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MEASUREMENTS ON HYDROCYANIC ACID ABSORBED BY CITRUS TISSUES DURING FUMIGATION[1,2]

E. T. BARTHOLOMEW,[3] WALTON B. SINCLAIR,[4] AND D. L. LINDGREN[5]

METHODS FOR the accurate determination of hydrocyanic acid (HCN) and studies of factors affecting the recovery of HCN from fumigated citrus tissues have been previously reported (*1, 2*).[6] The present paper is concerned with the results of the application of the principles derived from the earlier studies to further laboratory experiments, performed in conjunction with the fumigation studies of citrus trees under orchard conditions.

The effect of certain factors, such as oil sprays, the locality in which the trees were grown, and the temperature, age, and moisture content of citrus tissues at time of fumigation, have been studied in relation to the absorption and retention of HCN under both laboratory and field conditions. The comparative amounts of absorption and lengths of time of retention of HCN have also been studied in relation to maturity of leaves and fruits and in relation to their injurious or noninjurious effects. The results of laboratory experiments cannot always be applied directly to the solution of orchard fumigation problems, but they may serve as a basis for the formulation of field experiments.

The trees, leaves, and fruits used in the experiments described in this paper were of the Valencia-orange variety (*Citrus sinensis* Osbeck).

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[6] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

MATERIALS AND METHODS

The leaves used in these experiments were mature. The fruits were of different ages, but in most cases in this paper are referred to as either "green" or "mature." The term "green" refers to the color of the fruit; green fruits were always immature. Valencia oranges in California usually become orange yellow in color before they mature. A few fruits of this kind were used and are referred to as "immature yellow." The approximate age of the fruits in the different samples is indicated by the specifications given in connection with the tables and figures.

In the first of these experiments, the paired samples contained the same number of fruits, each fruit had approximately the same equatorial diameter, and each of the samples weighed approximately the same. The amounts of HCN recovered from these fruits were expressed as total recovery per sample. In the later experiments, the total surface area of the fruit in each sample was also determined, and the amounts of HCN recovered were then expressed in milligrams per unit of fruit surface.

At the end of the fumigation period, the fruits (both peel and pulp) in each sample were cut into 12 to 25 small pieces, the number of pieces depending upon the size of the fruit. These pieces were then placed in a distillation flask. When leaves were used, they were placed whole in the distillation flask. The distillations were made as already described in a previous report (*2*).

In one experiment, trees in the field were sprayed with oil, and samples of leaves and fruits from the sprayed trees, together with similar samples from unsprayed trees, were then brought to the laboratory and fumigated to determine the comparative amounts of HCN that each would absorb.

The Fumigation of Citrus Leaves and Fruits in the Laboratory.— When the leaf and fruit samples were brought to the laboratory, they were fumigated in a gastight metal fumatorium having a capacity of 100 cubic feet. This capacity was sufficient to make possible the fumigation of many samples at one time, which was often necessary during these investigations. At each fumigation, 8 ml of liquid HCN of at least 96 per cent purity was used in the fumatorium. (This amount of HCN for each 100 cubic feet in a gastight metal fumatorium in the laboratory has been found to be as effective for killing scale insects as 20 ml for each 100 cubic feet in a more or less porous tent in the field.) The liquid HCN was vaporized before it was forced into the fumatorium, where the air was kept in continuous motion by means of an electric fan. Unless otherwise stated, all fumigations were made at 75° F. The samples remained in the fumatorium for 40 minutes.

The Fumigation of Citrus Trees in the Field.—In the field, trees were fumigated at night or during the day and under different environmental conditions to determine the comparative amounts of HCN absorbed by leaves and fruits and the comparative lengths of time required for disappearance of the HCN from the tissues. The trees were fumigated by the usual commercial method (*9*). Each tree was covered with a canvas tent, and heated liquid HCN was vaporized into the tent near the bottom center of the tree. A 20-ml schedule (20 ml of liquid HCN per unit[7] of tree space) was used for all trees except one, for which an 18-ml schedule was used. The fumigation period for the field work was 45 minutes.

At the end of the fumigation period, the tent was removed from the tree, and the first samples of leaves or fruits were picked at once. The leaf samples (200 grams each) were placed directly into distilling flasks and covered with distilled water. The flasks were then stoppered, shaken thoroughly, and brought to the laboratory, where the leaves were distilled to recover the HCN which they had absorbed. The samples of fruits were brought to the laboratory, weighed, measured, cut into small pieces, placed in distilling flasks, and distilled for HCN recovery. Subsequent samples of leaves and fruits were taken at intervals from the same trees until the tissues were free, or nearly free, of HCN.

CONCENTRATIONS OF HCN IN FUMATORIUM AND IN TENTS DURING FUMIGATION PERIODS

HCN Concentrations in Fumatorium.—The concentrations of HCN in the fumatorium during each fumigation period were determined on 2-liter samples of air withdrawn at intervals of 1, 3, 7, 15, 30, and 40 minutes after fumigation began. As the air samples were withdrawn from the fumatorium, they passed through an 0.1 *N* solution of NaOH, upon which the HCN determinations were made. During this experiment 936 such determinations were made. The results are too numerous to give in tabular form but are shown in the form of a broken-line curve in figure 1.

Each point on the curve (fig. 1) represents the mean of 156 determinations made at the time interval indicated. The mean for the determinations made at the 1-minute interval was 1.45 mg of HCN per liter of space in the fumatorium; that for determinations at the 40-minute interval was 1.33 mg, or only 8.3 per cent less. The concentration of HCN in the fumatorium therefore remained nearly constant during each 40-minute fumigation period.

HCN Concentrations in Tents.—The concentrations of HCN in the tents during each fumigation were determined in the same manner as

[7] A unit equals approximately 100 cubic feet of space under a tent covering an average-sized citrus tree such as the trees used in these experiments.

those in the fumatorium. Two-liter samples of air were drawn from near the center of each tent at the intervals shown in table 1, and their HCN content was determined. The concentration of HCN in each tent at the different sampling intervals is also shown in this table.

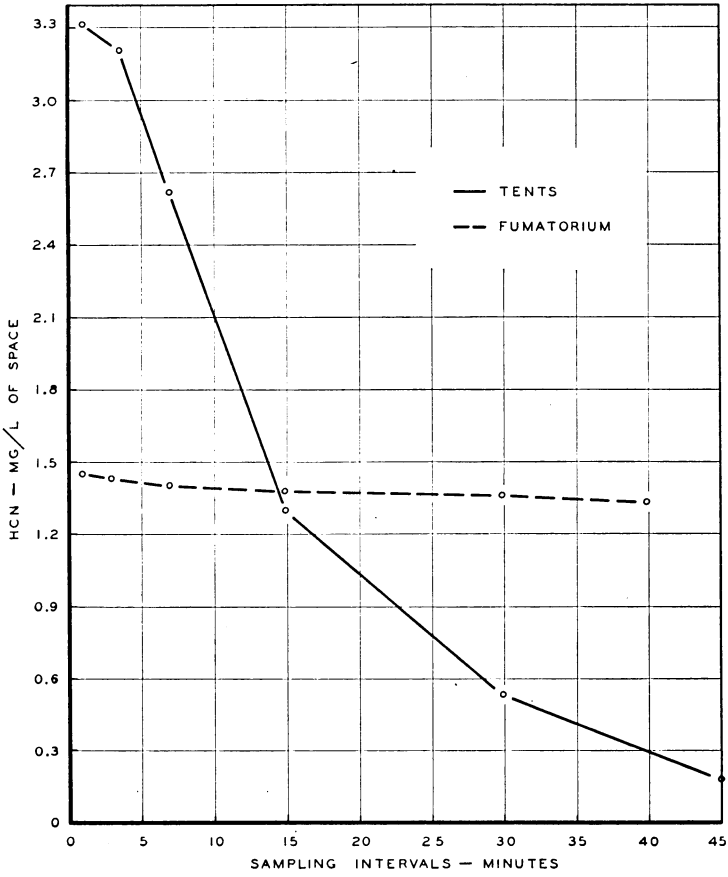

Fig. 1.—Average number of milligrams of HCN per liter of space under fumigation tents and in the fumatorium at given intervals during fumigation periods. The data for the tent curve were taken from table 1. Each point on the fumatorium curve represents the average of 156 determinations. The average fumigation dosage of liquid HCN for each 100 cubic feet of space was 20 ml under the tents and 8 ml in the laboratory fumatorium.

Although the initial amount of HCN forced under each tent was the same (20 ml per unit, except for tree 4, which was given only 18 ml), the amount to which each tree was exposed at different intervals during the fumigation period was noticeably different. These differences are shown by the HCN recoveries from the air samples taken at the given

intervals (table 1, cols. 1 to 8). Figures in column 1 of table 1 show very plainly that in several cases the HCN had not yet become distributed in the tent at the end of the 1-minute interval. Tent porosity, air temperature, humidity, and movement, and similar factors were no doubt responsible for this condition. The final concentrations at the end of the 45-minute fumigation periods (table 1, col. 8) ranged from 0.1 to

TABLE 1

CONCENTRATION OF GASEOUS HCN UNDER TENT, NEAR CENTER OF TREE, AT DIFFERENT INTERVALS DURING FUMIGATION*

Tree no.	HCN per liter of space								
	After 1 minute	After 3 minutes	After 4 minutes	After 7 minutes	After 15 minutes	After 30 minutes	After 40 minutes	After 45 minutes	Mean†
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
	mg	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
1	0.6	...	2.2	1.9	1.0	0.4	0.2	...	0.9
2	1.0	3.5	...	3.2	1.8	0.7	...	0.2	1.4
3	3.1	...	4.8	3.4	1.7	0.6	...	0.2	1.6
4	2.3	3.7	...	2.4	1.2	0.4	...	0.1	1.1
5	2.8	3.7	...	2.7	1.2	0.5	0.2	...	1.3
6	7.2	3.1	...	4.5	1.1	0.2	...	0.1	1.4
7	9.8	3.5	...	1.9	1.8	0.9	...	0.2	1.6
8	8.0	...	4.4	2.9	1.4	1.2	...	0.3	1.8
9	0.3	...	2.3	2.1	1.2	0.4	...	0.2	0.9
10	1.0	3.2	...	2.1	1.0	0.3	...	0.1	0.9
11	0.5	1.5	...	2.0	1.1	0.5	...	0.2	0.9
12	3.5	...	2.8	2.4	1.1	0.4	...	0.1	1.0
Mean	3.3	3.2		2.6	1.3	0.5	0.2		1.2

* Trees 1 to 5 and 9 to 12 were fumigated at night, September 20 to November 14, 1939, and July 23 to August 15, 1940, respectively; trees 6 to 8 were fumigated in the daytime (trees 6 and 7 at 9:45 a.m. and tree 8 at 10:30 a.m.), July 23 to 29, 1940. The fumigation schedule was 20 ml per unit except for tree 4, which was given only 18 ml per unit.

† Calculated from the formula $\frac{\Sigma MC \times T}{\Sigma T}$.

0.3 mg per liter of air sample withdrawn from the tents. The figures in this column show that an average of about 95 per cent of the HCN had been absorbed or had escaped from the tents by the end of the fumigation period.

