CONTENTS

THE PICRIC ACID METHOD FOR DETERMINING MINUTE AMOUNTS OF HYDROCYANIC ACID IN FUMIGATED INSECTS
WALTON B. SINCLAIR and R. C. RAMSEY

RELATION OF MORTALITY TO AMOUNTS OF HYDROCYANIC ACID RECOVERED FROM FUMIGATED RESISTANT AND NONRESISTANT CITRUS SCALE INSECTS
D. L. LINDGREN and WALTON B. SINCLAIR
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Resistance to fumigation with hydrocyanic acid gas (HCN) in the red scale, *Aonidiella aurantii* (Mask.), was first noted in 1914; and resistance in black scale, *Saissetia oleae* (Bern.), in 1915 (Quayle, 1938). Sufficient experimental data have accumulated to establish definitely the existence of two strains or races of red scale in the citrus areas of California: one race that is susceptible and another that is tolerant to HCN fumigation. These two races have been reared in the laboratory for the past seven years, under identical conditions in separate insectproof rooms, through an average of nine generations per year, or a total of some sixty generations; and they are still maintaining the original differential in susceptibility to HCN. As Dickson (1941) demonstrated, this difference in susceptibility depends on a single gene or group of closely linked genes in the X chromosome and therefore is sex-linked. The black scale has been less studied, because of the difficulties involved in rearing it from generation to generation; but, according to recent work by Lindgren and Dickson (1943), the resistant and the nonresistant black scale differ as much in their tolerance to HCN as do the two races of red scale, or even more.

In an effort to determine the basis for the difference in the reaction of the resistant and nonresistant races of scale insects to HCN fumigation, two lines of investigation were followed in the present experiments. In the first, the fumigation experiments, an attempt was made to evaluate separately the influence of HCN concentration and of exposure on the mortality of the two races of red scale. In the second, the sorption experiments, an attempt was made to correlate the mortality of fumigated resistant and nonresistant races of both red and black scales with the amounts of HCN sorbed (as measured by the amounts recovered); the effects of varied dosages, exposures, and pretreatments on sorption and mortality were studied.

MATERIALS AND METHODS

In the experiments here reported, studies were made of resistant and nonresistant mature adult female red scale, reared under controlled laboratory conditions, in insectproof rooms. The variation in age of the insects was less than 24 hours, since the scale crawlers were transferred to grapefruits from the stock cultures several times daily.

In the fumigation experiments, two series were conducted: in one the con-

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4 Associate Biochemist in the Experiment Station.
5 See “Literature Cited” at the end of the paper for complete data on citations, which are referred to in the text by author and date of publication.
centration of HCN was held constant and the time of exposure varied, whereas in
the other the exposure time was held constant and the HCN concentration
was varied. The fumigations were conducted in a 100-cubic-foot fumatorium
at 75° F. Counts to determine mortality were made 2 weeks after treatment.

Since a method was available (Sinclair and Ramsey, 1944) for recovering
small amounts of HCN accurately from fumigated insects, a series of experi­
ments was set up to determine how much cyanide could be recovered from
resistant and nonresistant red scale. Each insect was carefully removed from
the fruit, with little or no injury; according to earlier observations, the adult
red scale will survive and produce young for at least 36 hours after being
taken from the host. The sample used in the sorption experiments consisted
of 500 to 700 individuals. The insects were counted and weighed before
fumigation. The average weight of a nonresistant specimen was 0.212 mg:
of a resistant one, 0.214 mg. Two persons removed the insects from the fruit
(this work took 30 to 45 minutes) ; and, to eliminate any personal factor, the
two alternated in handling the resistant and nonresistant races. After being
weighed, the insects were placed in the bottom of petri dishes; and the samples,
one nonresistant and the other resistant, were fumigated in a 100-cubic-foot
fumatorium at 75° F. The relative humidity varied from 45 to 60 per cent
in the several experiments, no effect on sorption being noted within this range
of humidities. Immediately after fumigation, the insects were placed in the
distillation flasks, and the distillation process was begun. The two races of red
scale always received identical treatments before fumigation, after fumiga­
tion, and throughout distillation.

