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SULFURIC ACID AS A PENETRATING AGENT IN ARSENICAL SPRAYS FOR WEED CONTROL[1]

A. S. CRAFTS[2]

Sulfuric acid has been successfully used in the control of annual weeds and plant diseases in grain fields.[11] Åslander, in 1927,[1] reviewed briefly the earlier work and reported valuable experimental studies on the action of this chemical upon plants. He listed 53 weeds that have been killed and mentioned a few that did not respond to the acid. Most of the latter were perennials, grasses, and plants difficult to wet. He discussed the influence of soil moisture, relative humidity, and temperature upon the action of the acid and made histological studies on treated mustard leaves. Under the microscope he examined *Elodea* leaves in acid solutions. In all his work he compared sulfuric acid with iron sulfate. He found the acid much more rapid in its action upon the plant and explained its effectiveness in dry regions upon this basis. When the relative humidity was low, he found iron sulfate to crystallize on the leaves before penetration had taken place.

Sulfuric acid has proved useful in Arizona[3] against a number of weeds. Being produced as a convenient outlet for certain by-products of the smelting industry, it is relatively inexpensive. The chief drawback to its general use is its strongly corrosive action on metal equipment—a difficulty that must be overcome before it can serve the farmer in combating annual weeds.

More recently[5] sulfuric acid has been found useful as a penetrating agent in an acid arsenical spray that promises to become useful in controlling certain deep-rooted perennial weeds. The mechanism responsible for the action of this type of spray was described in 1927,[8] and further experiments were reported in 1930.[4] A later publication[5]

[1] Received for publication May 13, 1933.

[2] Assistant Botanist in the Experiment Station.

cites plot tests that indicate the critical concentrations of acid and arsenic. All these reports emphasize the important role of acid in the penetration of the spray solution. Since the success of this method so largely depends upon the operator's understanding of its mechanics, the underlying principles might well be studied more thoroughly. The present paper describes the reaction of the plant to sulfuric acid, provides data for evaluating the various factors involved, and discusses the relation of these factors to actual spray practice.

THE REACTION OF PLANT CELLS TO STRONG ACID

Åslander studied the effect of sulfuric acid on *Elodea* leaves. He found that it rapidly stopped protoplasmic streaming but in no case caused plasmolysis. Being convenient, fairly uniform, and easy to observe, the same material was used in the present studies. *Elodea* leaves, mounted in water between a flat slide and a cover glass, were examined at a magnification of 900 (Zeiss apochromat N.A. 1.3, 90x and 10x compensating oculars). At this magnification the rapid flow of protoplasm could be distinctly seen, the small chondriosomes were very clearly differentiated, and the relation of the protoplasm to the slowly moving plastids could be studied. The nuclei were clearly defined.

Figure 1*A* diagrams a normal leaf cell of *Elodea,* with arrows denoting the location and direction of streaming. The narrow strands of protoplasm stretching across the vacuole exhibit rapid motion; but close observation shows that the chondriosomes, in addition to being carried along in this smoothly flowing stream, also have impressed upon them the slight jerky motion of thermal agitation. The vacuoles in healthy cells appear clear and free from particles.

After selecting a favorable location, usually at the base of the leaf, where the plastids are not numerous and streaming is active, a drop of sulfuric acid was applied to the cover glass, and the cells were watched closely. With 0.1 N acid the killing process occurs slowly, so that the different stages can be studied. With stronger acid the process goes on more rapidly, but is not essentially different.

The first sign of acid injury is a gradual slowing down of the flow along the outer surface of the protoplasmic strands. The cause, apparently, is not increased viscosity, for the thermal agitation of the chondriosomes continues unaltered even after unidirectional movement has ceased. As this slowing down proceeds toward the center of the strand, the chondriosomes farther within lose their unidirectional motion until flow stops and they exhibit only the jerky Brownian movement. This movement continues for some time after streaming has stopped and the

plastids have come to rest. The strands of protoplasm appear to thicken somewhat, while the parietal layer lining the wall assumes a rough, swelled appearance. The protoplasm apparently increases in viscosity with time as the Brownian movement finally slows and stops, and all structures previously in motion come to complete rest. Meanwhile very minute particles appear in the vacuole. As they first become visible