The mean average concentrations of HCN in the tents for the fumigation periods are shown in column 9 of table 1. These values were calculated to give due weight to the time factor by using the formula suggested by Knight (*4*): the mean is estimated to be equivalent to $\frac{\Sigma MC \times T}{\Sigma T}$, where MC is the mean concentration for each time interval T. The same formula was used for calculating the mean concentrations of HCN in the fumatorium, although this was not really necessary, because concentrations varied only 8.3 per cent.

For comparison, a solid-line curve representing the average concentrations of HCN in the tents at the different times of sampling is shown in figure 1 with the broken-line curve for the fumatorium. The data for the tent curve were taken from table 1. The values for the 3- and 4-minute intervals were combined and averaged as for a 3½-minute interval, and the two values (trees 1 and 5) for the 40-minute interval were averaged with those for the 45-minute interval. This method of computation and the great difference in sampling values for the first three or four intervals may make the curve of questionable worth. It does, however, illustrate clearly the difference in decreases in concentrations of HCN in the air in the tents and in the fumatorium. The final decrease in the former was approximately 95 per cent; that in the latter, only 8.3 per cent.

THE EFFECT OF PRECONDITIONING TEMPERATURES ON THE ABSORPTION OF HCN BY GREEN AND MATURE FRUITS

Quayle and Rohrbaugh (*11*) found no significant difference in the kill of red scale fumigated at temperatures between 50° and 90° F. They demonstrated, on the other hand, that preconditioning of red-scale-infested lemon fruits at 50° for at least 4 hours before fumigation resulted in a higher percentage of kill than preconditioning at 90°, irrespective of the temperature at which the fumigation was made. They found, also, that rooted lemon cuttings preconditioned and fumigated at 50° at a relative humidity of 70 per cent were more severely injured than similar cuttings preconditioned and fumigated at 90° at the same relative humidity.

The relation of temperature to the degree of mortality of red scale and to the injury of lemon cuttings by HCN raised a question as to the effect of temperature on the absorption of HCN by citrus tissues. Experiments were therefore planned to determine the relation between preconditioning temperatures and the amounts of HCN absorbed by citrus tissues fumigated under controlled laboratory conditions.

These experiments were performed between September 26 and November 17, 1939. Both green and mature fruits were used. Fruits were picked the night before they were to be fumigated; they were weighed, and their equatorial diameters were determined. The weights of the 17 to 20 green fruits in each sample ranged from 1,374 to 1,531 grams, and their diameters ranged from 1⅞ to 2¼ inches; the weights of the 17 mature fruits in each sample ranged from 1,521 to 1,863 grams, and their diameters ranged from 1⅞ to 2 5/16 inches. Samples of green or mature fruits were preconditioned overnight (15 to 20 hours) in cabinets

maintained at temperatures of 43°, 50°, 65°, or at 80° F and at corresponding relative humidities of 78, 75, 70, and 50 per cent.

Available equipment permitted the recovery of HCN from only 2 samples at once. Under these conditions, 1 of the samples (green or mature) was preconditioned at 43° F and the other at 65° and the 2 were fumigated simultaneously. The next 2 samples were preconditioned the

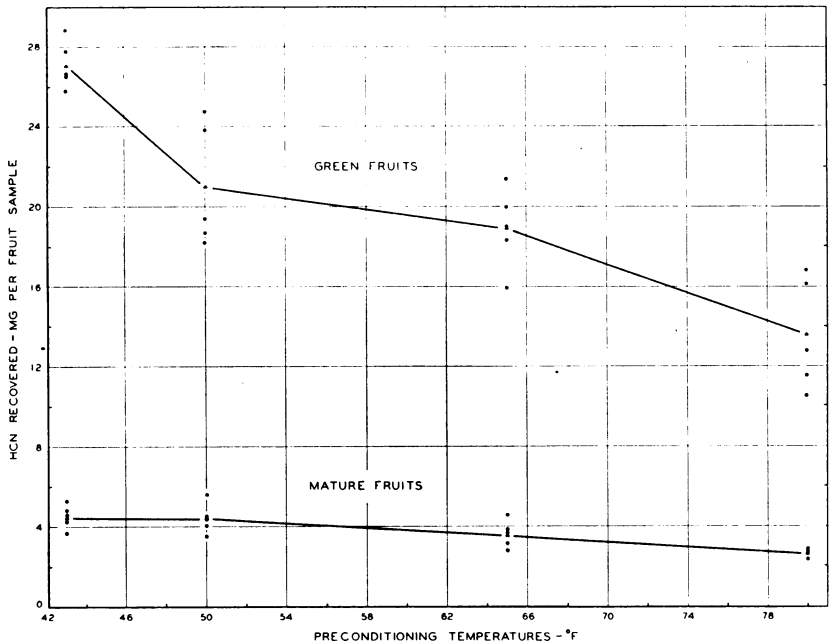

Fig. 2.—Amounts of HCN recovered from samples of green and mature Valencia-orange fruits preconditioned overnight in the laboratory at 43°, 50°, 65°, or 80° F and then fumigated in the laboratory fumatorium. The curves represent the means of the amounts of HCN recovered, while the scattered points indicate the amounts recovered from single samples. Note that the samples preconditioned at the lower temperatures absorbed more HCN than those preconditioned at the higher temperatures, and that the green fruits absorbed several times as much HCN as the mature fruits.

same day or sometimes a few days later, at 50° and 80°, respectively, before fumigation; 5 samples of green and 5 samples of mature fruits were tested at each preconditioning temperature.

After the required preconditioning period at the desired temperature, the paired fruit samples were placed in the fumatorium, fumigated, and subsequently distilled for HCN recovery. In these experiments all samples were fumigated at the same temperature, 75° F.

In order to determine the possibly injurious effects of the HCN, 6 additional fruits, which had been preconditioned at the same temperatures and relative humidities, were fumigated with each sample. After

the fumigation period, these fruits were placed in open paper bags and stored at 75° F for about 10 days to await the development of any injury that might have occurred. At the end of this storage period, the fruits were classified as good, or as slightly, moderately, or badly injured.

The relation of preconditioning temperatures to the amounts of HCN recovered from the green and mature fruits is shown in figure 2. The differences in the percentages of relative humidity that prevailed in the preconditioning cabinets did not appear to have any influence on the amount of HCN absorbed by the samples during fumigation. In addition to showing the mean values obtained at each preconditioning temperature for each kind of fruit, a point is given to represent the result of each individual test in order to show the diversity of results usually obtained when performing experiments with biological material grown under variable conditions.

The green fruits absorbed an average of 5.4 times as much HCN as the mature fruits. In general, the amount of HCN absorbed by the fruits decreased with increase in preconditioning temperature; the effect of the preconditioning temperature was not so great on mature fruits as on green fruits, however.

Although none was visible, there is a probability that some moisture condensed on the fruits that had been preconditioned at the lower temperatures (that is, at 43°, 50°, or 65° F) during the 40-minute fumigation at 75° in the fumatorium. HCN is readily soluble in water; it would therefore seem logical to conclude that the presence of the condensed moisture on the cooler fruits at least partially explains why more HCN was recovered from them than from the warmer fruits. On the other hand, the presence of a film of moisture, though not continuous, would tend to impede the entrance of HCN into the fruits; the total amount absorbed by the film and the fruit might thus be reduced (see "HCN Recovery from Mature Fruits Having Wet Surfaces," p. 389).

Perhaps it would be more logical to conclude that the cool fruits absorbed more HCN than the warm fruits because of the increased solubility of gaseous HCN at the lower temperatures. This explanation appears to agree very well with the gas law, which states that the lower the temperature, the greater the solubility of the gas. The greater viscosity of the water in or on the fruits at the lower temperatures would, of course, not interfere with the foregoing explanation. Even at the end of the 40-minute fumigation period, the fruits preconditioned at 43° F were noticeably cooler than those preconditioned at 80? The amount of HCN recovered from the fruits preconditioned at 43° was approximately twice that recovered from the fruits preconditioned at 80° (fig. 2).

The relation between preconditioning temperatures and HCN injury to the fruits is shown in table 2. Under the conditions of these experiments, the green fruits were much more severely injured than the mature fruits. On the other hand, the quantity or degree of injury to both green and mature fruits appears to bear little or no relation to preconditioning temperature. For example, with green fruits, the total percentage injured was nearly the same for those preconditioned at 43° F as for

TABLE 2

SEVERITY AND PERCENTAGE OF HCN INJURY TO GREEN AND MATURE VALENCIA-ORANGE FRUITS PRECONDITIONED 15 TO 20 HOURS AT DIFFERENT TEMPERATURES IMMEDIATELY PRECEDING FUMIGATION

Preconditioning temperature	Percentage* of fruits having different degrees of injury			
	Slight	Moderate	Bad	Total
Green fruits				
° F	*per cent*	*per cent*	*per cent*	*per cent*
43	6.7	23.3	26.7	56.7
50	32.3	22.6	9.7	64.6
65	40.0	10.0	3.3	53.3
80	33.3	26.7	3.3	63.3
Mature fruits				
° F	*per cent*	*per cent*	*per cent*	*per cent*
43	2.8	0.0	0.0	2.8
50	0.0	0.0	0.0	0.0
65	8.3	0.0	0.0	8.3
80	0.0	0.0	0.0	0.0

* Percentage figures are based on examination of lots of 30 fruits each. These fruits were preconditioned and fumigated at the same time as those from which the data in figure 2 were obtained.

those preconditioned at 80°. The percentages of badly injured fruits which had been preconditioned at the two lower temperatures were, however, somewhat larger than for those preconditioned at the two higher temperatures. Although the injuries to these fruits were superficial and were confined to the peel (see Quayle [*9*], fig. 339), fruits so injured could not be shipped as first class, and a large percentage of them would have to be discarded as culls.

Why there was a noticeably higher kill of red scale on lemon fruits and greater injury to rooted lemon cuttings when fumigated after preconditioning at 50° F than when fumigated after preconditioning at 90° (*11*), but a lack of appreciable difference in injury to green fruits fumigated after preconditioning at different temperatures (43°, 50°, 65°, and 80°), is a question that occurs naturally at this point. The answer must await further investigation.

THE EFFECT OF OIL SPRAY ON THE ABSORPTION OF HCN BY FRUITS AND LEAVES

The results of commercial pest-control work have indicated that citrus trees are somewhat less likely to be injured by HCN fumigation if they have recently been sprayed with oil. Some experiments were therefore performed to test this observation.