FUMIGATION EXPERIMENTS

Some light might be thrown on the basis for the difference in HCN suscepti­
bility of different races of scale insects, and hence on advisable commercial
fumigation practice in various districts, if the effects of concentration and of
exposure could be distinguished. Most experiments on resistant and non­
resistant scale insects have been concerned with concentrations and exposures
simulating those used in the field, little being done with high dosages and
short exposures or low dosages and long exposures. In one of the few experi­
ments designed to evaluate the effects of concentration and exposures sepa­
rately, Moore (1936) found: “The concentration to which the resistant red
scale in California is exposed is of double the value of the length of exposure
in effecting a kill. . . . The concentration to which nonresistant red scale in
California is exposed is of approximately equal value to the length of ex­
posure in effecting a kill.”

In comparing mortality curves of the two races of red scale, only the part
that covers the mortality range between 15 per cent and 98 per cent is of
interest. At either extreme of the typical mortality curve, the line flattens out:
with high dosages and long exposures, a 99 to 100 per cent kill of both races
will be obtained; with low dosages and short exposures there is no or very low
mortality of both; and if either of these two factors has a zero value, the
mortality will obviously be zero. But comparisons at the extremes would not
justify the conclusion that the two races do not differ in susceptibility to
HCN. Hence in the experiments reported in this paper, the values of concen­
tration and exposure chosen were such as could be expected to yield kills in the significant portions of the mortality curves.

In the first series of fumigations, the exposure was held constant at the very low value of 1 minute, and the concentration was varied from 4.0 mg to 9.6 mg per liter, values many times those used in commercial practice. The results were as follows:

<table>
<thead>
<tr>
<th>Milligrams of HCN per liter</th>
<th>Per cent mortality of nonresistant race</th>
<th>Per cent mortality of resistant race</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0</td>
<td>52</td>
<td>26</td>
</tr>
<tr>
<td>5.6</td>
<td>48</td>
<td>37</td>
</tr>
<tr>
<td>8.0</td>
<td>47</td>
<td>36</td>
</tr>
<tr>
<td>9.6</td>
<td>45</td>
<td>22</td>
</tr>
</tbody>
</table>

Although the two races showed differences in susceptibility to HCN, and all percentages were in the "significant" range, there was no increase in mortality of either race, even when the concentration was more than doubled. Judging from this result, with very short exposures only a limited amount of HCN is taken up by the insect.

In the second series, the exposure was increased to 10 minutes, a value high enough to reveal the effects of different concentrations. For the nonresistant race, concentration varied from 0.19 mg to 1.60 mg per liter, for the resistant from 0.4 mg to 3.9 mg, both ranges extending below and above that used in commercial practice (about 1.0 to 1.3 mg per liter).

In the third series, concentration was held constant at 0.4 mg per liter, and the exposure was varied from 2½ minutes to 25 minutes for the nonresistant scale and from 10 minutes to 90 minutes for the resistant.

The results for the second and third series are presented graphically in figures 1 and 2. The concentration-mortality and exposure-mortality curves in these figures have been converted to straight lines by Bliss's (1935) methods. Net mortality is plotted in probits on the vertical axis. The independent variable—concentration or exposure—is plotted on a logarithmic scale on the horizontal axis. In both figures, the lines are almost parallel, those for the nonresistant scale being slightly steeper than those for the resistant. These lines show clearly that a difference exists in the mortality of the two races—a difference affected by the concentration or the exposure.

This difference is further shown by the following data, which give the percentage increase in concentration and in exposure required to increase the mortality by 1 probit:

<table>
<thead>
<tr>
<th>Race</th>
<th>Percentage increase in exposure required to increase kill by 1 probit</th>
<th>Percentage increase in concentration required to increase kill by 1 probit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonresistant</td>
<td>89</td>
<td>80</td>
</tr>
<tr>
<td>Resistant</td>
<td>109</td>
<td>100</td>
</tr>
</tbody>
</table>

According to these data the percentage increase in exposure required to increase the mortality by 1 probit is greater for the resistant race than for the nonresistant. In other words, the nonresistant race is the one more affected by a given exposure increment.

