees	9.70	0.14	1 to 69.30
Check trees	9.30	1.11	1 to 8.38

Trees that were dusted with 90 per cent sulphur and 10 per cent lime and lead arsenate were affected just as were those that had been sprayed, as shown in Table 3, which is from another table in the same article by Yothers.

Yothers reported that mature-picked fruit can not be changed by immersion in a lead arsenate mixture.

In 1927 Hawkins and Barger (*10*) corroborated the results of previous workers in the use of arsenic on citrus and advised against its general application for the purpose of hastening fruit maturity.

In 1927 Copeman (*3*), in South Africa, investigated the use of arsenical and other materials on citrus and found that arsenic reduced the acid in the fruit but that there was no significant change in the sugar content. He concluded that the effect was systemic and that the fruit was affected through the leaves. The quantity of arsenic used per tree by Copeman was apparently less than that used by Juritz.

Takahashi, working in Japan, published a paper in 1928 (*16*) in which he reviewed most of the previous work on arsenic. He corroborated the findings of other workers as to reduction of acid and increase of sugar in navel oranges on arsenical-sprayed trees, as shown in Table 4, which is taken from the article cited.

TABLE 4.—*Effect of lead arsenate in spray on navel oranges as determined by Ikuro Takahashi in Japan in 1928*

Treatment of plots	Average weight of fruit	Fruits	Pulp	Percentage of juice to pulp	Specific gravity of juice	Principal components of juice			Ratio of sugar to acid
						Citric acid	Reducing sugar	Total sugar	
	Grams	*Number*	*Per cent*			*Per cent*	*Per cent*	*Per cent*	
Lead orthoarsenate, 2 pounds; lime sulphur (0.8 per cent), 50 gallons	222.1	5	76.73	64.55	1.050	0.4533	6.8646	8.0126	17.67
Check	216.1	5	76.18	66.53	1.048	.9769	4.7250	7.5630	7.74

Takahashi found that the effect persisted for more than one season and stated that small fruits were more affected than large fruits. No attempt was made to explain how arsenic affected citrus trees, although the opinion was expressed that it was through the root system.

In 1931 Copeman (*4*) published a very comprehensive study of the effect of arsenic on the ripening of fruit, in which he confirmed, with additional evidence, the points mentioned in his previous paper.

In 1929 Singleton (*15*), in Florida, stated that lead arsenate used in the proportion of one-half pound to 200 gallons had a very desirable effect on citrus fruit, but that when used at greater strengths it was quite unsatisfactory.

Work done in 1929 by the senior author and McBride, which was reported in part in 1931 (*13*), showed that when the fruit itself or the leaves of the branches immediately surrounding the fruit were sprayed the fruit was affected. Dipping the small, green fruit on an unsprayed tree in lead arsenate suspension affected it just as would the spraying of the foliage, and when the fruit was protected by a paper bag and the leaves around it sprayed the fruit was affected.

In 1932 Nelson and Mottern (*14*) reported the results of the analysis of oranges, sent to them by the Orlando laboratory, which had been sprayed with lead-arsenate bait spray. Their analyses showed that these were very low in acid and also low in sucrose. An important point, however, is the fact that in oranges heavily sprayed with arsenic the vitamin C content was reduced considerably. These writers also reported that the arsenic content of the edible portion of the orange was not changed by spraying the tree with heavy doses of lead arsenate.

METHOD OF INVESTIGATION

Work on this problem was undertaken with the idea of determining how arsenical insecticides affect trees and fruit. Other workers have pointed out clearly that arsenic does affect citrus fruit through the leaves or trees, but have told practically nothing about the extent of effect from various quantities.

In order to determine whether or not arsenic may produce the effect through the soil instead of the leaves, large quantities of lead arsenate were applied to the soil under orange and grapefruit trees, and analyses of the soil, fruit, and foliage were made at intervals for two and one half years afterwards.

In all the spraying work only half of a tree was sprayed, and the remaining half was used as a check. The unsprayed portions of these trees were compared with the adjoining trees that had not been sprayed, and no difference could be detected other than small differences attributed to the normal variability of trees. The unsprayed half of a tree was always used for a check on the sprayed half, and in this way individual tree differences were eliminated.

ARSENICAL DETERMINATIONS

Orange trees were sprayed with various quantities of acid lead arsenate, and at intervals of one month quantitative determinations were made of the water-soluble and insoluble arsenic remaining on the leaves.

RESPIRATION

Twigs from the same group from which the arsenical determinations were made were placed in respiration chambers and the quantity of liberated carbon dioxide measured. After this the relation between the arsenic present on the leaves and the quantity of carbon dioxide liberated was worked out.

CATALASE ACTIVITY

Other leaves from the same group were used for determining the quantity of catalase present in leaves on which the various quantities of arsenic were found.

FRUIT ANALYSIS

Fruit samples were taken for analysis from the trees that had been sprayed with arsenical insecticides, and determinations were made of the arsenic present, as well as of the relation between this chemical and the respiration and catalase of the leaves. When the fruit was quite small, approximately 25 to 30 mm in diameter, the hydrogen-ion concentration was measured electrically. As soon as enough juice was available the percentage of soluble solids was determined by a Brix hydrometer and the acid by titration with the standard alkali.

KIND OF CITRUS TREES USED

The work reported in this bulletin, except where otherwise stated, was carried on with orange trees. The principles involved in the effect of arsenic on grapefruit, tangerines, and other citrus fruits are probably the same as those involved in the effect on oranges, but the quantities of arsenic required to produce these effects may differ.

EFFECT OF LEAD ARSENATE ON CITRUS TREES WHEN APPLIED TO THE SOIL

There has been much theorizing as to the effect of the drip of arsenical insecticides on the soil beneath citrus trees. The writers were unable to find any published information on this point, although unpublished notes of work done in 1926 by W. W. Yothers, F. A. Wolf, and O. C. McBride reported the results of applications of arsenic to the soil under citrus trees. Paris green, white arsenic, and lead arsenate were used in quantities up to 1½ pounds per tree without any effect on fruit maturity as shown by either taste or chemical analyses. In the present work the quantity of arsenic applied to the soil was much greater than would result from the dripping of arsenical sprays over more than 250 years.