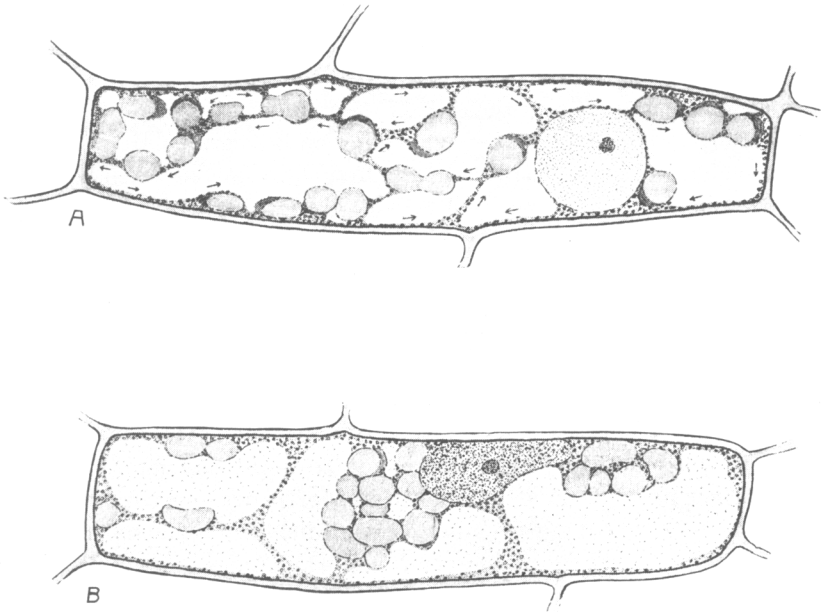

Fig. 1. The effect of sulfuric acid on *Elodea* cells; a comparison of *(A)* healthy, and *(B)* acid-killed cells.

they show very violent thermal motion; gaining in volume they become somewhat more sluggish. Their ultimate size approaches that of the chondriosomes, but they remain free moving within the vacuole. As the acid penetrates to the plastids, the green coloring matter turns to the light yellowish green, described by Åslander. These studies also substantiate Åslander's statement that no plasmolysis occurs in cells treated with sulfuric acid. The protoplasm is apparently killed and rendered permeable so rapidly that no withdrawal of water can take place. After killing with 4.0 N acid the protoplast slowly shrinks, resembling plasmolysis, but only after the cessation of streaming and the color change of the chlorophyll have shown that the cell is dead. Figure 1*B* shows a killed cell. The protoplasm, and especially the nucleus, appears granular. The plastids show the characteristic grouping described by Åslander. Chondriosomes are fixed within the protoplasm, while the vacuole is occupied by many minute, rapidly agitated particles.

STUDIES ON THE RATE OF KILLING OF PLANT CELLS BY STRONG ACID

Stiles and Jorgensen[13] found that plant tissues absorb hydrogen ions rapidly and that a simple exponential relation exists between absorption time and acid concentration.[14] The temperature coefficient for absorption within the range 0°–30° C was 2.2 for each 10°. On the basis of these two findings they suggest that this absorption is controlled by some chemical action in the cell.

Brenner,[2] conducting extensive studies on the permeability of plant cells to acids and bases, found that the toxicity of acids varied with the hydrogen-ion concentration.

Heilbrunn[6] reviewed the work of several investigators on the action of acids and alkalies upon protoplasm. He found general agreement on the observation that acids cause coagulation, an increase in the granules of the cell, and—in high concentrations—death.

These investigators were interested primarily in the reaction of plant tissues to relatively low concentrations of acid, and their experiments covered considerable periods of time. The present studies more directly concern the rapid killing of cells by concentrated strong acids.

The living protoplasm of the plant cell constitutes an extremely complex physico-chemical system adapted to a fairly uniform environment. Although buffered to a certain extent against changes in reaction of the surrounding medium, it cannot withstand strong acids of the concentrations used in weed sprays. Whereas the rate of absorption of dilute acid may be an exponential function of concentration[14] and therefore primarily chemical in nature, the rate of killing by more concentrated solutions depends upon three processes, diffusion of hydrogen ions through the cell walls, absorption of these ions by the cell-wall material, and reaction with the living protoplasm. The relative importance of these can be only surmised, since they cannot be well differentiated in the experiments with living tissues. They will be further considered as additional data are presented.