Experiments with Fruits.—Fruits for a preliminary experiment were picked at intervals between February 24 and 28, 1940. All fruits were

TABLE 3

AMOUNTS OF HCN RECOVERED FROM FUMIGATED OIL-SPRAYED AND UNSPRAYED DETACHED IMMATURE YELLOW VALENCIA-ORANGE FRUITS*

Paired samples	Fruits in each sample	Weight of fruit sample		HCN recovered per sample	
		Oil-sprayed	Unsprayed	Oil-sprayed	Unsprayed
nos.	*number*	*grams*	*grams*	*mg*	*mg*
573 and 574	12	1,586	1,609	3.3	7.9
575 and 576	14	1,709	1,692	3.7	7.8
577 and 578	14	1,720	1,698	4.5	8.3
579 and 580	14	1,721	1,685	2.9	5.6
581 and 582	14	1,725	1,600	4.2	7.4

* Fruit samples were picked between February 24 and 28, 1940, and paired. One of each of the paired samples was sprayed with a 1 per cent light-medium oil immediately after picking; the other served as an unsprayed control. Two days later both samples were fumigated in the fumatorium at the same time.

completely yellow in color but immature. Samples consisting of 12 to 14 fruits each were paired. One sample of each pair was sprayed with a 1 per cent light-medium oil by means of a precision sprayer; the other (unsprayed) sample served as a control. Two days after the fruits had been sprayed, the sprayed and unsprayed samples were fumigated in pairs in the fumatorium, the same concentration of HCN being used in each test. Table 3 shows the comparative amounts of HCN recovered from the sprayed and unsprayed samples. It is interesting to note that only about half as much HCN was recovered from the oil-sprayed as from the unsprayed fruits. Since all the fruits were yellow in color, it was to be expected that only a relatively small amount of HCN would be absorbed and recovered.

The results of this experiment were of sufficient interest to warrant the expansion of these studies to include spraying tests under field conditions. A row of Valencia-orange trees in a plot at the Citrus Experiment Station was selected. Four of the trees were sprayed very heavily with a 1½ per cent light-medium oil of the same viscosity as that used in the preceding experiment but of a different brand. Approximately 25 gallons of spray were applied to each tree. (In ordinary commercial

practice not over 20 gallons would be used on trees of this size.) Five other trees in the same row were kept for controls. Great care was exercised to prevent the spray from falling on the control trees. The spray was applied March 5, 1940, between 10:00 and 11:00 a.m., and the first samples of sprayed and unsprayed fruits were picked about 4:00 p.m. the same day. The fruits were preconditioned overnight in the laboratory at 73° F before being fumigated and distilled. Several similar sam-

TABLE 4

AMOUNTS OF HCN RECOVERED FROM IMMATURE YELLOW VALENCIA-ORANGE FRUITS PICKED FROM OIL-SPRAYED AND UNSPRAYED TREES AND THEN FUMIGATED*

Paired samples†	Weight of fruit sample		Total surface area of fruit sample		Date of fumigation, 1940	HCN recovered from fruit samples per 1,000 cm^2 of surface	
	Oil-sprayed	Unsprayed	Oil-sprayed	Unsprayed		Oil-sprayed	Unsprayed
nos.	*grams*	*grams*	cm^2	cm^2		*mg*	*mg*
583 and 584..	1,559	1,562	1,596.3	1,586.7	March 6	3.3	3.0
585 and 586..	1,557	1,563	1,586.7	1,573.3	March 6	4.4	3.9
587 and 588..	1,553	1,557	1,621.3	1,605.9	March 7	3.8	3.7
589 and 590..	1,548	1,553	1,575.7	1,606.9	March 7	3.7	3.2
591 and 592..	1,545	1,585	1,567.5	1,578.1	March 8	3.1	3.3
593 and 594..	1,548	1,597	1,575.7	1,577.1	March 8	3.7	3.4
607 and 608..	1,710	1,711	1,723.7	1,726.1	March 15	3.1	3.4
609 and 610..	1,655	1,697	1,706.7	1,712.8	March 15	3.2	3.2
611 and 612..	1,781	1,763	1,662.5	1,625.9	May 13	1.8	2.3

* Fruits were picked in the afternoon preceding fumigation and were preconditioned overnight in the laboratory at 73° F. The trees had been sprayed on March 5, 1940, with 1½ per cent light-medium oil.
† Samples consisted of 15 fruits each, except samples 611 and 612, which consisted of 12 fruits each.

ples were picked and treated in the same manner on the dates indicated in table 4. All fruits were immature yellow. In this experiment the HCN recoveries are expressed as milligrams per unit of fruit surface instead of for the whole sample, as in the preceding experiment.

As already pointed out (table 3), the fruits that were brought to the laboratory before being sprayed absorbed much less HCN than similar unsprayed fruits. The results in table 4 show, however, that when the fruits were sprayed in the field and then brought to the laboratory and fumigated, they absorbed as much HCN as the unsprayed control fruits. The reason for this difference is not known. It is possible that the film of 1 per cent light-medium oil applied to the fruits in the laboratory by means of the precision sprayer was thicker than that of the 1½ per cent light-medium oil applied to fruits in the field by means of the commercial power sprayer. Special care was taken to spray all outside fruits thoroughly, and only outside fruits were used in the experiments.

Apparently the length of time that the oil remained on or in the fruit did not influence the penetration of HCN, because the amount of HCN

recovered on March 6 was approximately the same as that recovered on March 15. The recoveries from the sprayed and unsprayed samples on May 13 were about equal but were smaller than the recoveries in March, probably because the fruits had matured. For comparative differences in the amounts of HCN absorbed by mature and immature fruits, see figure 2 (see, also, figs. 3, 4, 5, and 9).

The amounts of HCN recovered from the fruits in these particular experiments are below the concentrations of HCN per unit weight that would ordinarily cause injury of any great extent to the fruit at this

TABLE 5

AMOUNTS OF HCN RECOVERED FROM MATURE VALENCIA-ORANGE LEAVES PICKED FROM OIL-SPRAYED* AND UNSPRAYED TREES AND THEN FUMIGATED

Paired samples†	Fumigation		HCN recovered from leaves per 200-gram sample	
	Date, 1940	Hour	Oil-sprayed	Unsprayed
nos.			*mg*	*mg*
595 and 596	March 11	9:00 a.m.	39.7	42.4
597 and 598	March 11	1:00 p.m.	36.7	43.0
599 and 600	March 12	8:00 a.m.	44.4	41.0
601 and 602	March 12	1:00 p.m.	42.6	42.0
603 and 604	March 13	8:20 a.m.	30.0	33.1
605 and 606	March 13	1:30 p.m.	37.5	37.5

* The trees had been sprayed on March 5, 1940, with 1½ per cent light-medium oil.
† Each sample consisted of 200 grams of leaves. The samples were fumigated in pairs (sprayed and unsprayed) in the fumatorium immediately after picking.

stage of maturity. There was, accordingly, no injury to the portions of the unsprayed samples reserved for observation, but 6.25 per cent of similar portions of the oil-sprayed fruits showed slight injury. While this was not a great deal of injury, it was enough to show that the oil spray, under these experimental conditions, was injurious rather than protective in effect.

Experiments with Leaves.—The 200-gram samples of leaves used in these experiments were picked from the same oil-sprayed and unsprayed trees from which the fruits had been picked. (Counts have shown that the number of mature leaves in a 200-gram sample varies from 250 to 270.) HCN recoveries are expressed as milligrams per 200-gram sample (table 5) rather than as milligrams per unit of surface area, as was done with the fruits (table 4). The thicker the leaf, the greater is the weight per unit area; on this basis the samples used might have had different surface areas. It is very probable, however, that the surface areas of the samples were similar, because the samples consisted of a composite of leaves picked at random from all the sprayed trees and from all the unsprayed trees, respectively.

In this particular experiment the oil spray did not have a retarding effect on the penetration of HCN into the leaves. Several years ago Quayle (*8*) observed that sprays made from "heavy" oils protected orange trees from HCN injury even when fumigated with unusually high dosages of HCN (220 per cent schedule). As a result of further investigation on this problem, Quayle and Ebeling (*10*) stated that:

> Hydrocyanic acid is not absorbed by the oil, consequently there may be less absorption by the tree where the surface is covered with oil; if so, a higher concentration of HCN could be used in the air surrounding the insect, without injury to the tree. Some tests have shown that an oil-sprayed tree is less likely to be injured by fumigation than a tree under the same conditions which has not been sprayed (Quayle, 1922) [(*7*)]. Other comparative tests have shown that there is little or no increased protection from oil spray coverage.

The spray oils in use at the present time are lighter than those formerly used, but the opinion still prevails among growers and commercial operators that oil sprays tend to protect citrus trees against HCN fumigation injury. It is generally admitted, however, that there have been many cases in which the oil spray had no apparent protective effect.

THE EFFECT OF MOISTURE CONTENT OF TISSUES ON THE ABSORPTION OF HCN

It is important to determine the relation between the moisture content of citrus leaves and fruits and the amount of HCN which they absorb during fumigation, for this relation is not only fundamental to the understanding of the physiological effects of HCN in the tissues but also important from a practical viewpoint. Many growers and fumigators are of the opinion that during the summer months, other factors being equal, considerably more injury will result to citrus trees if they are fumigated when the soil is wet than when it is comparatively dry. During the winter months, however, there appears to be little or no relation between injury to the tissues and soil moisture, for the trees can be successfully fumigated after a rain just as soon as the ground will permit the fumigators to operate. The two following experiments were performed to determine any relation that might exist between the moisture content of leaves and fruits and the amount of HCN they would absorb when fumigated under laboratory conditions.

HCN Recovery from Fumigated Fresh and Partially Wilted Mature Leaves.—The purpose of this experiment was the determination of the comparative amounts of HCN that may be absorbed by fresh and by partially wilted mature Valencia-orange leaves when fumigated in the fumatorium. About 525 grams of mature leaves were picked at random from 6 to 8 trees, brought to the laboratory, and thoroughly mixed. One

100-gram sample of these leaves was used for determining total moisture content; 2 samples of 200 grams each were fumigated in the fumatorium after the moisture content of 1 sample had been reduced.

The moisture content of the leaves in the partially wilted sample was reduced the desired amount (usually 15 per cent of the fresh weight) by spreading the leaves in a ¼-inch-mesh wire tray, 20 inches long, 13 inches wide, and 2 inches deep. The tray was supported in the upper end of a carton about 3 feet high and open at top and bottom. The carton was placed over a hot plate, with a 3-inch space between the bottom of the

TABLE 6

AMOUNTS OF HCN RECOVERED FROM FUMIGATED FRESH AND WILTED MATURE VALENCIA-ORANGE LEAVES*

Paired samples	Moisture in leaves (fresh-weight basis)		HCN recovered from leaves per 200-gram sample	
	Fresh	Wilted	Fresh	Wilted
nos.	*per cent*	*per cent*	*mg*	*mg*
613 and 614	57.0	47.0	45.7	41.6
615 and 616	57.2	42.2	43.0	41.7
617 and 618	57.5	42.5	41.6	42.1
619 and 620	56.6	41.6	44.2	45.1
621 and 622	57.9	42.9	41.3	41.1
623 and 624	55.8	40.8	46.3	44.6

* Tests were made May 14 to 24, 1940. About 525 grams of mature leaves were picked at random from 6 to 8 trees for each test. One 200-gram sample of these leaves was kept in an airtight container while another 200-gram sample was being wilted (about 20 minutes). A third sample of 100 grams was used for moisture determination. The fresh and wilted samples were fumigated simultaneously in the fumatorium.

carton and the floor to insure good ventilation. By weighing the leaves at intervals, the desired loss in moisture content could be determined. Fresh leaves were kept in an airtight container during this process (about 20 minutes). Paired samples of fresh and wilted leaves were then fumigated at the same time.