A seedling orange and a grapefruit tree more than 30 years old were chosen for the experiment. The treated and check trees were close to each other and on the same type of soil, which was Norfolk sand, well drained and having the average amount of humus.

The soil was sprayed at intervals of from one to two weeks with a hand-operated pressure apparatus fitted with a rod and nozzle. Sixty applications of one-half pound of lead arsenate (30 pounds in all) in 7½ gallons of water were made beneath the grapefruit tree, and 14 applications (7 pounds in all) beneath the orange tree.

METHOD OF ANALYSIS

To obtain the arsenic from the soil for analysis, the distillation method (*2, p. 35*) was used. Fifty grams of soil was found to be the most satisfactory quantity for the test. After the solution had been obtained from the distillation, the quantity of arsenic in the solution was determined by the modified Gutzeit method. The pH value of each sample was determined by the Welch hydrogen-ion-concentration apparatus.

PENETRATION INTO THE SOIL

The first analysis was made just after the 60 applications to the grapefruit tree and the 14 applications to the orange tree had been completed. The results are given in Table 5.

TABLE 5.—*Effect of arsenic in the soil on the fruit of grapefruit and orange trees, Orlando, Fla., 1931*

Variety	Parts of arsenic trioxide per million of soil		pH of soil	Fruit analyses		
	1 to 2 inches depth	8 to 10 inches depth		Acid	Solids	Ratio of solids to acid
				Per cent	*Per cent*	
Grapefruit	2,000	6.0	5.0	2.03	10.9	5.37
Check grapefruit	.04	.04	5.0	2.29	11.6	5.06
Orange	700	6.0	4.9	1.21	10.6	8.8
Check orange	.04	.04	5.0	1.14	9.2	8.1

After 30 pounds of acid lead arsenate ($PbHAsO_4$) had been applied to a slightly acid soil under the grapefruit tree, the first 2 inches of soil had arsenic present (as arsenic trioxide, As_2O_3) in the proportion of 2,000 parts per 1,000,000. During the 1½-year period over which this material had been applied there had been 115.15 inches of rainfall, and arsenic was found down as deep as 14 inches in the proportion of 3 parts of arsenic trioxide per 1,000,000 of soil. This makes it certain that the arsenic was well diffused among the roots, for almost all the roots of these citrus trees are found in the first foot of soil.

The orange tree under which 7 pounds of lead arsenate had been applied had proportional quantities, 700 parts per 1,000,000, in the upper 2 inches of the soil about its roots.

EFFECT ON FOLIAGE AND FRUIT

In the case of both the grapefruit and orange trees, the trees were absolutely normal, and only a trace of arsenic was found in the leaves. The flushes of growth appeared regularly, and the foliage was of normal size and color. The fruit was not affected, for by analyses the acid and solids were found to be but little different from those of the check trees. The color and size of the fruit were similar to those of the untreated trees near by.

EFFECT ON UNDERGROWTH

There were no differences in the growth of vegetation under the sprayed and unsprayed trees. With a few exceptions, there were the same plants, in the same state of growth, beneath both the sprayed and the unsprayed trees. Under the grapefruit tree where there were 2,000 parts of arsenic trioxide per 1,000,000 of soil, the following species were present: Spanish needle (*Bidens leucantha* L. Willd.), Mexican tea (*Chenopodium ambrosioides* D.), and ragweed (*Ambrosia bidentata* Michx.). Under the orange tree, where there were 700 parts of arsenic trioxide per 1,000,000 of soil, Spanish needle, Mexican tea, and crab grass (*Eleusine indica* Gaertn.) were growing normally. All of these plants grew under the check tree also. Because of the protection afforded by the lead arsenate there was little insect injury on the plants where the soil was treated.

LOSS OF ARSENIC FROM THE SOIL

Lead arsenate, when applied to the soil as in the foregoing experiments, is not readily leached out by the normal rainfall of Florida. A year after the previous analysis a second one was made of the soil beneath the grapefruit tree, and 1,800 parts of arsenic trioxide per 1,000,000 in the 1 to 2 inch range were found, and 5 parts at the 8 to 10 inch depth. Even though this large quantity of arsenic was present around the roots of the tree for over a year, the tree was normal and set a good crop of fruit. The vegetation beneath the tree was the same as during the previous year.

It is very improbable that any arsenical injury would ever result from the dripping of the spray material after an application of fruit-fly bait. When it is considered that 1 pint of a spray containing 8 pounds of lead arsenate to 200 gallons of water is sufficient for a tree, the 30 pounds used on the soil beneath the grapefruit tree would make enough spray material for 6,000 applications.

APPLICATION OF LEAD ARSENATE TO TREE AND FRUIT

TIME THE INSECTICIDE REMAINS ON THE TREE AFTER SPRAYING

The first and most important consideration in determining the effect of arsenical insecticides on orange leaves or foliage is an exact analysis to find the quantity of arsenic, soluble and insoluble in water, that remains on the leaves at various intervals after spraying.

Samples of leaves were taken from a representative part of the tree and were selected from such trees as would give a cross section of the entire treated or untreated plot. Ten grams of green weight was found to be the most satisfactory sample and these samples were always run in duplicate. The determination of arsenic was made by the official Gutzeit method (*2, p. 306*).

To determine the water-soluble arsenic, the leaves were kept for 10 minutes in 200 c c of distilled water, and this solution was then carefully filtered off and analyzed. The leaves and filter paper were then used to determine the water-insoluble arsenic.

To determine the total arsenic trioxide (As_2O_3), the arsenic was dissolved from the leaves with 2 per cent hydrochloric acid at room temperature (21° to 26° C., or 69.8° to 78.8° F.). The leaves, cut in half-inch strips, were kept in this solution for at least 10 minutes. At the end of this time they had usually turned yellow or brown. To check this method of removing arsenic from the leaves, many of the samples were afterwards digested and the remaining arsenic extracted by the distillation method (*2, p. 35*). The additional arsenic found was less than 1 per cent of the total arsenic of the sample.