Before discussing the experimental work with plant materials in acid solution, we might well consider briefly the various terms used to designate acidity. Normality is a measure of the titratable acid in a solution. Hydrogen-ion concentration is a measure of the equilibrium number of hydrogen ions in a solution, and is directly related to the dissociation of the solute. Until recently pH has been defined as the logarithm of the reciprocal of the hydrogen-ion concentration of a solution and as such could be computed from the dissociation of the solution as determined

from conductivity. According to modern theory, pH is defined as the logarithm of the reciprocal of the hydrogen-ion activity of a solution. Since activity is a function of free energy, pH is now computed from E.M.F. data and is usually determined by means of a hydrogen electrode or similar equipment.

In describing the killing of plant tissue with acids, use of the pH function allows a compact and accurate presentation of data. Though values computed from conductivity measurements are not accurate, the errors introduced by their use are probably no greater than those of the

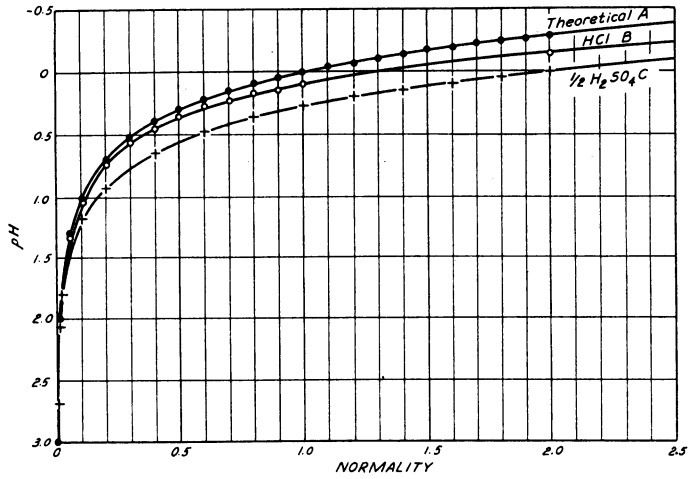

Fig. 2. The relation between normality and hydrogen-ion concentration in strong acid solutions.

determination of killing rate. Figure 2 presents curves computed from the data on hydrochloric and sulfuric acids in tables XIX and XX of Van Nostrand's Chemical Annual.[10] Curve *A* is the theoretical hydrogen-ion concentration at 100 per cent dissociation; curve *B*, the apparent concentration of hydrogen ions in hydrochloric acid solutions; and curve *C*, the values for ½ H_2SO_4.

In order to study more accurately increasing concentrations of acid as affecting the time rate of plant-tissue killing, a series of tests were made with *Elodea* leaves and leaf sections of wild morning-glory and grass. In the first test (December, 1931), thin sections of morning-glory leaf were placed in a series of solutions, and the time of killing, as denoted by the color shift of chlorophyll, was taken. The data on this test are presented graphically in figure 3 (curve *A*). The first four solutions were dilutions of HCl; the remaining fifteen, phthalate buffer solutions. The pH values of the HCl dilutions were computed from curve *B*, figure

2. The buffer solutions were checked with a quinhydrone electrode. Since a logarithmic function of hydrogen-ion concentration is used, it was deemed best to plot these values against log time.

According to these tests the time required to kill plant tissues with strong acids depends on the hydrogen ions in the solution. When plotted