Table 6 shows that under these experimental conditions there was no significant difference between the amounts of HCN absorbed by fresh and by wilted leaves. The results of this experiment indicate that if there is any great difference in injuries when fumigations are conducted under dry and under wet conditions, the injury to the tissues is not wholly dependent upon the amount of HCN absorbed. These fresh and wilted leaves had been detached and were fumigated in the laboratory, however; results may therefore not be indicative of what would have happened had fresh and wilted leaves been fumigated in the field while attached to the trees.

HCN Recovery from Fumigated Turgid and Nonturgid Green Fruits.—The tests on turgid and nonturgid fruits were similar to those on fresh

and wilted leaves. For each test, 2 samples of fruit were collected in the late afternoon. The fruits of 1 sample were cut with stems about 12 to 18 inches long; those of the other sample were clipped without stems. The stems of fruits of the first sample were immediately submerged in water and recut to a length of about 6 or 8 inches, then transferred to Erlenmeyer flasks containing tap water. Upon being brought to the laboratory, the sample with the stems was placed in a glass-walled humidity cabinet; the one without stems was put in an open paper bag and set in the same room outside the cabinet. The temperatures and humidities to which the different samples were exposed between the time of collection and fumigation are shown in table 7.

The paired samples were fumigated the following day. Just before fumigation the stems were removed from the turgid sample, and the fruits in both samples were weighed and measured, so that the comparative total surface areas of the 2 samples could be determined. The fruits with stems had become very turgid; the others had become slightly wilted.

The results recorded in table 7 show the comparative amounts of HCN absorbed by, and recovered from, the turgid and nonturgid samples of fruit. No significant difference is noticeable between the 2 samples of each pair. There was, however, a marked difference in the amounts of HCN recovered from the different samples within each of the two groups —turgid and nonturgid. The mean concentrations of HCN in the fumatorium during each fumigation period were much the same, yet the amounts of HCN recovered gradually increased at each successive fumigation. The prefumigation and HCN-recovery treatments were the same for all samples; therefore no explanation can be given for these results, unless one may say that during this period the fruits were undergoing some physical or chemical change which made them more susceptible to HCN absorption. The results of these fruit tests (table 7) are similar to those for the leaves (table 6) in that there was no appreciable difference in the amounts of HCN absorbed by turgid and nonturgid tissues.

After each fumigation, aliquot samples of turgid and nonturgid fruits were placed in paper bags and stored in a room at 75° F for about 10 days. They were then examined for the presence of HCN injury and were classified as uninjured or as slightly, moderately, or badly injured. The comparative effects of the HCN on the turgid and nonturgid fruit samples, expressed in mean percentages, are shown in table 8. Although there were no significant differences in the amounts of HCN recovered from the samples, 84 per cent of the turgid fruits were injured, as compared with only 29 per cent of the nonturgid fruits.

TABLE 7

AMOUNTS OF HCN RECOVERED FROM TURGID AND NONTURGID GREEN VALENCIA-ORANGE FRUITS FUMIGATED IN PAIRS IN THE FUMATORIUM*

Paired samples†	Preliminary storage conditions for fruits				Total weight of fruits		Total surface area of fruits		Mean concentration of HCN in fumatorium per liter of space	HCN recovered from fruit per 1,000 cm² of surface	
	Relative humidity		Temperature								
	Turgid	Nonturgid	Turgid	Nonturgid	Turgid	Nonturgid	Turgid	Nonturgid		Turgid	Nonturgid
nos.	*per cent*	*per cent*	*° F*	*° F*	*grams*	*grams*	*cm²*	*cm²*	*mg*	*mg*	*mg*
743 and 744	90	54	75	75	1,778	1,568	2,421	2,241	1.4	3.8	3.7
745 and 746	84	54	74	74	1,742	1,511	2,376	2,215	1.4	4.3	4.9
747 and 748	88	50	75	74	1,624	1,501	2,279	2,175	1.3	5.2	5.6
749 and 750	89	49	73	70	1,632	1,431	2,188	2,075	1.3	6.2	6.6
751 and 752	90	49	71	71	1,555	1,424	2,251	2,083	1.4	8.1	7.1

* Tests were made August 21 to September 6, 1940.

† Samples 743 to 748 consisted of 35 fruits each; samples 749 to 752 consisted of 30 fruits each. Fruits of 1 sample of each pair were made turgid by placing with stems in water in a humid chamber for 15 to 20 hours; fruits of the other sample of each pair were made nonturgid by storing in open paper bags for the same period of time at the temperatures and relative humidities indicated. Paired samples were fumigated in the fumatorium simultaneously.

HCN Recovery from Mature Fruits Having Wet Surfaces.—It is well known that HCN has a great affinity for water, and that the two are mutually miscible in all proportions. Since this is true, it was decided to determine the comparative amounts of HCN that could be recovered from fumigated fruits having wet and dry surfaces.

TABLE 8

INJURY TO TURGID AND NONTURGID GREEN VALENCIA-ORANGE FRUITS, CAUSED BY FUMIGATION WITH HCN*

Fruit samples	Fruits uninjured	Fruits injured			
		Slightly	Moderately	Badly	Total
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Turgid	15.8	23.7	0.0	60.5	84.2
Nonturgid	71.4	16.7	4.8	7.1	28.6

* The values given are the mean percentages for 5 samples each of turgid and nonturgid fruits.

TABLE 9

COMPARATIVE AMOUNTS OF HCN RECOVERED FROM WATER-SPRAYED AND UNSPRAYED MATURE VALENCIA-ORANGE FRUITS FUMIGATED IN THE FUMATORIUM*

Paired samples†	Weight of fruits		Total surface area of fruits		HCN recovered from fruits per 1,000 cm^2	
	Water-sprayed	Unsprayed	Water-sprayed	Unsprayed	Water-sprayed	Unsprayed
nos.	*grams*	*grams*	cm^2	cm^2	*mg*	*mg*
625 and 626	1,663	1,653	1,681	1,678	3.9	5.3
627 and 628	1,682	1,693	1,710	1,720	3.8	4.9
629 and 630	1,673	1,692	1,727	1,733	2.9	4.7
631 and 632	1,683	1,669	1,700	1,714	2.1	3.8
633 and 634	1,670	1,753	1,728	1,737	2.1	3.1
641 and 642	1,839	1,835	1,816	1,792	1.9	3.4

* Tests were made between May 27 and June 18, 1940.
† Each sample consisted of 15 fruits selected at random from 6 to 8 trees. As soon as the fruits of each pair of samples had been picked, weighed, and measured, those of 1 sample were sprayed with water; the others were left unsprayed. Both samples were fumigated at the same time.

Paired samples of mature Valencia-orange fruits were picked and brought to the laboratory for weighing and for determining of total surface areas. Fruits of 1 sample were then sprayed with water; those of the other sample were left unsprayed. A small amount of a wetting agent was added to the spray water, so that the surface of the sprayed fruits would be covered with a film of water. The fumigation was done immediately after the spraying was completed, and the sprayed and unsprayed samples were both fumigated at the same time. When the samples were taken from the fumatorium, the sprayed fruits were still moist. These fruits were washed with distilled water before they were cut, and this water was then added to that in the distillation flask.

The results of this experiment (table 9) show that less HCN was recovered from the water-sprayed fruits than from the unsprayed fruits. The presence of the film of water on the surface of the water-sprayed fruits appears to be the most plausible explanation for these results. The thickness of the water film was, of course, not known; but the thicker the film, the greater should be the amount of HCN absorbed by it, and the smaller the amount passing into the fruit during the 40-minute fumigation period. The results indicate that because of the water-film barrier, a comparatively small amount of HCN entered the sprayed fruits. The thickness of the water film and the partial pressure of gaseous HCN in the fumatorium therefore largely determined the amounts of HCN recovered from the water-sprayed fruits and their water films.

During the warm months of the year, fumigators cease fumigation when visible moisture begins to collect on the leaves and fruit, for fumigation is then likely to result in injury to the tissues. The results of this experiment substantiate previous evidence to the effect that injury under such conditions is caused, not by the presence of the moisture on the fruits and foliage, but by unusual gas pressure due to the decrease in permeability of the tent wall (*9*). It seems probable that the condensed moisture on the leaves and fruits would not be sufficient to retard materially the entrance of HCN into the tissues, especially in the presence of unusually high gas pressure. Injury under these conditions would probably be comparable to the injury resulting to the fruits that were made very turgid by placing them with their stems in water in a humid chamber overnight before fumigation (table 7).

More fundamental work should be done on this problem before definite conclusions are drawn, however. For example, the partial pressure of HCN under a damp fumigation tent at the end of a 45-minute fumigation period may not be relatively high, because the excessive moisture on the tree may absorb the HCN as rapidly as it would have passed out through the walls of a comparatively dry tent (*9*). On the other hand, it seems probable that the initial pressure of gaseous HCN in a moist tent would be unusually high for at least the first few minutes. A condition of this kind, even if of short duration, might mean the difference between injury and noninjury to the tree.

THE RECOVERY OF HCN FROM FUMIGATED GREEN FRUITS FROM COASTAL AND INLAND AREAS

When fumigated with HCN, citrus trees are more subject to injury during the fall months (September, October, and November) than at any other time of the year. Practical experience in southern California has shown, also, that when fumigated during this susceptible period,

under comparable conditions, citrus trees in the coastal areas are usually more easily injured than those in the inland areas.

This situation led to the formulation of plans for determining the comparative amounts of HCN absorbed by green Valencia-orange fruits from coastal and from inland areas when fumigated in the laboratory under controlled conditions. Furthermore, it was planned to correlate the amount of HCN absorbed, with the severity of injury to the tissues.

Samples were taken daily for four periods of 4 days each between December 5, 1939, and January 27, 1940. On each day 1 sample was picked in the coastal area and 1 in the inland area and brought to the laboratory for measuring and weighing. The fruits were preconditioned at 65° F for 15 to 20 hours before fumigation. Paired samples of fruits from each area were fumigated at the same time, and HCN recoveries were made in the usual manner. Samples collected in December, 1939, contained 23 fruits each, of which 17 were used for HCN determinations; but in January, 1940, the fruits being larger, each sample contained only 20 fruits, of which 14 were used for HCN determinations. The 6 extra fruits in each sample were stored at 70° after fumigation and were later observed for injury.

The fruits from the coastal area were picked from one grove located at Santa Ana and from three other groves within 3 miles of, but in different directions from, Santa Ana. All fruit samples from the inland area came from two plots at the University of California Citrus Experiment Station.

These experiments were repeated and refined during the fall and winter of 1940–41. The three groves selected in the coastal area were located near those of the previous year; the three groves in the inland area were within 3 to 8 miles of Riverside. In each grove, plots containing 26 trees were selected, and all samples were taken from these plots at 2- to 3-week intervals, from October 8, 1940, to February 11, 1941. Methods of sampling, preconditioning, and fumigating were the same as those of the previous year, except that additional fruits were picked at each sampling period for maturity determinations (expressed by the percentage of soluble solids and acids in the juice and by the ratios of these two). The purpose of the maturity determinations was to investigate any possible relation between the maturity of the fruits and the amounts of HCN they would absorb. Each sample for HCN determination contained 15 fruits, and all fruits in a given pair of samples (inland and coastal) were similar in size.