The greatest quantity of soluble arsenic was present immediately after spraying, and during the first month two-thirds of this disappeared. After this there was only a gradual decrease. The rate of disappearance of arsenic from citrus trees is shown in Table 6 and in Figure 1.

TABLE 6.—*Milligrams of arsenic trioxide per 10 g*[1] *of fresh leaves at monthly intervals after one application of spray consisting of 8 pounds of lead arsenate to 200 gallons of water, Orlando, Fla., 1931*

Time after spraying, months	Arsenic trioxide (As_2O_3)		Cumulative rainfall	Time after spraying, months	Arsenic trioxide (As_2O_3)		Cumulative rainfall
	Water-soluble	Water-insoluble			Water-soluble	Water-insoluble	
	Mg	*Mg*	*Inches*		*Mg*	*Mg*	*Inches*
0	0.34	2.76		4	0.004	0.02	17.19
1	.08	1.39	3.02	5	.008	.33	20.01
2	.04	.82	5.00	6	.005	.19	22.21
3	.01	.53	12.04				

[1] g is the abbreviation for gram or grams.

The trees had been sprayed with 8 pounds of lead arsenate to 200 gallons of sirup-sugar mixture. During the six months there had been 22.21 inches of rain.

Analyses were made from time to time of leaves from trees that had been sprayed 1 year or 18 months previously, and arsenic was still found in quantities such as 0.001 mg per 10 g of leaves. There was very little difference in the solubility or rate of disappearance when either sirup and sugar or lime sulphur was added to the lead arsenate mixtures.

ARSENICAL CONTENT OF WOOD, BARK, BLOSSOMS, AND SMALL FRUIT OF SPRAYED TREES

In an effort to determine what happens to the arsenic that is sprayed on citrus trees, analyses of thoroughly acid-washed bark and wood from limbs 1 inch in diameter, as well as blossoms and small, green fruit one-fourth to three-eighths inch in diameter, were made.[3] In 201 out of 204 samples in which live bark and wood given 15 applications of 1 quart of fruit-fly bait spray were analyzed, the bark showed more arsenic than the wood. The arsenic in the bark ranged from 0 to 4.5 mg per kilogram of bark. In the wood, from 0 to 2.05 mg of arsenic was present per kilogram of wood. In the analyses of orange blossoms from similarly sprayed trees arsenic was found to be

[3] MCBRIDE, O. C., MARLOWE, R. H., and BASSETT, I. P. GUTZEIT DETERMINATIONS FOR ARSENIC IN CITRUS FRUIT JUICES, LEAVES, TWIGS, AND BLOSSOMS. Unpublished manuscript on file in Bureau of Entomology, U. S. Department of Agriculture.

present in quantities ranging from 0 to 6.9 mg of arsenic trioxide per kilogram of sample. In the small fruit the quantity of arsenic present ranged from a trace to 0.16 mg of arsenic trioxide per kilogram of sample.

In all the analyses of bark, wood, blossoms, and small fruit made above, the samples were taken from trees to which no arsenic had been applied for at least four months. This shows that in the case of the wood and the blossoms and small fruit which were produced the next season after the sprays were applied, the arsenic had been translocated from the sprayed parts.

EFFECT OF ARSENICALS ON RESPIRATION OF ORANGE LEAVES

Respiration, one of the most vital processes carried on by living plants, was studied to determine how it was affected by various

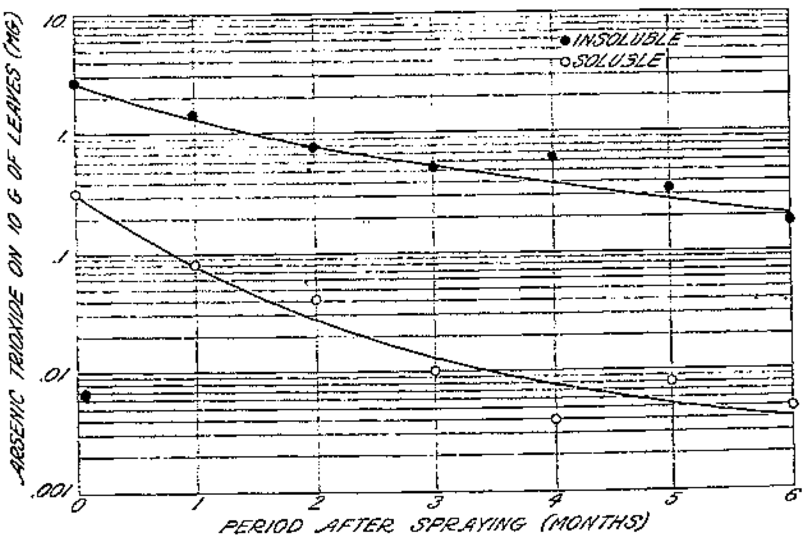

FIGURE 1.—Quantities of arsenic trioxide per 10 g of fresh leaves at monthly intervals after one spraying of 8 pounds of lead arsenate in 200 gallons of water. Semilogarithmic

quantities of arsenic. The fact that arsenic stimulated respiration in Elodea had been established by Lyon (*12*). In this case he submerged Elodea in an arsenical solution and found that respiration was stimulated up to 160 per cent of normal.

In the present work the rate of respiration was measured by determining the quantity of carbon dioxide liberated in a closed jar at a constant temperature, 25° C. (77° F.). The gas, carbon dioxide, was absorbed in sodium hydroxide solution (tenth normal) and titrated against hydrochloric acid (tenth normal) by the use of the double-indicator method.

When a test was to be made, twigs having from three to five leaves were cut under water, and the cut end was put in a vial of water. During the 2-day run the whole assembly was kept in a jar with the sodium hydroxide in the bottom. The sodium hydroxide was titrated once daily. The entire experiment was run in a dark room, and the twigs were kept at a temperature that varied less than one-half degree during the time of the experiment. After each run was completed, the area of leaves used was determined by a planimeter, and the weight of carbon dioxide liberated per square inch per 24 hours was used in comparison. In all cases the determinations were made in triplicate and the mean of the three used. The results of these observations are shown in Table 7 and Figure 2.