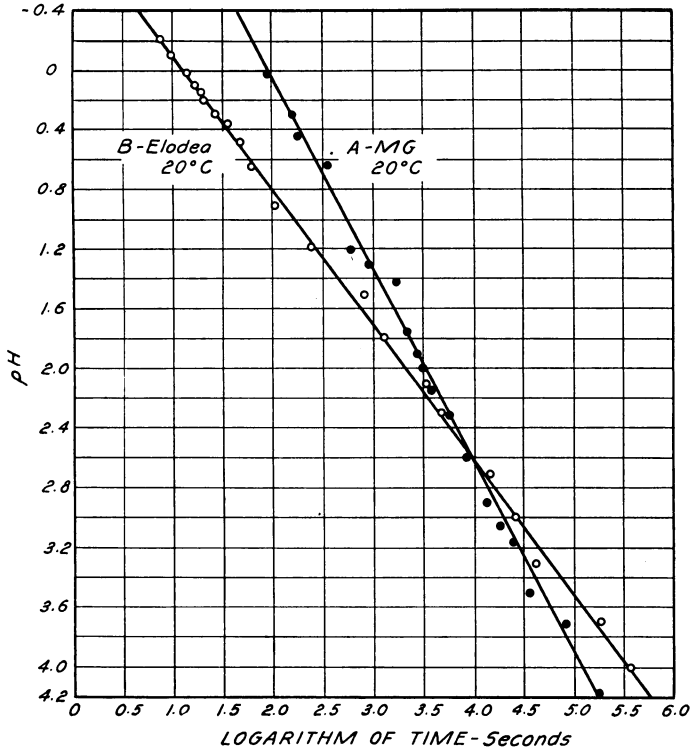

Fig. 3. The effect of hydrogen-ion concentration upon the time required for killing plant tissue with strong acids.

against the hydrogen-ion concentration computed from conductivity experiments, the data give a straight-line relation, indicating that a function related to mobility as well as activity, plays a primary part in the killing process. Though pH may not be a strictly correct symbol for this co-log function of the hydrogen ions, a new phraseology would only cause confusion. The term will be used therefore with the understanding that it does not apply strictly to the activity function.

Further tests were run, with *Elodea* leaves as indicator material. Leaves of comparable age and size were selected and a large number were pulled from the stems and stored in fresh tap water at the beginning of each test. The acid solutions were standard dilutions of CP

H_2SO_4 with distilled water and were not buffered. A large excess of solution was used each time, so that the acid was not exhausted. Each value represents the average of ten separate readings. The solutions were kept

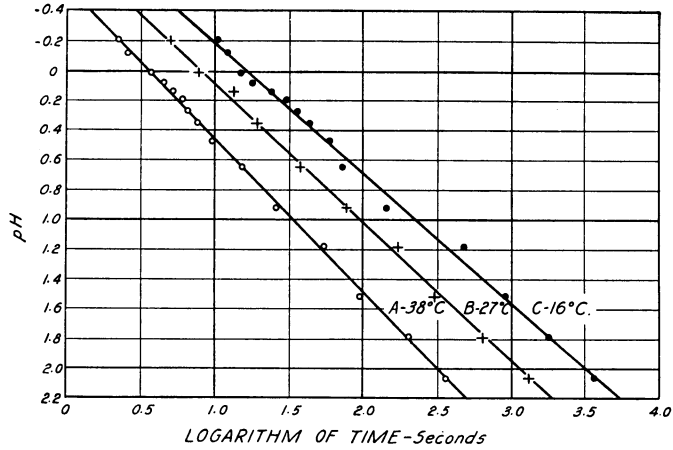

Fig. 4. The relation of temperature to rate of killing of *Elodea* leaves in sulfuric acid.

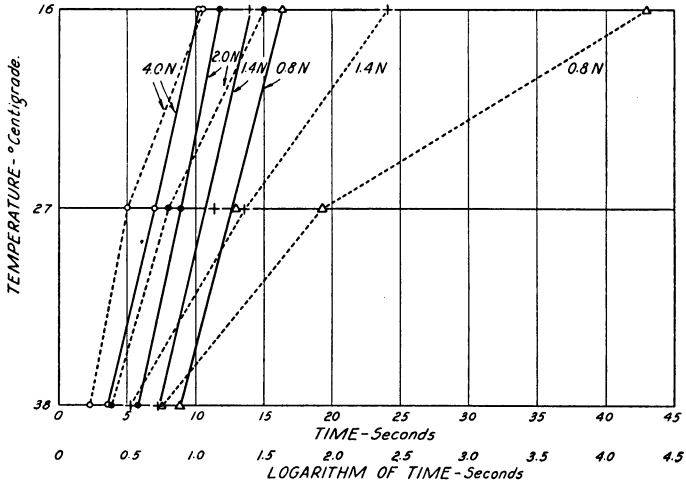

Fig. 5. Time-temperature and log time-temperature curves for killing of *Elodea* leaves in sulfuric acid. Broken lines represent time in seconds. Solid lines represent logarithm of time in seconds.

at a constant temperature, and the leaves were constantly agitated during the test. These data appear as curve *B*, figure 3. Killing in the less acid solutions is a slow process and the selection of a stage comparable with those chosen in the more concentrated solutions is attended by a considerable personal error. Furthermore the individual leaves or sections exhibit wide variations in killing rate. In spite of this, when the

values are averaged they fall close to the line as shown in figure 3. This seems to strengthen the assumption that the relation is exponential.