The data also show that the percentage increase in HCN concentration required to increase the kill by 1 probit is greater for the resistant red scale
Fig. 1.—A comparison of the effect of different concentrations of HCN upon the mortality of mature adult nonresistant and resistant red scale. The exposure time was 10 minutes.

Fig. 2.—A comparison of the effect of different lengths of exposure upon the mortality of mature adult nonresistant and resistant red scale. The concentration of HCN was 0.4 mg per liter.
than for the others. Clearly, again, with the mature adults, the nonresistant race is the one more affected.

To sum up: at least under the conditions of these experiments, both HCN concentration and exposure to HCN are more important in effecting an increase in the mortality of the nonresistant than of the resistant red scale.

SORPTION EXPERIMENTS
RECOVERY OF HCN FROM RESISTANT AND NONRESISTANT RED SCALE

Methods for accurate determination of HCN and studies of factors affecting HCN recovery from citrus tissues have been published by Bartholomew and Raby (1935) and Bartholomew, Sinclair, and Janes (1939). In an attempt to correlate injury of citrus tissues with the amount of HCN recovered after fumigation, Bartholomew, Sinclair, and Lindgren (1942) reported: “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.” This information immediately raises the question whether the differential in mortality of resistant and nonresistant red scale can be correlated with the amount of HCN absorbed by the two races.

Many theories regarding red-scale resistance to HCN have been published. Moore (1933) states: “With the data accumulated to date, it would appear that the difficulty of killing the resistant red scale was due to the difficulty of reaching the insect through its scale (waxy covering) rather than to any distinct immunity of the insect to hydrocyanic acid.” But as Lindgren (1941) has shown, the same differential in resistance between the two races exists in the crawler, which has no waxy covering, as in the adult insect.

Haas (1934), studying the composition of red scale, found no relation “between the ability of the insects to resist fumigation and their organic or inorganic iron or (ash) phosphorus content.” Since citrus scale insects contain rather large amounts of copper, he suggested that a reduced copper content may be related to fumigation resistance.

Quayle (1938) made no attempt to explain the mechanism of resistance to HCN, but did suggest some possible causes: “The question as to whether it is due to a difference in the waxy covering, or whether this is more tightly sealed to the surface in the case of the red scale, to a difference in respiratory rate, to a difference in nervous response, or to some other factor or factors, is not investigated.”

Although Carpenter and Moore (1938) were not working with scale, their findings regarding the sorption of HCN by various other insects may be related to the problem of resistance within species. They concluded: “The amount of hydrocyanic acid sorbed varies with the species; those insects which are generally known to be difficult to kill were found to sorb smaller quantities of hydrocyanic acid than those species which are easily killed.”

In the next paragraph are quoted observations made by Hardman and Craig (1941) in their studies on resistant and nonresistant red scale.
In each race the spiracles close within 3 to 5 minutes after the cyanide reaches them. In the resistant race the spiracles remain closed as long as HCN is present for at least 30 minutes. In the nonresistant race the spiracles remain closed for only about 1 minute and then open and death follows in a short time if the cyanide concentration is lethal. The resistant scale can survive a lethal concentration of cyanide for at least 30 minutes. ... There seems no doubt but that the relative ability to maintain closure of the spiracles is sufficient to explain the difference in resistance to HCN of the two races.

This conclusion is apparently based on the assumption that HCN does not penetrate the integument, but can obtain access to the scale tissues only through the spiracles.

Figure 3 graphically presents the amounts of HCN recovered from resistant and from nonresistant red scale fumigated at 75° F with a concentration of 16 mg of HCN per liter. As will be noted, the nonresistant scale sorbed much larger amounts of HCN than the resistant, at all exposures from 1 minute to 10 minutes. The points follow in line very well, considering that each exposure time is the result of a separate fumigation. At a 1-minute exposure, 50 per cent more HCN was recovered from the nonresistant scales than from the others; and at a 10-minute exposure 80 per cent more. These great differences indicate that the amount of HCN recovered must be related to the difference in kill of the two races.