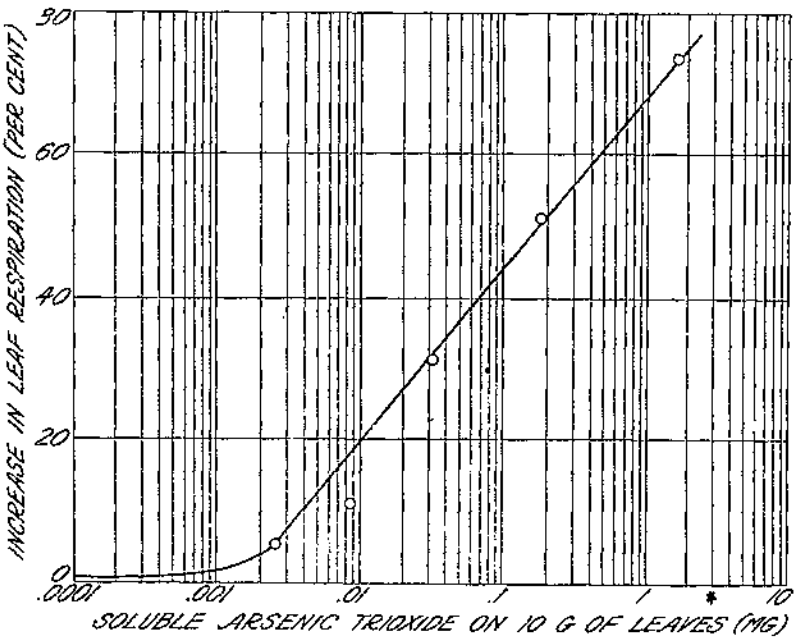

FIGURE 2.—Effect of lead arsenate in various quantities on the respiration of orange leaves, expressed in percentage of carbon dioxide liberated in the dark. Semilogarithmic

TABLE 7.—*The effect of lead arsenate in various quantities on the respiration (carbon dioxide liberated in the dark) of orange leaves*

Treatment	Determinations made	Average soluble arsenic trioxide per 10 g of green leaves	Carbon dioxide liberated per square inch per day	Increase in liberation of carbon dioxide
	Number	*Mg*	*Mg*	*Per cent*
Unsprayed, check	27	0	1.68	----
Sprayed with dilute arsenical solution	3	.0026	1.78	6.0
	5	.0086	1.89	12.5
	20	.0300	2.23	32.7
Sprayed with concentrated arsenical solution	7	.1930	2.54	51.2
	2	1.75	2.91	72.0

As can readily be seen, water-soluble arsenic in very small quantities increases respiration. When only enough arsenic is present on orange leaves to stimulate respiration 5 to 10 per cent (less than 0.01 mg soluble arsenic trioxide on 10 g of green leaves) there is no injury; the leaves even become more glossy and take on a more intense shade of green. When the quantity is increased the stimulation is increased in direct proportion, and when the stimulation goes from 50 to 75 per cent above normal the leaves become yellow, and when the quantity is increased beyond that point the leaves usually drop.

When compounds of arsenic of different solubility were used, as when lead arsenate was compared with some form of a soluble arsenic compound, such as sodium arsenate, it was found that the stimulation was in direct proportion to the quantity of soluble arsenic on the leaves.

It has been pointed out that arsenic remains on trees for long periods after the spraying has been done. It is here shown that when soluble arsenic is present on citrus leaves the respiration is stimulated. Other experiments extending over the entire growing season have shown that the respiration of arsenical-sprayed trees

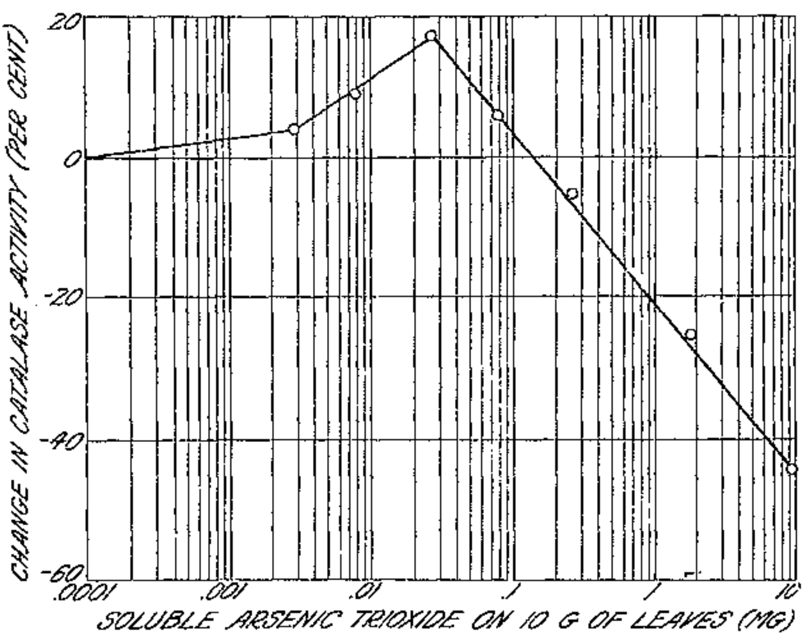

FIGURE 3.—Effect of lead arsenate in various quantities on the catalase activity of orange trees. Semilogarithmic

was constantly stimulated as long as lead arsenate was present on the leaves.

EFFECT OF ARSENICALS ON CATALASE ACTIVITY OF ORANGE LEAVES

One of the standards used recently as a measure of the state of growth or condition of plants is the quantity of catalase present. In general, other workers have found that leaves that are growing vigorously show a high catalase content as compared with those that are yellow or are growing poorly. Determinations were made both on a small scale from twigs sprayed by hand and from the trees that were given normal applications in the field.