The effect of temperature is very apparent in the field work with acids. A temperature series was run with *Elodea* leaves. The pH-log time relations are shown in figure 4. The straight-line relation is again apparent.

The relation between temperature and time of killing for the different concentrations appears in figure 5. When temperature is plotted against log time, this relation becomes linear.

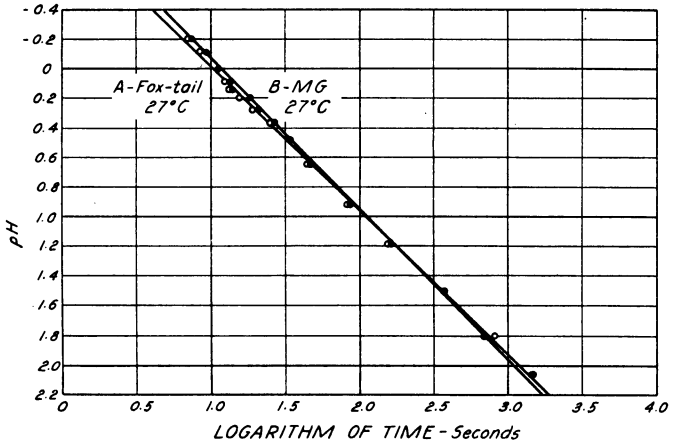

Fig. 6. The effect of hydrogen-ion concentration upon the time required to kill leaf cells of morning-glory and foxtail with sulfuric acid.

An average temperature coefficient of 2.3 has been calculated between the temperatures 17°–27° C and 27°–37°. This indicates that the chemical phases of the killing process predominate in the consumption of time. Diffusion, however, is too important a factor to be neglected. When the rates at different concentrations are considered individually, the temperature coefficients are found to decrease with increasing concentration. Possibly increased hydration of cell walls adds to the distance and hence to the time required for diffusion. Further study shows that the process is too complicated for an accurate mathematical analysis; but the importance of temperature in determining penetration of acids is clearly shown.

This method of study was applied to weeds encountered in the field by substituting leaf sections of morning-glory and foxtail for *Elodea* in the same procedure. As shown by figure 6, the results are nearly the same. The two morning-glory curves (figs. 3 and 6) differ considerably from

each other. The leaf material used in the first (fig. 3*A*) was taken from the field in December, 1931; that used in the latter (fig. 6*B*) from the greenhouse in January, 1933. In the first test the temperatures were not accurately controlled nor the times so carefully taken as in the second.

The time rate of killing plant tissue in an unlimited supply of acid has been shown, by these last experiments, to be very rapid. Whereas 0.5 N acid killed these cells in one minute or less, it has recently been found[5] that 1.0 N acid or stronger is needed to bring about satisfactory penetration in the field and that a volume of solution sufficient to wet the foliage thoroughly is required. Apparently factors other than concentration enter the problem of killing with acid. Among the most important is the effect of reaction with plant buffers.

THE PLANT BUFFER SYSTEM

The action of plant buffers was noted in some previous work on morning-glory.[4] Further studies have since been made.

The plant body is a complex organization, including among its many components organic acids and their salts, proteins and their various derivatives, basic nitrogenous compounds, colloidal carbohydrate material, and other substances, all of which may act as buffers. Preliminary experiments on the titration of plant tissue—acid mixtures with alkali showed that little or no acid is lost from the reacting mixture when tissues are treated with acid. Although some CO_2 may pass off, the addition of a quantity of alkali, equivalent to the original acid will always bring the reaction back approximately to the starting point.

The buffer capacity of ground morning-glory tissue was studied by titrating with alkali in a hydrogen cell. Ten grams of freshly ground foliage were placed with 40 cubic centimeters of 0.1 N hydrochloric acid on a water bath at 25° C. When the tissue was completely discolored, the mixture was placed in the cell of a hydrogen electrode; then, with constant mechanical stirring, the hydrogen-ion concentration was measured at 30° C as 0.1 N sodium hydroxide was added in small quantities. Figure 7 shows the titration curve along with a similar curve for pure hydrochloric acid. Curve *A* represents the plant titration curve, *B* the titration of 40 cc of 0.1 N hydrochloric acid, to which were added 10 cc of water, with 0.1 N sodium hydroxide. The ground plant tissue had an initial pH of 5.87 and a dry weight percentage of 22.2.