*According to personal correspondence these authors, by subsequent experiments, have shown that “the integument is permeable to HCN since scale insects can be killed by HCN when it has no access to the spiracles.”*
The values reported in figures 3, 4, 5, and 6 designate the amounts of HCN recovered from the insect samples immediately after fumigation. They do not necessarily represent the total HCN sorbed during the fumigation period. With the method used in this study, the HCN recovered from a given insect sample would be equal to the total amount sorbed only if the body tissues of the insect were not capable of chemically reacting with the HCN. If, however, the body tissues of the insects are capable of chemically reacting with or fixing some of the HCN sorbed during a fumigation, then the amounts afterward recovered from the sample are proportionately reduced. The difference in the amounts recovered from the resistant and nonresistant scale may be due to the difference in the amounts fixed by the two races. But the quantity of HCN chemically changed or fixed by the tissues during fumigation is probably very small as compared with the total sorbed, so that the HCN recovered from the samples immediately after fumigation is believed to represent adequately the comparative sorption capacities of two samples fumigated under identical conditions. This theory agrees with the previously mentioned results obtained by Carpenter and Moore (1938); the latter used values representing the total sorption of HCN at a given gas pressure and including both the free HCN in the tissues and the HCN that may have been chemically combined.

In earlier experiments with dosage mortality, a concentration of 4.0 mg of HCN per liter and a 2 1/2 minute exposure killed 99.6 per cent of the nonresistant scales, 91.3 per cent of the resistant. One can assume, accordingly, that at an exposure of 2 or 4 minutes and a concentration of 16 mg of HCN per liter all (or nearly all) of the two races would be killed. Then the differences in sorption of gas obtained (fig. 3) are due either to the first minute or two the insects (still alive) are exposed to the HCN, or else to something besides the mechanism of respiration. That the first of these two explanations is not the correct one is indicated by the trend of the curves: the curves tend to diverge as the exposure increases, and the amounts absorbed per gram of scale also increase with the exposure and approach a saturation value for a given concentration of HCN. If the difference in sorption cannot be explained by respiratory processes carried on while the insect is living, it may be due to some inhibitor or to some chemical reaction that takes place in the resistant scale and changes the HCN to some other compound. Plumb\(^7\) suggests the following explanation: “May it not be that this ability to resist fumigation is based on the development of a counter-mechanism within the respiratory enzymes; so that inhibition of the enzyme by CN\(^-\) is blocked?” Although such a mechanism may be a factor in the resistance of red scale to HCN, the theory does not explain the entire picture, since it assumes that the HCN is present within the tissues of the resistant red scale but is prevented from acting. As shown by the experiments discussed above, there is an actual difference in the amounts of HCN recovered—a fact indicating that the HCN either does not get into the resistant scale so readily as into the nonresistant, or else is changed over when it does get in.

To determine whether this difference in HCN sorption is due to the first minute or two when the scales (still alive) are exposed to the HCN, several series of experiments were conducted: The scales were first killed by HCN,\(^7\) Plumb, George H., personal correspondence with H. J. Quayle, February 15, 1942.
lack of oxygen, or high temperature, with exposures previously determined to
be lethal. The two races showed no difference in tolerance to high temperature
or lack of oxygen. Scales killed in either of these ways were immediately
removed from the hosts (grapefruits) and fumigated. Those killed by HCN
were held for 5 hours before fumigation; 4 hours had been found by careful
checking to be sufficient for complete dissipation of the sorbed HCN. An­
other series of tests (table 1, series 5) was conducted to determine how much
HCN was sorbed by insects dead and completely dried; the interval after
death was 19 days. A check series (no. 1, table 1) was run without pretreat­
ment. In all series, scales were placed in the distillation flasks immediately
after fumigation, and the HCN recovered. Table 1 gives the results.