The quantity of oxygen liberated from hydrogen peroxide by the catalase of the leaves was used as an index of the catalase activity. In making this determination five disks 1 cm^2 each were cut from leaves washed in tap and distilled water and ground to a smooth paste with an equal quantity of calcium carbonate ($CaCO_3$) and a few drops of water. This was washed into a flask with 15 to 20 ml of distilled water and allowed to stand at 25° C. (77° F.) for one hour. Then it was connected to the apparatus where 20 c c of neutralized hydrogen peroxide (H_2O_2) was introduced and the quantity of oxygen evolved in five minute measured at one-half minute intervals. All the determinations were made in a water bath at 25° C.

The quantity of soluble arsenic on the leaves from which the disks were taken was determined by the Gutzeit method. The results of the experiment are shown in Table 8 and Figure 3.

TABLE 8.—*The effect of lead arsenate in various quantities on the catalase activity of orange leaves*

Treatment	Determinations made	Soluble arsenic trioxide per 10 g of fresh leaves	Oxygen liberated in 5 minutes	Increase or decrease in catalase activity
	Number	*Mg*	*Ml*	*Per cent*
Unsprayed, check	29	0	32.4	----------
Sprayed with dilute arsenical solutions	4	.0027	33.8	4.3
	4	.0075	35.4	9.3
	10	.028	38.0	17.3
	6	.081	34.3	5.9
Sprayed with concentrated arsenical solutions	10	.255	30.7	−5.2
	10	1.808	24.3	−25.0
	2	9.600	17.9	−44.8

It can readily be seen that the catalase activity changes according to the quantity of soluble arsenic present. The lighter application stimulated the catalase activity. When quantities less than 0.05 mg of soluble arsenic trioxide on 10 g of green leaves were present the catalase activity was increased. In heavy applications, where the arsenic trioxide ranged from 0.255 to 10.0 mg, catalase activity was decreased or entirely stopped. In these cases the leaves were usually injured so badly that they fell. Extensive experiments showed that catalase activity could be increased not over 10 per cent and no injury result. Such stimulation usually followed the presence of about 0.01 mg of soluble arsenic trioxide per 10 g of green leaves. The quantity that caused the most favorable stimulation of respiration caused the most favorable stimulation of catalase activity also.

RELATION BETWEEN LEAF PROCESSES AND CONDITION OF THE FRUIT

The relation between the respiration of orange leaves during the growing season and the percentage of acid in the mature fruit is very definite. The respiration was measured several times during the growing season, and the average increase or decrease over the check was recorded in terms of per cent. The acid was determined at maturity and considered as a per cent increase or decrease over the check. The check in each case was the unsprayed part of the same tree from which the respiration studies and fruit analyses had been made. This relation is shown in Table 9 and Figure 4.

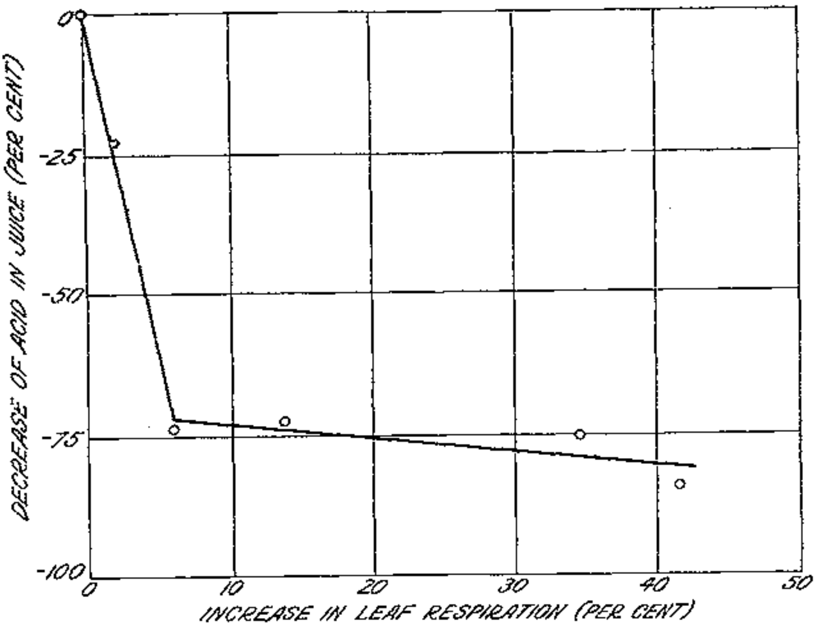

FIGURE 4.—Relation between respiration of orange leaves during the growing season and the acid in the fruit at maturity

TABLE 9.—*The relation between the respiration of orange leaves during the growing season and the acid in fruit at maturity*

Treatment[1]	Increase over normal in respiration of leaves[2]	Decrease from normal in acid in fruit juice
	Per cent	*Per cent*
Check, no treatment	0	
Lead arsenate, 1 pound; water, 50 gallons	6.5	74.1
Do	14.3	73.4
Lead arsenate, 2 pounds; water, 50 gallons	34.6	75.1
Do	41.5	85.3

[1] 1 application June 4, 1931. [2] Average of 4 monthly analyses.

As can quite readily be seen, when the respiration is increased the acid in the fruit is reduced.

Besides the relation between the acid and respiration, there appears to be an agreement between the catalase activity of the orange leaves and the soluble-solids content of the fruit juice at maturity. Table 10 and Figure 5, indicating this relation, show a maximum increase in soluble solids of 1.5 per cent. Differences as small as this, however, should be the result of many determinations under various conditions before they can be considered significant.

TABLE 10.—*Relation between soluble solids of orange juice at maturity and the catalase activity of leaves during the growing season*

Treatment[1]	Increase in catalase activity[2]	Increase or decrease in soluble solids
	Per cent	*Per cent*
Check, no treatment	0	
Lead arsenate, 1 pound; water, 50 gallons	7.6	1.5
Lead arsenate, 2 pounds; water, 50 gallons	21.5	0
Do	28.5	-.5

[1] 1 application June 4, 1931. [2] Average of 4 monthly analyses.