This ground plant tissue had an appreciable buffering capacity; only when the medium surrounding the cells differs considerably from that of the tissue itself does death occur. Although the reaction returned to neutrality when an equivalent amount of alkali was added, 23.5 cc suf-

ficed to take it back to pH 4.0, approximately the point of lethal action. Therefore, 16.0 cc carried it on to the neutral point; and, presumably, a like amount of acid would take it back to pH 4.0 again. This, then, is a measure of the effective buffer capacity of ground morning-glory tissue against the acid solutions used. Since the initial pH of the plant tissue was 5.8, the difference in titration between this and 4.0 represents the acid consumed in killing the tissue. For 10 grams of tissue, 12.5 cc of 0.1 N acid were required. This fact accounts for the large volume of acid required in treating the mass of tissue on a given area of land.

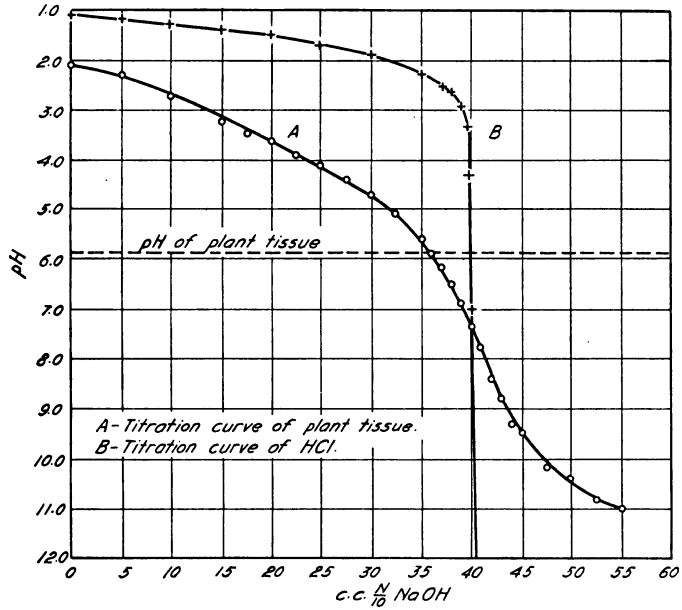

Fig. 7. Titration curves for ground morning-glory tissue and pure HCl.

THE EFFECT OF THE CUTICLE UPON PENETRATION

The other important factor in the rate of killing is the effect of the plant cuticle upon diffusion. According to popular notion, the plant cuticle is an impervious layer, whose function is to prevent evaporation of water or to bring water loss entirely under the control of the stomatal mechanism. Numerous experiments have shown, however, that the cuticle on the leaves of many plants permits a fairly rapid loss of water when the stomata are closed. Rudolph[12] found that, with the air at rest, as much as one-third of the normal water loss is cuticular. Using acids and plant poisons to study penetration of the cuticle by solutes, he found ready movement of these reagents into the leaves. The time re-

quired to kill leaves of different species with sulfuric acid varied greatly and, generally speaking, the leaves having the thicker cuticle require the longer time. All the leaves that he tried, however, were killed after some time, and his experiments as well as many of the author's show that hydrogen ions can diffuse through the leaf-cuticle of many common weeds. The rate is somewhat lower than for *Elodea* or for sectioned material.

Experiments on the rate of penetration of sulfuric acid into morning-glory leaves of different ages have shown that in general the younger leaves, even if their cuticle is thinner, are less pervious than the older ones. Because their waxy surface is so difficult to wet, intimate contact is not obtained. Thus the quality of the surface layer as well as its thickness enters the problem. Under California conditions, the older leaves of many weeds are attacked by red spiders; and these have often been observed to die more quickly than uninjured leaves. There are, apparently, two reasons: namely, puncture of the cuticle, which allows ready entry of the acid; and accumulation, by the webs and débris of the insects, of more acid per unit area than occurs on healthy leaves. So long as the leaves are not dead and dry, this insect injury apparently enhances the penetration and effectiveness of the acid sprays. Only when the live area available for treatment is considerably reduced are the effects of the application inhibited.