The results of the 1939–40 determinations are shown in figure 3. Each value is the average of 2 samples and represents milligrams of HCN recovered per unit of fruit surface. In general, the coastal fruits ab-

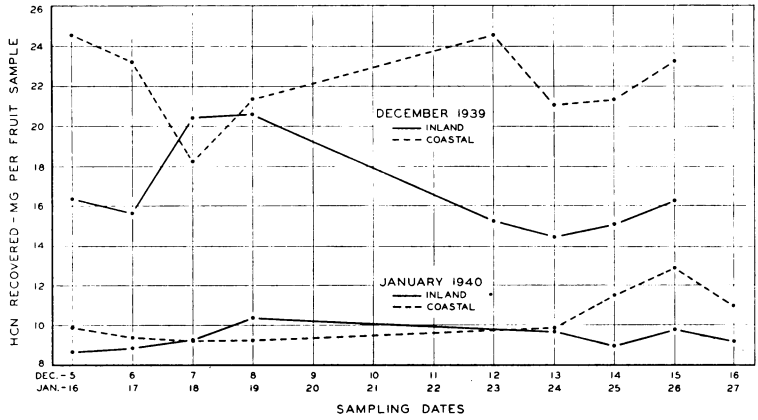

Fig. 3.—Amounts of HCN recovered from Valencia-orange fruits brought from groves in the inland and coastal areas and fumigated in the laboratory fumatorium in 1939–40. Each point on the curves represents the average of the amounts recovered from 2 samples of 15 fruits each. Compare these curves with those in figures 4 and 5, which show the results of a similar experiment in 1940–41.

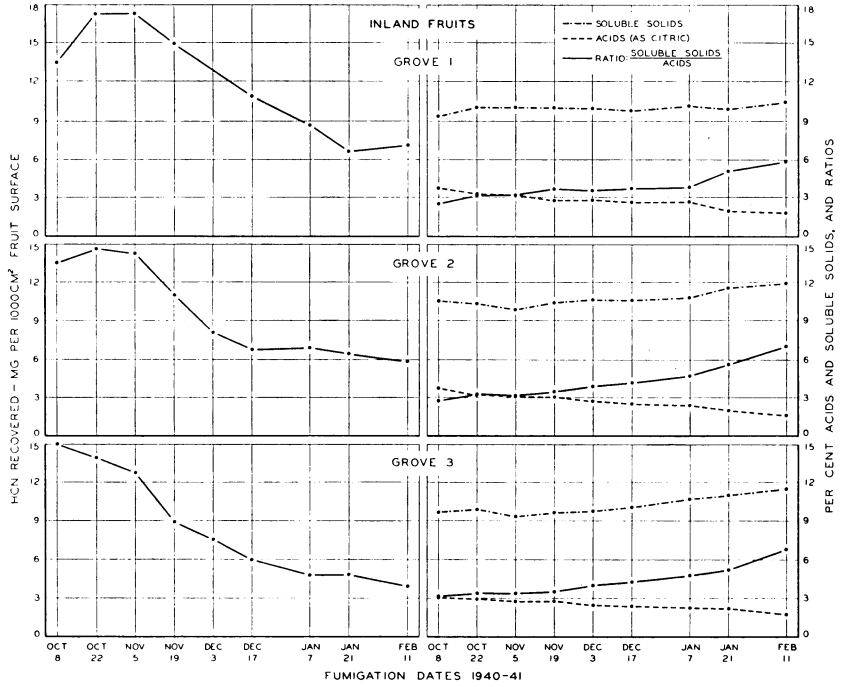

Fig. 4.—The amounts of HCN recovered from Valencia-orange fruits brought from groves in the inland area and fumigated in the laboratory fumatorium in 1940–41. Note the decreasing amounts of HCN recovered as the fruits became more mature. The increasing maturity of the fruits is shown by the curves for acids, soluble solids, and ratios. Compare these values for fruit samples from the inland area with those for the samples from the coastal area, shown in figure 5. Each point on the curves represents the average of the amounts recovered from 2 samples.

sorbed more HCN than the inland fruits. On only two dates, one in December and one in January, was there plainly less absorption of HCN by coastal fruits than by inland fruits; but there were two dates in January on which the absorptions for the fruits from the two areas were practically the same. The crossing of the curves and the individual differences shown in amounts of absorption within a given month are probably due to the condition of the fruits from the different groves at

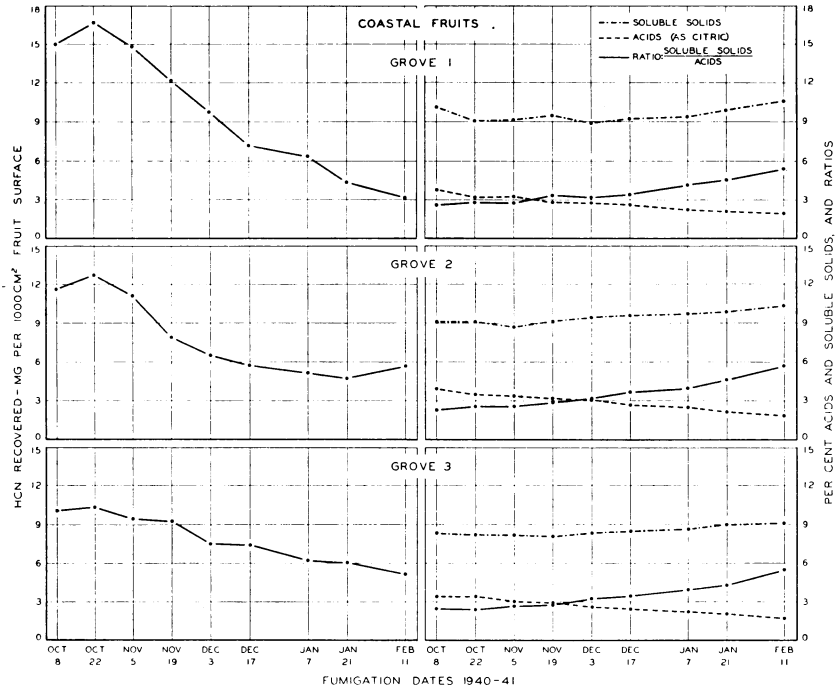

Fig. 5.—The amounts of HCN recovered from Valencia-orange fruits brought from groves in the coastal area and fumigated in the laboratory fumatorium in 1940–41. Note the decreasing amounts of HCN recovered as the fruits became more mature. The increasing maturity of the fruits is shown by the curves for acids, soluble solids, and ratios. Compare these values for fruit samples from the coastal area with those for the samples from the inland area, shown in figure 4. Each point on the curves represents the average of the amounts recovered from 2 samples.

the time they were fumigated. The absorption of less HCN by January fruits than by December fruits verifies the general observation that the more mature the fruits, the less HCN they will absorb.

The results of the 1940–41 determinations for inland and for coastal fruits are shown in figures 4 and 5, respectively. These figures show recoveries of HCN and determinations of fruit maturity for each of the three groves in the two areas separately. After the second determination

(October 22), as the fruits became more mature, there was a gradual diminution in the amounts of HCN absorbed. There was, on the other hand, a gradual upward trend in percentages of soluble solids and in ratios of soluble solids to acids, with a decrease in titratable acidity. The inland samples, as a whole, absorbed an average of 15 per cent more HCN than the coastal samples (just the reverse of what occurred in 1939–40). In only 6 of the 54 paired determinations was more HCN absorbed by the coastal than by the inland samples. The explanation for the reversal of results is not known.

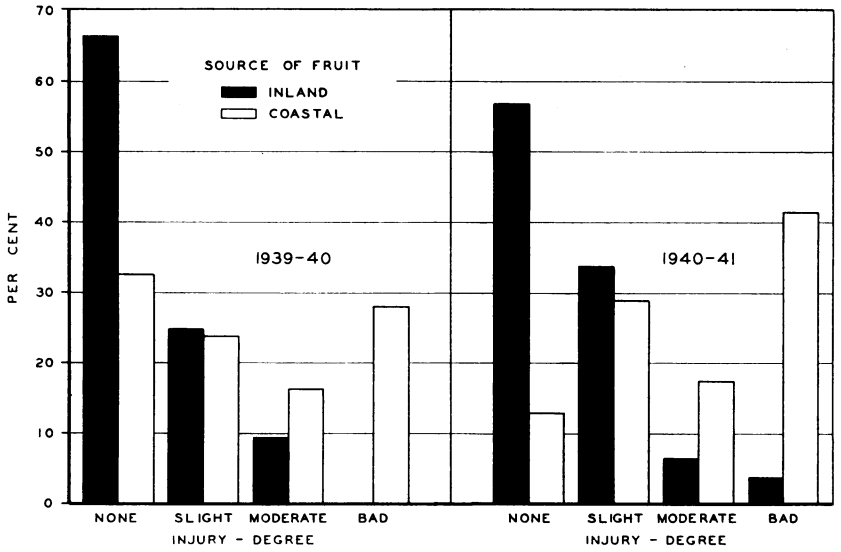

Fig. 6.—Comparative percentages of uninjured and HCN-injured fruits from inland and coastal areas for the seasons of 1939–40 and 1940–41 (see figs. 3, 4, and 5). None of the inland fruits were badly injured in 1939–40.

Since all determinations to date have shown that the more mature the fruit in any particular grove becomes, the less HCN it will absorb, the results of the 1939–40 determinations were tentatively explained on the basis that the inland fruits were more mature than those from the coastal area and therefore absorbed less HCN. Such an explanation will not hold for the 1940–41 results, however. Figures 4 and 5 show that at the time determinations were made, inland fruits were, again, more mature than coastal fruits (as shown by ratios of soluble solids to acids) but absorbed more rather than less HCN. Perhaps such reversals of results should be expected, however, with materials that are subject to a wide variety of biological and climatic factors.

Despite the fact that the inland fruits absorbed less HCN than the coastal fruits in 1939–40 and more HCN than the coastal fruits in

1940–41, the comparative amounts of HCN injury on the fumigated inland and coastal fruits, as shown in figure 6, were consistent. Injury, in both years, was much more evident on the coastal than on the inland fruits: For example, in 1939–40, 68 per cent of the coastal fruits but only 34 per cent of the inland fruits were injured; in 1940–41 the figures were 87 and 43 per cent, respectively. The results of 1940–41 are of special interest because the inland fruits absorbed more HCN that year than the coastal fruits, yet were much less severely injured.

THE FIXATION OF HCN BY FUMIGATED GREEN FRUITS

Experiments were planned to determine at what rate HCN would be liberated from fumigated fruits when a definite volume of air was drawn over them. It was important to know if the fumigated fruits continued to liberate HCN with an increase in the time of aspiration, or if the release of the HCN occurred within a definite time during aspiration.

Four experiments were made between September 19 and October 2, 1940. Eight samples, consisting of 25 green fruits each, were used in each experiment and were fumigated simultaneously. Fruits were sorted to have as nearly as possible the same weight and total surface area. The weight of the samples ranged from 1,349 to 1,528 grams—a difference of 179 grams; and the total surface areas ranged from 1,836.4 to 1,950.7 sq. cm—a difference of 114.3 sq. cm. Since the experiments required 32 samples (800 fruits), the total variation in the weight and surface area of the individual samples was relatively small and sufficiently close for comparative purposes.