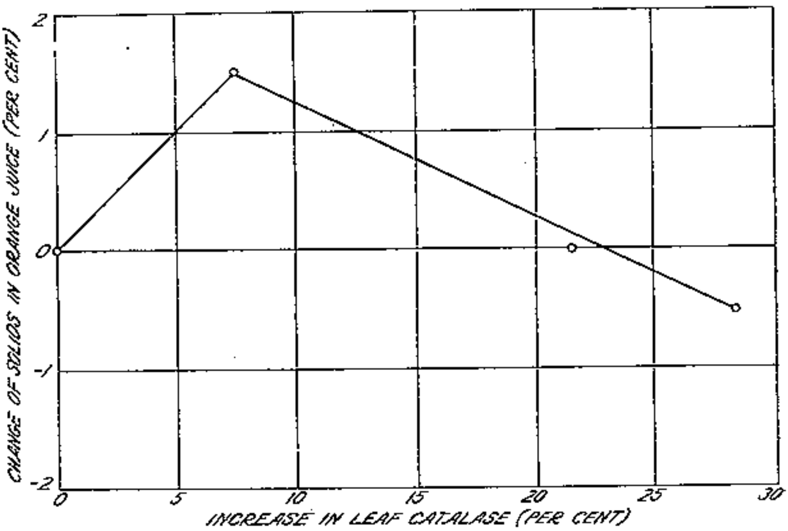

FIGURE 5.—Relation between soluble solids of orange juice at maturity and catalase activity of the leaves during the season

EFFECT OF ARSENICALS ON ORANGES WHEN LEAVES AND FRUIT WERE SPRAYED

As stated previously, it has been known for a considerable time that arsenic greatly changed fruit composition. In order to determine just how much effect various quantities of arsenic did have on fruit composition, many analyses have been made both of the insecticide on the tree during the growing season and of the fruit at various stages of development. In most cases only the total quantity of arsenic remaining on the leaves and the composition of the fruit at maturity are shown.

METHOD OF MAKING DETERMINATIONS

The hydrogen-ion determinations were made by means of a Welch quinhydrone apparatus. Early in the season, when the fruit was small and the quantity of juice was insufficient for a titration, a half fruit was covered with quinhydrone and the contact and bridge placed in the macerated material. When the fruit contained more juice the acid determinations were made by titration against sodium hydroxide

(0.156 N) and the soluble solids determined by a Brix hydrometer. The latter methods are the standard methods used for making the

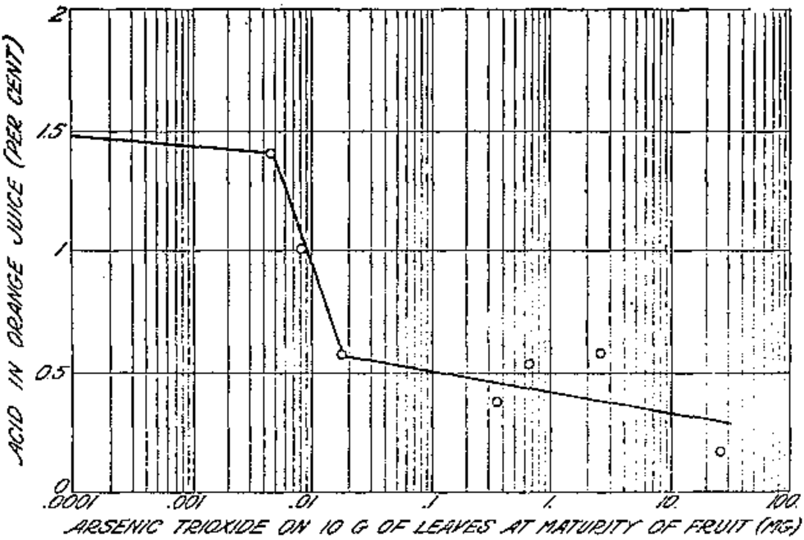

FIGURE 6.—Effect of lead arsenate on the acid in orange juice when arsenic trioxide has been present on the leaves in varying quantities during the growing season. Semilogarithmic

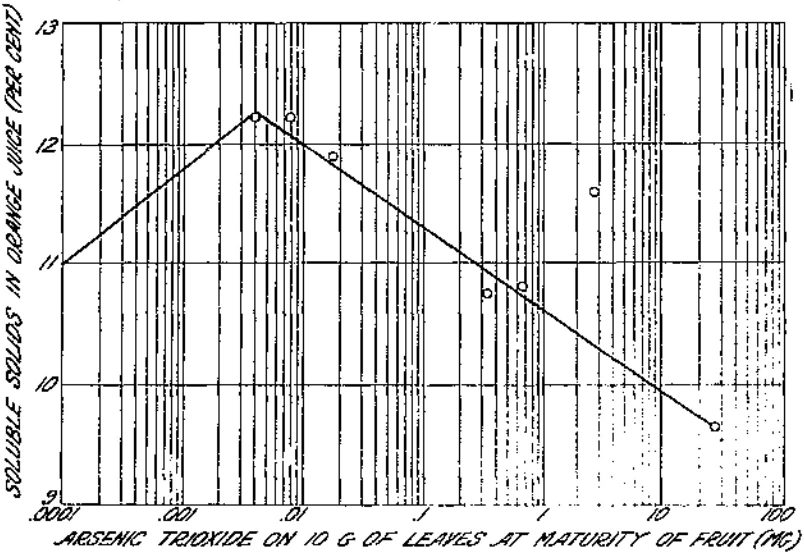

FIGURE 7.—Effect of lead arsenate on the soluble solids in orange juice when arsenic trioxide has been present on the leaves in varying quantities during the growing season. Semilogarithmic

maturity determinations called for by the State laws of Florida (5) The results of these analyses are shown in Table 11 and Figures 6 and 7

TABLE 11.—*The effect of lead arsenate on the acid, solids, ratio, and pH of orange juice when it has been present on the leaves in varying quantities throughout the growing season*