Another popular notion is that sprays of this nature enter the leaves through the stomata. Rudolph found no stomatal penetration in his experiments, and many tests have shown that it does not occur under normal conditions. Considering the surface tension of aqueous solutions, we see that a great pressure difference would be required to infiltrate leaves with an acid spray. Since the stomatal cavities do not become injected after spraying, and since the acid injury appears over large areas, irrespective of the presence or distribution of stomata, this action apparently does not occur.

Åslander thought that his histolological studies indicated stomatal penetration since "destruction of the cells" was "first noticeable in the neighborhood of these openings." But though the guard cells may be more easily penetrated by the acid and so more rapidly killed, this fact does not indicate that the acid actually entered the leaf through the stomata. As Åslander states further on, "It also seems to penetrate through the epidermal cells." Only if the surface tension of the solution in contact with the cuticle were greatly lowered could the solution possibly enter the stomata in any quantity. Under field conditions it probably does not enter in this manner at all.

PENETRATION OF SULFURIC ACID INTO MORNING-GLORY LEAVES

In order to apply the information thus far gained to the problem of penetration of acid solutions under field conditions, further studies have been made. These include experiments on the rate of injury of

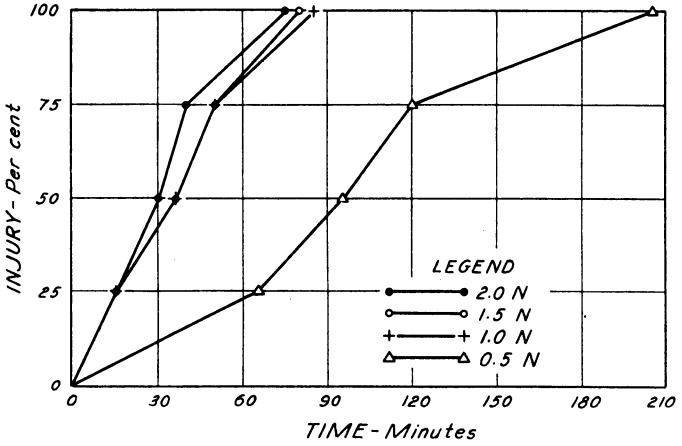

Fig. 8. The effect of immersing morning-glory leaves in sulfuric acid solutions of different concentrations.

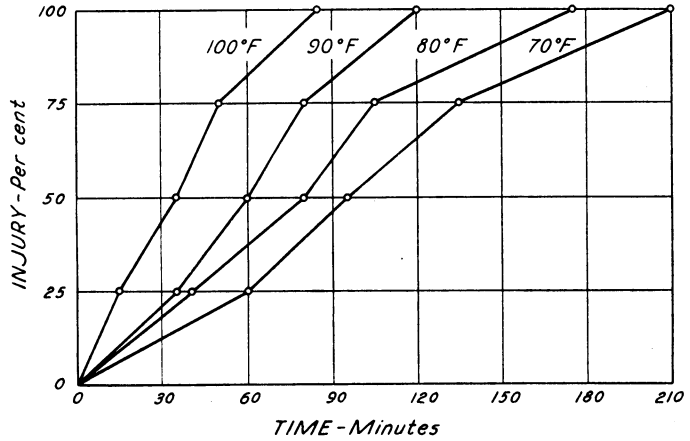

Fig. 9. The relation of temperature to injury time in sulfuric acid treatment.

morning-glory shoots immersed in acid baths of varying concentration, and studies on the rate and type of injury developing on shoots that have been dipped in the various acid solutions and subsequently exposed to the air with their cut ends in tap water. Figure 8, presenting the results at 38° C in graphic form, serves to emphasize the difference

between 0.5 N sulfuric acid and the three higher concentrations with respect to time required for a given amount of injury.