In each experiment, before fumigation, all samples were preconditioned overnight at 71° F and 70 per cent relative humidity. After the fumigation period, the HCN absorption was determined immediately on 2 of the 8 samples, to serve as checks. Each of the other 6 samples was placed in a Pyrex-glass desiccator (6-liter capacity). All 6 desiccators were connected to individual absorption bottles containing 100 ml of *N* NaOH solution; these, in turn, were connected to a suction pump through a Greiner rotameter gauge for measuring the total volume of air passing through the desiccators and, subsequently, through the alkaline solutions in the bottles. (Preliminary experiments showed that all HCN liberated by the fruit in such tests would be caught by the NaOH solution in the absorption bottles.) Incidentally, the lower end of the inlet tube extended almost to the bottom of each desiccator, so that the air would pass over the fruit on its way to the outlet in the top.

The volume of air flowing over each sample of fruit was 33.3 liters per hour. This means that, after correction for fruit volume, there was

a complete change of air in each 6-liter desiccator approximately every 8 minutes. At this rate of change of air, any HCN liberated by the fruit should have been carried over and absorbed by the NaOH in the absorption bottles.

At the end of chosen periods of aspiration (table 10), 2 desiccators and their absorption bottles were disconnected, and the rate of aspiration for the remaining samples was then readjusted so that it would be unchanged from the original rate. HCN determinations (in milligrams per unit of fruit surface) were at once made on the 2 fruit samples. The

TABLE 10

AMOUNTS OF HCN RELEASED BY CONTROL AND ASPIRATED SAMPLES OF FUMIGATED GREEN VALENCIA-ORANGE FRUITS*

Experiment no.	Amounts of HCN released					
	Controls	Samples aspirated for:†				
		4 hours	24 hours	28 hours	33 hours	48 hours
	mg	*mg*	*mg*	*mg*	*mg*	*mg*
1	13.5	2.6	2.7	...	...	1.4
2	8.6	1.7	1.8	...	2.1	...
3	12.2	1.8	1.8	1.8	...	...
4	12.1	2.6	2.6	...	...	3.3

* Experiments were performed between September 19 and October 2, 1940.

† Each value for a given experiment represents the average amount of HCN released by 2 control samples or by 2 aspirated samples, and is based on the amount of HCN released per 1,000 cm^2 of fruit surface. No sample was aspirated more than once. For the average amounts of HCN remaining in the aspirated samples at the end of the aspiration periods, plus the amounts that these samples released while being aspirated, see figure 7.

NaOH solution in each disconnected absorption bottle was diluted to 1 liter in a volumetric flask. Aliquot portions of 150 ml were taken from these flasks for the titration of HCN, which, in turn, was used to calculate the total HCN in the absorption liquids.

Each value in table 10 represents the average amount of HCN given off by 2 separate samples of fruit. No sample was aspirated more than once. Values in this table show that, under the conditions of these experiments, the amounts of HCN liberated from the fruits and caught in the absorption bottles were comparatively small, and that the HCN was practically all liberated during the first 4 hours of aspiration.

The average total amounts of HCN recovered from the 2 samples of fruit and their absorption liquids in each experiment are shown in figure 7. The curves in this figure indicate that the total amounts of HCN recovered decreased with each increase in the length of time of aspiration, and that the amounts recovered after the first 4 hours were comparatively small. The amounts of HCN in the absorption liquids plus the amounts that remained in the fruits at the end of any given aspira-

tion period were small in comparison with the total amounts of HCN absorbed by the fruits during the 40-minute fumigation period (shown at the zero aspiration time in fig. 7). The results show very plainly that under these conditions a comparatively large proportion of the HCN absorbed had been fixed or so changed that it could not be recovered by the usual methods. The curves in the figure show that as much as 85

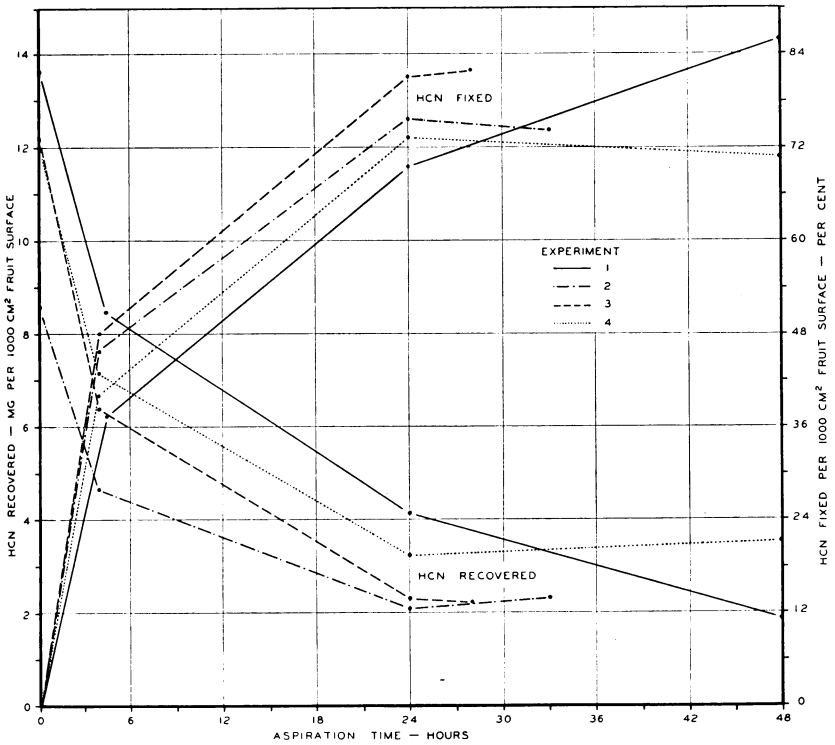

Fig. 7.—Amounts of HCN recovered from samples of green Valencia-orange fruits immediately after fumigation in the laboratory fumatorium and from similar samples and their respective absorption liquids after different intervals of aspiration. The figure also shows the percentages of HCN fixed in fruit samples by the end of each aspiration period. Each point on the curves represents the average of 2 samples.

per cent of the absorbed HCN had been fixed or changed in those samples that were not tested until the end of the 48-hour aspiration period.

The fixation of HCN by green fruits was further investigated by determining the HCN in fruit samples immediately after fumigation (to serve as controls) and by placing other samples, which had been fumigated at the same time, in 5-liter Pyrex flasks. The flasks were sealed with rubber stoppers covered with tin foil. In each stopper there was an inlet tube that extended to the bottom of the flask and an outlet tube

that terminated at the bottom of the stopper; both tubes had glass stopcocks. Each sample consisted of 25 green fruits of approximately the same age and size as those used in the preceding experiments. The samples were sealed in the flasks for 48 hours. After this period the flasks were connected by means of glass tubing to absorption bottles containing 100 ml of *N* NaOH. Air was drawn through the flasks and bottles at the rate of 60 liters per hour for 2 hours. The HCN was then determined in the fruit samples and in the absorption liquids. The contents of each absorption bottle were diluted to 1 liter in a volumetric flask, and 150-ml aliquots were used for HCN determinations. These experiments were performed during the latter part of September, 1940.

TABLE 11

FIXATION OF HCN BY FUMIGATED GREEN VALENCIA-ORANGE FRUITS SEALED IN GLASS CONTAINERS*

Experiment no.	HCN recovered from:†			HCN fixed by sealed fruits
	Control fruits	Absorption liquid	Sealed fruits	
	mg	*mg*	*mg*	*mg*
1	16.5	0.0	0.0	16.5
2	23.4	0.0	3.0	20.4
3	22.6	0.0	2.1	20.5

* Sealed for 48 hours.
† Each value represents the total amount recovered from a single fruit sample or from the corresponding absorption liquid.

The amounts of HCN recovered from the control fruits, the absorption liquids, and from the sealed fruits, and the amounts of HCN fixed by the sealed fruits are shown in table 11. No HCN was found in the absorption liquid in any of the aspiration bottles. Any HCN that had escaped from the fruits into the flasks had been reabsorbed during the 48-hour period. No HCN could be recovered from 1 of the fruit samples that had been sealed in a flask for 48 hours, and only 3.0 and 2.1 mg, respectively, could be recovered from the other 2 sealed samples.

The results of these experiments confirm those of the aspiration experiments, showing that green Valencia-orange fruits readily fix HCN to the extent that it can no longer be recovered as HCN by the steam-distillation method.

THE RECOVERY OF HCN FROM LEAVES AND FRUITS OF FUMIGATED TREES

To state even approximately how much HCN a citrus tree will absorb during a fumigation period is difficult, owing to many factors, including the physiological condition of the tree at the time of fumigation and

those factors already described as affecting the concentration of HCN under the tent (see "HCN Concentrations in Tents," p. 375). Nevertheless, important information has been obtained on this problem by conducting experiments to determine the amounts of HCN that citrus leaves and fruits will absorb during the 45-minute fumigation period and how long they will retain the HCN when the trees are fumigated under different conditions in the field. Such information is of vital importance in studying the causes of HCN injury to the tissues, and no information of this kind has been available up to the time of these experiments.

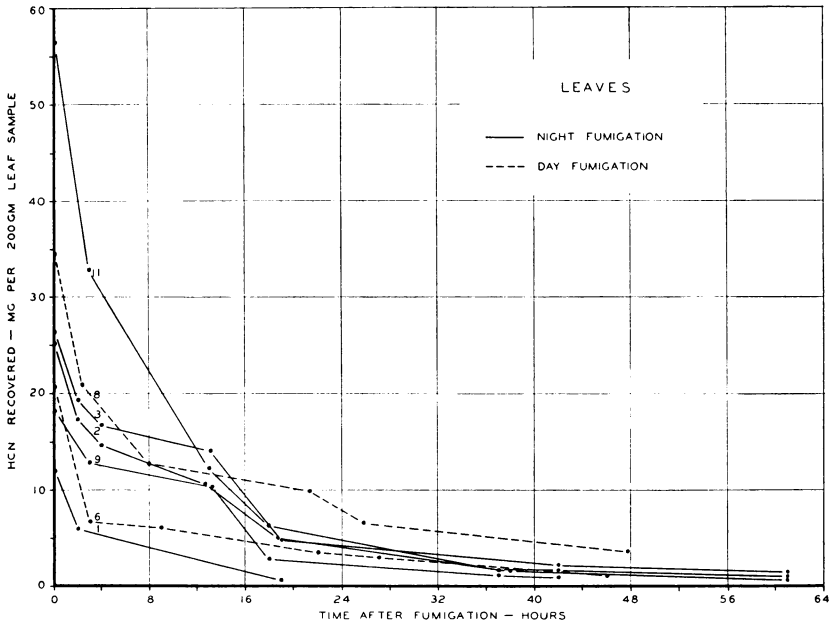

Fig. 8.—Amounts of HCN recovered from 200-gram samples of mature Valencia-orange leaves from trees fumigated at night and from trees fumigated during the day. Note differences in initial recoveries and in lengths of time the leaves retained the HCN. The numerals on the curves designate tree numbers. Each point on the curves represents the average of the amounts recovered from 2 samples.