Spray treatment	Total arsenic trioxide per 10 g of green leaves at picking time	Acid in juice	Soluble solids in juice	Ratio of solids to acid	pH of juice
	Mg	*Per cent*	*Per cent*		
Check, no treatment	0	1.47	11.0	7.5	3.64
Lead arsenate, 2 pounds; water, 50 gallons, 1 year previous	.004	1.41	12.20	8.6	3.35
As above, 2 applications	.008	1.01	12.20	12.0	3.65
As above, 6 applications	.018	.59	11.90	20.2	3.99
Lead arsenate, 1 pound; water, 50 gallons, 6 months previous	.344	.38	10.74	28.3	5.17
Lead arsenate, 2 pounds; water, 50 gallons	1.679	.54	10.81	20.0	4.66
As above, several applications	[1] 2.50	.59	11.60	19.7	3.99
30 pounds lead arsenate, applied to 1 tree	[1] 25.0	.17	9.65	56.7	5.00

[1] When arsenic was present in these quantities the leaves were injured and many of them fell, so a part of the arsenical effect was lost. These analyses were from leaves still on the trees.

HYDROGEN-ION CONCENTRATION

There was a very definite relation between the hydrogen-ion concentration of fruit at maturity and the quantity of arsenic used on the tree.

ACID IN FRUIT JUICE

Small quantities of arsenic reduced the acid in oranges very decidedly. When as much as 0.01 mg of arsenic (as arsenic trioxide) was left on 10 g of leaves at maturity, which was the quantity that would probably be left after a spraying of 8 ounces per 200 gallons of water, the maximum change was produced. Ten or one hundred times this quantity made no appreciable difference on the acid content of the fruit.

SOLUBLE SOLIDS IN FRUIT JUICE

Gray and Ryan (*9*) state that the sugars in fruit were not significantly changed, but work by Juritz (*11*) showed that there was a considerable decrease in sugars. While these points are apparently in disagreement, the data indicated that widely different quantities of arsenic were used by the two workers. Copeman stated that the sucrose was decreased but that there was no significant change in the total sugars (*3*). In the Florida work it was found that a very slight quantity of arsenic, 0.004 to 0.008 mg of arsenic trioxide, reduced the acid in the fruit juice considerably; the same quantity actually increased the soluble-solids content in fruit at maturity. When more than this quantity of arsenic was present on leaves at maturity the soluble solids were reduced in direct proportion to the quantity of arsenic present. When as much as 1 mg was present at maturity there was approximately a 10 per cent reduction in the soluble-solids content of juice of mature fruit.

RATIO OF ACID TO SOLUBLE SOLIDS

Since the effect of 0.008 mg of arsenic trioxide on orange leaves at fruit maturity was shown in the reduced acid content of fruit juice and slightly increased soluble-solids content, the ratio of acid to solids was obviously changed. When more than this quantity of arsenic was present the solids were decreased; but because of a very great decrease in the acid also, the ratio was sometimes extremely high.

The writers are of the opinion that the fruit composition is changed only when the majority of the actively growing leaves on the tree at the time the fruit is growing have arsenic on them. For example, when arsenic was applied only to the old leaves of an orange tree it produced no effect, but later when it was applied to the young leaves the fruit composition was changed considerably. When lead arsenate was applied as late as eight months before some Temple oranges were analyzed and only the old leaves were present, there was no effect on the fruit composition, but where the new leaves had appeared before the lead arsenate was applied there was a very marked change in the composition of the fruit. When the leaves that have arsenic on them drop from the tree their effect on the fruit will be lost. If this happens during the first season, the next crop will be normal, but if the leaves stay on for more than a year the next crop will be affected also.

ARSENICAL ANALYSES OF FRUIT JUICE

Only a trace of arsenic was found [4] in a liter of orange juice when a tree had been given thirteen 1-quart applications of fruit-fly bait spray at 10-day intervals. This mixture contained 8 pounds of lead arsenate per 200 gallons. Eighteen daily applications of lead arsenate of the same quantity and the same strength were applied with the same result.

Arsenic as arsenic trioxide (As_2O_3) was found to the extent of 0.01 to 0.16 mg per liter of juice when 17 applications of 5 gallons of bait spray were made at 10-day intervals. Since the quantity of arsenic found in a liter of juice when the trees were very excessively sprayed was only a small fraction of a minimum medicinal dose, the juice from fruit trees normally sprayed could be in no way toxic for human consumption.

CHANGE OF pH OF ORANGE JUICE DURING GROWTH

The hydrogen-ion concentration was normally low when the fruit was small, but as the fruit grew the acid increased very rapidly. When the trees were treated with arsenic the change took place, but the acid content did not ever get so high as in untreated fruit. After the acid had reached its maximum it began to decrease, and on the trees that had been sprayed with arsenic the change was much more rapid than on those unsprayed. This is shown in Table 12 and Figure 8.

TABLE 12.—*Effect of lead arsenate when it is present on the leaves during the growing season, on the rate of development and disappearance of acid in oranges*

[Trees sprayed June 6, 1930]

Month of analysis	Arsenic trioxide per 10 g of sprayed leaves	pH of arsenical-sprayed fruit	pH of unsprayed fruit	Month of analysis	Arsenic trioxide per 10 g of sprayed leaves	pH of arsenical-sprayed fruit	pH of unsprayed fruit
	Mg				*Mg*		
June	3.10	4.55	4.60	September	0.54	4.08	3.28
July	1.47	3.03	2.99	October	.62	4.32	3.72
August	.86	3.53	3.07	November	.33	5.32	4.25

[4] McBride, O. C., Marlowe, R. H., and Bassett, I. P. Op. cit.

EFFECT OF ARSENIC NOT SYSTEMIC

It has been stated by other workers that the effect of arsenic on citrus trees was systemic. Gray and Ryan (*9*) stated that arsenic affected the entire tree if only a part was sprayed. This was also the opinion of Juritz (*11*). Yothers (*17*) reported that only the sprayed part of the tree was affected. In the present work the writers corroborated Yothers's statement that the effect was not systemic, since all the data obtained were the result of spraying half-trees and using the unsprayed part as a check.