TABLE 1

RECOVERY OF HCN FROM RESISTANT AND NONRESISTENT RED SCALE
(All samples fumigated at 16.0 mg HCN per liter for 10 minutes at 75° F and
45 to 60 per cent relative humidity)

<table>
<thead>
<tr>
<th>Series no. and treatment before sorption fumigation</th>
<th>HCN recovered per gram of scale (fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant race</td>
</tr>
<tr>
<td>1. Check: live scale, no pretreatment.</td>
<td>0.171</td>
</tr>
<tr>
<td>2. Scale previously killed by lack of oxygen.</td>
<td>0.286</td>
</tr>
<tr>
<td>3. Scale previously killed by high temperature.</td>
<td>0.184</td>
</tr>
<tr>
<td>4. Scale killed by HCN fumigation 5 hours before sorption fumigation.</td>
<td>0.423</td>
</tr>
<tr>
<td>5. Scale killed by HCN fumigation 19 days before sorption fumigation; completely dried out.</td>
<td>0.120</td>
</tr>
</tbody>
</table>

As will be observed from this table, when the scales are killed by lack of
oxygen or by high temperature, the resistant ones sorb less HCN than the
nonresistant; but the difference is not so great as when the insects are alive.
If the scales are killed by HCN 5 hours before the sorption test, the reverse
is true: the resistant ones take up more HCN than the others. If the insects
are dead and entirely dried, there is no difference in the amount of HCN
absorbed.

From these observations one might deduce that the difference in recovery
is due to some chemical change of HCN in the tissues of the scale. To explain
the reversal obtained when the scales are fumigated and killed by the HCN
before the recovery tests, one may infer some upset of the process whereby the
resistant scales are enabled either to sorb less HCN than the nonresistant
or else to change the HCN over to some other form. This theory would account
for the resistant scales’ taking up as much HCN as the nonresistant scales,
but does not explain why after being killed beforehand with HCN they
actually take up more. Because such differences are obtained in HCN
sorption by dead resistant and nonresistant scales, and because the curves
continue to diverge with increasing sorption up to saturation values (fig. 3)
long after the scales are known to be dead, one may conclude that the differ­
ential in HCN sorption is not entirely due to the mechanism of respiration.

Apparently, therefore, under these experimental conditions, the sorption
of HCN by resistant and nonresistant red scale involves, also, such principles as rate of diffusion of the gas through the external and into the internal tissues, or the chemical reaction of bodily constituents with HCN so as to fix it and chemically change it into nontoxic substances. That such physical and chemical factors should be considered in the sorption of HCN by insects, especially in high concentrations, is essential for the elucidation of this problem.

In another series of experiments with resistant and nonresistant red scale, a 40-minute exposure was used with various concentrations of HCN. Figure 4 shows the results graphically. Again the nonresistant scale insects consistently take up more HCN than the others. The lowest concentration used in this series of experiments was 1.8 mg per liter, which is only slightly higher than that used in the field in commercial practice. At this concentration the nonresistant red scales sorbed 52 per cent more HCN than the resistant; and at 4 mg HCN per liter, 45 per cent more HCN.

In comparing the sorption curves (figs. 3 and 4) with the mortality curves (figs. 1 and 2), one immediately notes the similarity between them. Indications are that the amount of HCN recovered from the two races of red scale is directly related to the mortality. In like manner Carpenter and Moore (1938), working with various species of insects, concluded that "the ease of killing with hydrocyanic acid does not depend so much upon its actual toxicity, which is, of course, great for all forms of animal life, as it does upon how
readily the hydrocyanic acid is sorbed by the insects.” Evidently, high sorption of HCN is associated with ease of killing. The degree of correlation cannot, however, be calculated from these and other published data; only after performing similar experiments upon many species of insects can one generalize. To illustrate, even when two species of insects sorb the same amount of HCN under identical conditions, the lethal dosage for each species may be very different.

**Fig. 5.—**Recovery of HCN from resistant and nonresistant black scale. The fumigation concentration was 16 mg of HCN per liter, the temperature 75° F, and the relative humidity 45 to 60 per cent.

**RECOVERY OF HCN FROM RESISTANT AND NONRESISTANT BLACK SCALE**

In 1915 (Quayle, 1938) black scale, *Saissetia oleae* (Bern.), in the Charter Oak district of Los Angeles County, proved resistant to HCN. Lindgren and Dickson (1943) found differences in the tolerance to HCN of black scale reared in the laboratory under identical conditions.