The experiments on the fumigation of Valencia-orange trees under field conditions were conducted between September 20 and November 14, 1939, and between July 23 and August 15, 1940. Equipment was not available to make recoveries of HCN on samples of leaves and fruits picked from the same tree at the same time. This meant that at a given fumigation, either leaves or fruits had to be chosen for the determination of the HCN.

The first samples of leaves and fruits were taken from the trees just as soon as the fumigation tents had been removed. Other samples were

taken at intervals to determine the length of time the HCN would remain in the tissues. The samplings were continued until the tissues yielded only a few milligrams of HCN or none at all.

Figures 8 and 9 show the relation between the time intervals after fumigation and the amounts of HCN recovered from the leaves and fruits. There was wide variation in the amounts of HCN recovered from the samples of mature leaves picked immediately after the tents were removed. The differences in the slopes of the curves also show that the

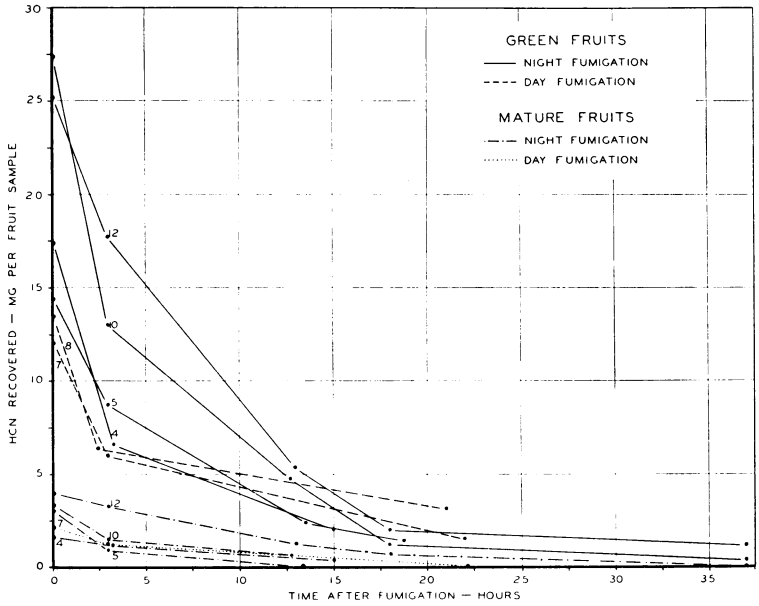

Fig. 9.—Amounts of HCN recovered from samples of green and of mature Valencia-orange fruits from trees fumigated at night and from trees fumigated during the day. Note the differences not only in initial recoveries and in lengths of time the HCN was retained, but also in amounts of HCN recovered from green and from mature fruits. Curve 12 for mature fruits is extended to the 37-hour point, but the fruits contained no HCN at this time and only 0.71 mg at the time of the 18-hour test. The numerals on the curves designate tree numbers.

rates of loss of HCN from the individual trees were vastly different. The results of these experiments and of those of other investigators (*6, 9, 11*) show the effect on HCN absorption of the physical factors which influence the concentration of HCN in the gaseous phase under the tent.

HCN Recovery from Leaves.—It is obvious that any reduction in the amount of HCN under the tent during a fumigation period would be reflected in the amount recovered from the leaves. This is well illustrated by results obtained from trees 1, 2, and 3 (fig. 8): The first 200-gram

samples of leaves picked from tree 1 immediately after fumigation gave an average yield of 12.2 mg HCN, while those picked 19 hours later yielded only 0.6 mg. In contrast, the leaf samples from trees 2 and 3 gave initial yields of 25.4 and 26.6 mg HCN, respectively, and similar samples picked from the same trees 61 hours later yielded 0.7 and 1.6 mg, respectively. The leaf samples from tree 1 did not yield so much HCN initially as those from trees 2 and 3, nor did they retain it so long.

Tree 1 was fumigated at 6:52 p.m., September 20, 1939, at a temperature of 80° F and a relative humidity of 50 per cent. The mean concentration of HCN under the tent during the fumigation period was 0.9 mg per liter of space (table 1, p. 377). The air was very still during the fumigation period, but a strong, dry north wind arose 20 minutes after the removal of the tent from the tree. The low relative humidity permitted excessive gas leakage through the tent wall during the fumigation period, and the rapid air movement soon after the tent was removed quickly dissipated the HCN liberated from the tissues after the removal of the tent. This type of environmental condition during the fumigation of citrus trees under field conditions is conducive to low absorption of HCN, and, conversely, to low recoveries from the fumigated leaves.

Tree 2 was fumigated at 6:50 p.m., September 27, and tree 3 at 6:35 p.m., October 4, 1939. The temperatures at the time of fumigation of trees 2 and 3 were 65° and 64° F, and the relative humidities were 80 and 87 per cent, respectively. The mean concentrations of HCN under the tents during the fumigation periods for trees 2 and 3 were, respectively, 1.4 and 1.6 mg per liter of space under the tent (table 1).

Mention should be made also of the fact that by the time the third samples of leaves were collected from tree 2 (4 hours after fumigation), and the second samples from tree 3 (2 hours after fumigation), the leaves had become damp with condensed moisture. This condition no doubt influenced the length of time that these leaves retained the HCN.

Trees 9 and 11 were fumigated at the same hour in the evening as trees 2 and 3 and at approximately the same temperatures and relative humidities, on July 31 and August 12, 1940, respectively. The mean concentrations of HCN in the tents over trees 9 and 11 were both 0.9 mg per liter of space (table 1). In spite of the fact that the mean concentrations of HCN in the tents over these trees was the same, the leaf samples from tree 11 yielded much more HCN and retained it much longer than those from tree 9 (see fig. 8).

Sufficient data on which to base an explanation for these results are not available. Tree 9 was fumigated just before an irrigation, and tree 11 soon after an irrigation. The leaves on tree 9 did not appear to wilt during the day, but the leaves on an adjoining grapefruit tree of about

the same size and in the same row wilted visibly during the warm part of the day. Tree 11 was fumigated after the irrigation water had had time to penetrate but while the soil was still wet on the surface. The leaves on tree 11 had become damp by the time the second samples were taken at 10:30 p.m. Whether these conditions were responsible for the fact that the leaves from tree 9 absorbed much less HCN than those from tree 11 cannot be stated. In this connection, however, it may be pointed out that the moisture content of fresh and partially wilted leaves and of turgid and nonturgid fruits fumigated in the laboratory did not appear to govern the amount of HCN that they absorbed (see tables 6 and 7); and that during the warm months of the year, a grove or a portion of a grove fumigated while the soil is wet is much more likely to be injured than one fumigated while the soil is comparatively dry.

The information on the fumigation of these trees is given in detail to show that although they were all fumigated with the same schedule of HCN (20 ml per unit), the mean concentration of HCN per liter under the tent was not the same during each fumigation period. These differences, together with the comparative differences in physiological, climatic, and other factors operating at the time of fumigation, were sufficient to cause variation in the total absorption and length of retention of HCN by the tissues.

Another interesting relation is observed when recoveries of HCN from leaves of trees fumigated during the day are compared with those from leaves of trees fumigated at night. Trees 6 and 8 (fig. 8) were fumigated at 9:45 a.m., July 23, and at 10:30 a.m., July 29, 1940, at temperatures of 97° and 75° F and at relative humidities of 31 and 58 per cent, respectively. The mean concentrations of HCN in the tents over trees 6 and 8 during the fumigation period were 1.4 and 1.8 mg per liter of space, respectively (table 1). As shown in figure 8, the leaves from tree 6 absorbed and retained less HCN than those from tree 8. The amounts of HCN yielded by the leaves from the trees fumigated during the day were, in general, comparable to those from trees fumigated at night.

The comparative results of the night and day fumigations were of special interest because the trees fumigated at night suffered very little or no injury, while those fumigated during the day were severely injured, especially on those portions of the trees that received direct sunlight. The direct sunlight probably made the cutinized surfaces of the leaves more permeable to HCN, although this effect was not registered in the HCN yields of these leaves, as compared with the yields of those fumigated at night. It is possible that the leaves fumigated during the day absorbed more HCN than those fumigated at night, but that photochemical action, either on the HCN or on the tissues, caused them to fix

excessive amounts of the absorbed HCN, which could not be recovered. However this may be, it was very evident that photochemical or other changes had made the tissues very susceptible to HCN injury.

The results of this experiment are of interest because they at least indicate that the stomata are not an important factor in governing the rate of entrance of HCN into citrus leaves. These results with citrus do not confirm the findings of Clayton (*3*), who worked with tomatoes and *Tradescantia zebrina* (*Zebrina pendula*) and concluded that the amount of HCN absorbed by the leaves depended upon the width of the stomatal openings. They do, however, substantiate the results of Stone (*12*) and Moore (*5*), who concluded that other attributes of the tissues were more important than the stomata in regulating the absorption of HCN. The excessive injury to the leaves fumigated during the day may have been due to the much greater physiological activity of the tissues during the day than at night, although this explanation does not appear to harmonize with the fact that rooted lemon cuttings were more severely injured at low than at high temperatures (*11*).

HCN Recovery from Fruits.—Other trees were fumigated for the purpose of studying the absorption and retention of HCN by fruits. Trees 4 and 5 were fumigated at night on October 18 and November 11, 1939; trees 7 and 8, during the day of July 26 and 29, 1940; and trees 10 and 12, at night on August 5 and 15, 1940, respectively. Paired samples of green and mature fruits were tested from all the trees, except tree 8, from which only green fruits were used. The range in concentrations of HCN in the tents during the fumigation periods and the range in temperatures and relative humidities were similar to those for the preceding experiment, in which leaves instead of fruits were tested. Green and mature fruits for each pair of samples were selected for uniformity in size and similarity in weight.

Experimental results are illustrated in figure 9, in which the total milligrams of HCN recovered from the fruit samples are plotted against the time intervals after fumigation. Results are expressed in total milligrams of HCN recovered from each sample rather than per unit of surface area, because the method for determining the surface area of the fruits had not been worked out at the time the first tests in this series were made.

The lack of uniformity in the amounts of HCN recovered from the fruits in this experiment (fig. 9) is similar to that for the leaves (fig. 8). Again the effects of environmental and other factors are evident. The main point of interest in this experiment, however, is the comparatively large difference between the amounts of HCN recovered from the samples of green and of mature fruits. An average of 6.3 times as much HCN

was recovered from the green fruits as from the mature fruits. These results compare very favorably with those of the preconditioning experiment (fig. 2), in which 5.4 times as much HCN was recovered from the green fruits as from the mature fruits. With reference to the comparative amounts of HCN absorbed by green and by mature fruits, the same relation held, whether the fruits were fumigated during the day or at night. Traces of HCN could be recovered from some of the leaf samples 61 hours after fumigation, but the maximum period for recovery of HCN from the fruit samples was 37 hours after fumigation.

The results of these fruit experiments again emphasize the importance of the physiological condition of the tissues in governing the amount of HCN that they will absorb and the extent to which they will be injured, if at all.