RELATION BETWEEN TIME ARSENICAL SPRAY REMAINED ON LEAVES AND ITS EFFECT ON CITRUS FRUIT

Juritz (*11*) and Copeman (*3; 4, p. 144*) have stated in their works that the effect of arsenic persisted for more than one year. All the work done by these men was carried on under arid conditions.

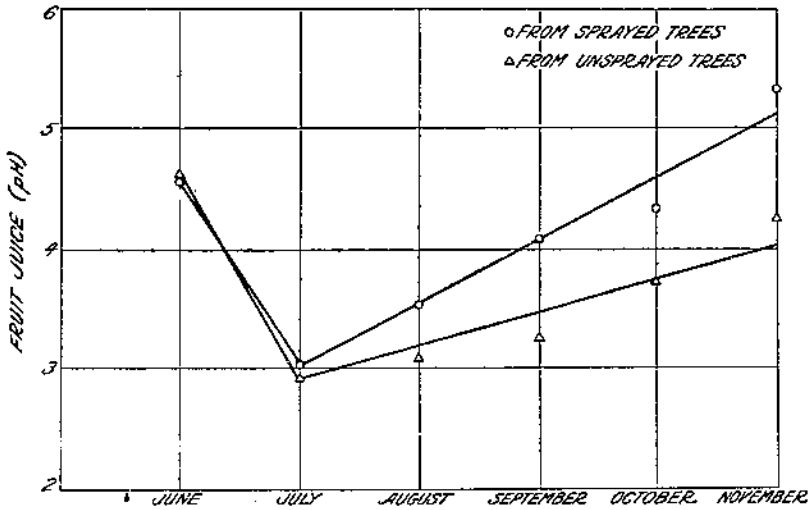

FIGURE 8.—Effect of lead arsenate on the rate of development and disappearance of acid in oranges when it is present on the leaves during the growing season. The trees were sprayed June 6

Experiments conducted in Florida, where the rainfall is approximately 55 inches per year, showed that the effect also persisted for about the same length of time. Fruit from trees heavily sprayed in May showed the effect throughout their growing season and until the fruit was harvested 10 or 12 months later. Fruit from trees sprayed several times during the growing season showed only a slight effect 18 months after the last spraying. At the end of 2½ years, however, fruit from sprayed and unsprayed trees was identical.

It was found that when applications of arsenic were made to actively functioning leaves the effect was almost immediately shown in the fruit. When an application of lead arsenate was made to Lue Gim Gong oranges as late as September, an analysis made one and one-half months later showed that the acid was reduced to 39 per cent of normal as shown by the checks.

SUMMARY

No noticeable effect on the fruit, tree, or undergrowth of a grapefruit tree was produced by 60 spray applications of one-half pound each of lead arsenate in 7½ gallons of water to the soil under the tree. The applications were made over a period of one and one-half years, and at the end of that time there were 2,000 parts of arsenic trioxide per million in the first 2 inches of soil, while only a trace was present in the leaves. Likewise, there was no effect on the fruit, tree, or undergrowth of an orange tree after 14 applications of one-half pound of lead arsenate each in 7½ gallons of water, even though there were 700 parts of arsenic trioxide per million in the first 2 inches of soil.

One year after the last application an analysis of the soil under the grapefruit tree was again made, and 1,800 parts per million was found in the first 2 inches of soil, and even at that time the tree, fruit, and undergrowth were normal.

When lead arsenate was sprayed on orange leaves a diminishing supply of water-soluble and insoluble arsenic was present for a year later. A tree sprayed with 8 pounds of lead arsenate per 200 gallons of sirup-sugar mixture had 0.34 mg of soluble arsenic as arsenic trioxide and 2.76 mg of insoluble arsenic on 10 g of green leaves immediately after spraying. At the end of six months the water-soluble arsenic had decreased to 0.005 mg and the insoluble to 0.19 mg.

An analysis of the wood, blossoms, and small fruit showed that arsenic was evidently translocated to these parts of the tree, for arsenic was present in quantities ranging from a trace to 6.9 mg of arsenic trioxide per kilogram of sample.

Soluble arsenic (measured as arsenic trioxide) present on leaves in quantities of 0.01 mg per 10 g of leaves stimulated respiration slightly, but when more than 1 mg was present on 10 g of leaves the respiration increase ranged from 50 to 75 per cent and the leaves usually fell.

When soluble arsenic was present at 0.01 mg of arsenic trioxide per 10 g of leaves the catalase activity was stimulated, but when quantities greater than this were present the catalase activity was decreased until, when as much as 1 to 10 mg was present, it was almost stopped.

When arsenic was present on the leaves, not so much acid formed in the fruit, and what was formed disappeared more rapidly than in the unsprayed fruit. When as much as 0.01 mg total arsenic trioxide was present on 10 g of green leaves at maturity, the maximum reduction without injury to the tree in fruit acid was produced. The hydrogen-ion concentration of fruit juice from trees that had been sprayed was much lower than that of juice from unsprayed trees.

The solids of fruit juice were somewhat increased when as little as 0.008 mg of arsenic trioxide was present, but when more than this quantity was present the solids were decreased. In this case also, approximately the same quantity of arsenic that stimulated the catalase activity caused the solids in the juice to be increased.

The ratio of acids to solids was very markedly affected by arsenic on the leaves. When as much as 0.01 mg of arsenic trioxide per 10 grams of leaves was present the ratio was only a little above normal, but a slight increase over this caused the ratio to rise to 20 to 1. When so much arsenic was present that the leaves dropped, the arsenic was eliminated from the tree and the fruit was less affected.

When the trees were sprayed seventeen times with 5 gallons per application a maximum of 0.16 mg of arsenic trioxide per liter of juice was found.

Experiments made by the writers have demonstrated that the effect of arsenic is not systemic, only the sprayed part of a tree being affected.

The effect of spraying with arsenicals is shown almost immediately and persists to a slight degree for about 18 months. After 2½ years, however, it has entirely disappeared.

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