At the time the sorption tests were being conducted, no black scales were being reared in the laboratory. Specimens were obtained in the field, however, from two groves—one grove known from previous tests (Lindgren and Dickson, 1943) to have resistant and the other nonresistant black scale. Conditions of the experiment were the same as for red scale. Figure 5 gives results of the sorption tests. At the 1-minute exposure there was no difference in the amount of HCN recovered from the two races of black scale; but in all the other exposures tried, the differences are great. Since the black scales obtained
in the field were large (4 to 6 mg each), perhaps a 1-minute exposure was not long enough to get much cyanide into the scale's body; the HCN recovered may be due to surface absorption. With black scale, as with red, more HCN is recovered from the nonresistant than the resistant race.

**RECOVERY OF HCN FROM WALNUT-HUSK-FLY PUPAE**

According to A. M. Boyce and R. B. Korsmeier, pupae of the walnut husk fly, *Rhagoletis completa* Cress., are very difficult to kill with HCN; concentrations higher than 30 mg of HCN per liter, with 1-hour exposure, did not kill 100 per cent of the pupae. It was decided, accordingly, to determine how much HCN could be recovered from such pupae. The findings might have some bearing on the relation of HCN sorption to resistance in scale insects.

Early in the work, this amount was found to vary with the relative humidity before and during the fumigation. Figure 6 shows the amount of HCN recovered from husk-fly pupae preconditioned and fumigated at a humidity of 15 to 20 per cent, and from another group preconditioned and fumigated at 70 to 90 per cent. Clearly, much more HCN is recovered from the former group. Comparing figure 4 with figure 6, one notes that less HCN is recovered from husk-fly pupae than from the resistant or nonresistant red scale. The red scale is also more susceptible to HCN than are the husk-fly pupae.

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*Unpublished data on file at the University of California Citrus Experiment Station.*
SUMMARY

Fumigation of nonresistant and resistant red scale indicates that the resistant race, with the various concentrations and exposures tried, is more difficult to kill than the nonresistant. Any added increment of either concentration or exposure has a greater effect on the kill of the nonresistant scale than on the others.

More HCN is recovered from fumigated nonresistant than from fumigated resistant red scale.

With red scale first killed by heat or lack of oxygen, and then fumigated, more HCN is recovered from the nonresistant than from the resistant race; but the difference is less than in insects fumigated without pretreatment.

With refumigated red scale previously killed by HCN fumigation but not dried up, more HCN is recovered from the resistant than from the nonresistant race. With red scale fumigated when dead and dried, equal amounts of HCN are recovered from the resistant and from the nonresistant race.

More HCN is recovered from fumigated nonresistant than from fumigated resistant black scale.

More HCN is recovered from walnut-husk-fly pupae if they are held and fumigated at low rather than high humidity.

More HCN is recovered from red scale than from walnut-husk-fly pupae, on a weight basis.

ACKNOWLEDGMENTS

The writers are grateful to Professor H. J. Quayle for his valuable counsel and to Mr. R. C. Ramsey for aid in certain phases of the experiments.
LITERATURE CITED

BARTHOLOMEW, E. T., and E. C. RABY.

BARTHOLOMEW, E. T., WALTON B. SINCLAIR, and BYRON E. JANES.

BARTHOLOMEW, E. T., WALTON B. SINCLAIR, and D. L. LINDGRÉN.

BLISS, C. I.

Carpenter, E. L., and William Moore.

Dickson, R. C.

Haas, A. R. C.

Hardman, N. F., and RodERICK Craig.
1941. A physiological basis for the differential resistance of the two races of red scale to HCN. Science 94(2434):187.

Lindgrén, D. L.
1941. Factors influencing the results of fumigation of the California red scale. Hilgardia 13(9):491–511.

Lindgrén, D. L., and R. C. Dickson.

Moore, William.

Quayle, H. J.

Sinclair, Walton B., and R. C. Ramsey.