DISCUSSION

The results previously reported in this field of investigation (*2*) and those reported in this paper are the only ones which give quantitative information on the amounts of HCN absorbed by citrus tissues during the fumigation period and on the length of time that recoverable HCN remains in the tissues after the fumigation period. The discussion of these experimental results is concerned largely with the factors which may have influenced absorption and retention of the HCN. No attempt has been made to bring into the discussion all the results obtained by other workers, which may have a bearing on the data described in this paper. The work of Woglum (*13*) is mentioned here because it probably has a more extensive and direct application than any of the other published data on the effects of HCN on citrus tissues.

Woglum did not make quantitative determinations on the absorption and retention of HCN by the plant tissues, but he did make extensive observations on the presence or absence of injury to citrus trees subjected to different temperatures, moistures, amounts of light, and so forth, before, during, and after fumigation. As a result of his experiments he concluded that "it is necessary to consider the prefumigation and postfumigation environments of fumigated plants as well as that during the actual treatment."

In the present experiments, all paired fruit samples contained equal numbers of fruits, so chosen that they were, as nearly as possible, of the same age and size. The weights of the 2 samples of each pair were therefore approximately the same. This procedure minimized sample differences and placed the HCN recoveries on a reasonably comparable basis. In the major portion of this work, where fruits were concerned, the HCN recoveries were placed on a still more comparable basis by measuring the total surface area of the fruits in each sample and ex-

pressing the amounts of HCN recovered as milligrams per unit of fruit surface. The results obtained by this method show that the recoveries of HCN per 1,000 sq. cm of fruit surface ranged from approximately 5.5 mg for young fruits (about 4.5 cm in diameter) to approximately 1.0 mg for fruits that were fully mature (6 to 8 cm in diameter), a wide and interesting difference.

The HCN recoveries from mature leaves are expressed as total milligrams per 200-gram sample. The total surface areas of the fumigated samples of leaves were not determined, but since these experiments were completed, the surface areas of several 200-gram samples have been measured with a photoelectric area determinator made by the American Instrument Company. These determinations show that the average 200-gram sample of mature leaves has a total surface area (both sides of leaves) of 13,500 sq. cm. This figure is only approximate; the difference between the total surface areas of 2 samples may be as great as 10 per cent. On the basis of a total surface area of 13,500 sq. cm per 200 grams of mature leaves, the average recovery of HCN from the samples of "unsprayed" and "fresh" leaves (tables 5 and 6) was 3.1 mg per 1,000 sq. cm. The average recovery per unit area from samples of mature fruits was much less than this, and that from immature fruits much greater.

The results of earlier studies by other research workers on the fumigation of plants (including citrus) with HCN, before efficient methods for the recovery and determination of minute amounts of HCN were available, indicated that the degree of injury to HCN-fumigated tissues was proportional to the amount of HCN absorbed. There is considerable evidence, however, from the experimental results presented in this paper, that this is not the case when citrus tissues are concerned.

It is true that green fruits in the present studies were found to absorb more HCN than mature fruits; that experimental results already published (*2*) showed that immature leaves absorbed more HCN than mature leaves; and that, in both cases, the tissues that absorbed the most HCN were those most severely injured. It seems probable, however, that these results were not entirely due to the comparative amounts of HCN absorbed but to some other factor or factors. For example, there was practically no difference in the amounts of HCN recovered from day- or night-fumigated leaves, from day- or night-fumigated fruits, or from turgid or nonturgid fruits; and in 1940–41 the coastal fruits absorbed less HCN than the inland fruits; yet the first-mentioned leaves or fruits of all four of these experiments were more severely injured than the others. Other experiments have shown that green fruits from a given grove may absorb less HCN but be more severely injured than similar fruits from a different grove, which absorbed more HCN.

Such results as these strongly indicate that the extent of injury to fumigated citrus tissues is governed principally by such factors as sunlight and by the physiological condition of the tissues rather than by the amount of HCN absorbed. The importance of the physiological condition of the tissues was indicated by the earlier work of Woglum (*13*).

In the course of these studies it has been of special interest to find that as soon as the color of the fruit changes from green to yellow or orange, there is usually a noticeable decrease in the amount of HCN absorbed during fumigation either in the laboratory or in the field (figs. 2 and 9). The amount of HCN absorbed is apparently not entirely controlled by the presence of chlorophyll or by the conditions which accompany photosynthetic activity, however, because some green fruits will absorb more than others of a similar age and size from a different grove or even from the same grove. The physiological conditions which influence the absorption of HCN by citrus tissues remain to be determined by future studies.

The curves in figures 8 and 9 show that, after fumigation at night under field conditions, recoverable HCN may remain in mature fruits for 20 to 25 hours, in green fruits for 35 to 40 hours, and in mature leaves for at least 60 hours. Without further data, it is difficult to suggest an explanation for these differences. Adsorption, tissue composition and structure, climatic conditions, and the fixation of HCN by the tissues are all important factors in governing the length of time that recoverable HCN will remain in the tissues.

The experiments on the fixation of HCN (see "The Fixation of HCN by Fumigated Green Fruits," p. 395) showed that fumigated green fruits sealed in flasks had fixed almost all of the sorbed HCN by the end of 48 hours. Possibly the mature leaves, which retained recoverable HCN for the greatest length of time, were less active physiologically than the green fruits and thus fixed less HCN. On this basis, however, the mature fruits, which were presumably less active physiologically than the green fruits, should have retained their HCN longer than the green fruits; this they did not do.

In earlier studies (*2*), it was shown that during the fumigation period, gaseous HCN penetrated not only to the inner surface of the peel but also into the pulp of the fruit. Although the depth of penetration into the pulp was not determined, it was several times the thickness of a mature leaf. Because of the comparative thinness of the leaves, it would seem that they should have lost their HCN sooner than the fruits, which was not the case. The tissues of the leaf are more compact than those of the fruit peel, and those of the green fruit are more compact than those of the mature fruit. Therefore, the most plausible explanation for the

difference in lengths of time that the HCN remained in the leaves and in green and mature fruits appears to be that the more compact the tissues, the longer they will retain HCN.

SUMMARY

Some of the factors influencing the absorption and retention of HCN by citrus tissues have been determined by conducting fumigation experiments in a gastight metal fumatorium in the laboratory and in regulation canvas tents in the field.

The concentrations of HCN remained nearly constant in the fumatorium but, as might be expected, varied greatly in the tents during the fumigation periods (fig. 1 and table 1).

Considerably more HCN was absorbed by fruits preconditioned overnight at 43° F before fumigation than by those preconditioned at 80°, and green fruits absorbed an average of 5.4 times as much HCN as mature fruits (fig. 2).

Under laboratory conditions the absorption of HCN by fruits was retarded by the application of oil spray, but both fruits and leaves sprayed under field conditions absorbed as much HCN as unsprayed fruits and leaves (tables 3, 4, and 5). In the laboratory none of the fruits were injured by the HCN; in the field none of the unsprayed, but about 6 per cent of the oil-sprayed fruits were injured.

Less HCN was absorbed by leaves and fruits on trees that had not been recently irrigated than by those on trees that had been recently irrigated (fig. 8, curves 9 and 11), but there was no appreciable difference in the amounts of HCN absorbed by turgid and nonturgid leaves and fruits fumigated in the laboratory (tables 6 and 7). The turgid fruits were more severely injured than the nonturgid fruits. Fruits sprayed with water and a spreader and fumigated at once, absorbed less HCN than similar fruits whose surfaces were dry (table 8).

In 1939–40, green fruits from inland areas absorbed less HCN than green fruits from coastal areas (fig. 3); but in the similar experiment in 1940–41, the inland fruits absorbed more HCN than the coastal fruits (figs. 4 and 5). The coastal fruits were much more severely injured than the inland fruits both years (fig. 6).

Green fruits fixed or chemically changed absorbed HCN so that it could not be recovered and determined by the usual methods (fig. 7).

Leaves and fruits of trees fumigated during the day absorbed approximately the same amounts of HCN as those fumigated at night, but were much more severely injured. In these experiments recoverable HCN was retained by mature leaves for at least 60 hours, by green fruits 35 to 40 hours, and by mature fruits 20 to 25 hours (figs. 8 and 9). An

average of 6.3 times as much HCN was recovered from the green fruits as from the mature fruits.

The stomata are apparently not important in governing the rate of entrance of HCN into citrus leaves and fruits.

The physiological condition of the tissues rather than environmental influences or the amount of HCN absorbed seems to determine whether they will or will not be injured by HCN after fumigation at night; injury after day fumigation appears to result from the effects of sunlight, which raises the temperature and influences the physiological condition of the tissues.

The results of laboratory fumigations may, but do not always, indicate the results that will be obtained when the fumigations are made under field conditions.

LITERATURE CITED

1. BARTHOLOMEW, E. T., and E. C. RABY.
1935. Photronic photoelectric turbidimeter for determining hydrocyanic acid in solutions. Indus. and Engin. Chem., analyt. ed. **7**:68–69.
2. BARTHOLOMEW, E. T., WALTON B. SINCLAIR, and BYRON E. JANES.
1939. Factors affecting the recovery of hydrocyanic acid from fumigated citrus tissues. Hilgardia **12**(7):473–95.
3. CLAYTON, E. E.
1919. Hydrogen cyanide fumigation. Bot. Gaz. **67**:483–500.
4. KNIGHT, HUGH.
1925. Factors affecting efficiency in fumigation with hydrocyanic acid. Hilgardia **1**(3):35–56.
5. MOORE, WILLIAM.
1916. Studies in greenhouse fumigation with hydrocyanic acid: Temperature and moisture factors influencing the injury of plants during fumigation. Minnesota State Ent. Rept. **16**:93–108.
6. PRATT, F. S.
1930. Fumigation gas concentrations under tents. California Citrog. **15**:440, 455–57.
7. QUAYLE, H. J.
1922. Resistance of certain scale insects in certain localities to hydrocyanic acid fumigation. Jour. Econ. Ent. **15**:400–4.
8. QUAYLE, H. J.
1927. Spray and fumigation combination for resistant red scale. Jour. Econ. Ent. **20**:667–73.
9. QUAYLE, H. J.
1938. Insects of citrus and other subtropical fruits. 583 p. (See specifically p. 478 and 488.) Comstock Publishing Company, Inc., Ithaca, New York.
10. QUAYLE, H. J., and WALTER EBELING.
1934. Spray-fumigation treatment for resistant red scale on lemons. California Agr. Exp. Sta. Bul. **583**:1–22.
11. QUAYLE, H. J., and P. W. ROHRBAUGH.
1934. Temperature and humidity in relation to HCN fumigation for the red scale. Jour. Econ. Ent. **27**:1083–95.
12. STONE, G. E.
1913. The influence of various light intensities and soil moisture on the growth of cucumbers, and their susceptibility to burning from hydrocyanic acid gas. Massachusetts Agr. Exp. Sta. Ann. Rept. **25**(Part I):61–72.
13. WOGLUM, R. S.
1920. Fumigation of citrus plants with hydrocyanic acid: conditions influencing injury. U. S. Dept. Agr. Bul. **907**:1–43.

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