These tests were run at a series of temperatures including 21.3°, 27.0°, 32.3°, and 38° C, corresponding closely to 70°, 80°, 90°, and 100° F,

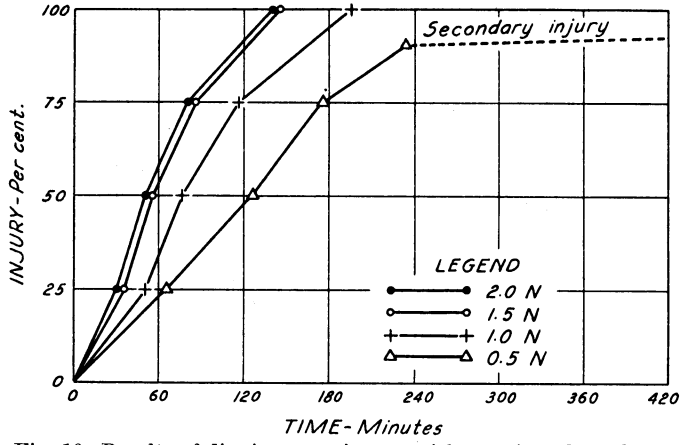

Fig. 10. Results of dipping experiments with morning-glory shoots. Injury to dipped shoots in open air.

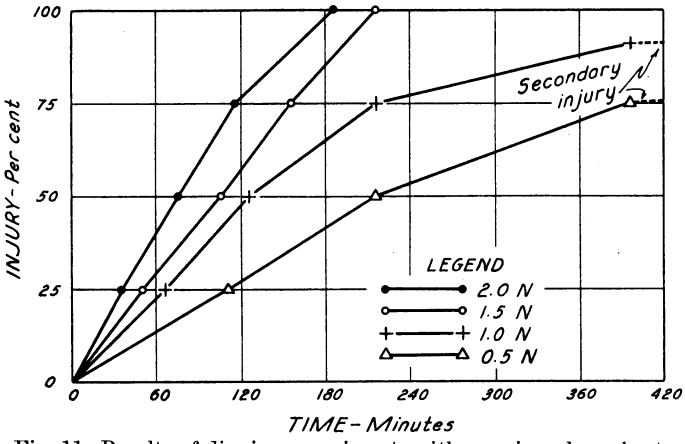

Fig. 11. Results of dipping experiments with morning-glory shoots. Injury to dipped shoots in saturated atmosphere.

within which range most sprays are applied at Davis. Since the temperature series are almost parallel throughout, only one set is presented (fig. 9). This is typical of all of the results and shows the effect of higher temperatures in accelerating the acid injury.

The effects of dipping shoots in these different acid solutions and then allowing the injury to develop as the shoots stand with their cut ends in water are more readily comparable with those obtained on sprayed

plants in the field. Figure 10 represents such a series. The slower rate of injury shows how plant buffers reduce the acid concentration as injury takes place. Evaporation tends to counteract this effect. In order to separate these two factors a separate series was run in a saturated atmosphere; the data are presented in figure 11.

Comparing figures 10 and 8, we see the influence of plant buffers in reducing the amount of acid available. As the lower concentrations in the former case were not present in sufficient amount to cause complete injury, the shoots remained mottled and finally died from secondary effects. Comparing figures 10 and 11, we see the effect of evaporation upon rate of injury. In the higher concentrations this is less noticeable than in the lower, where it is pronounced. The mottled partial killing with 0.5 N acid is very evident in the field and does not give satisfactory results.[5] The ideal situation is to have practically a saturated atmosphere after spraying and to use a sufficiently concentrated acid solution to cause rapid killing. These tests show that at least 1.0 N acid is required.

QUANTITATIVE RELATIONS

Since, with the solutions of lower acid concentration, the absolute quantity of acid available for reaction evidently becomes limiting, the quantitative phases of the problem must be considered in some detail. The growth of perennial weeds in the field varies between wide limits, so that each infestation must be treated as a separate problem. For a study of this sort it seems best to work near the upper limits. Square-yard plots were located in a dense area of morning-glory, and the top growth was removed by clipping the stems at the ground level. The foliage, rolled up, was taken to the laboratory. There each roll was weighed; sprayed thoroughly with water, by means of a knapsack sprayer, the same type of application being used as in field-plot tests; rolled up again; and reweighed. The data appear in table 1.

In spraying this material, an attempt was made to apply as much water as possible with no loss by run-off. The plots represented stands varying from a minimum growth giving complete coverage (plot 5) to a dense heavy mass (plots having 1,000 grams or more per square yard). Obviously, the variation in coverage was great; a similar variation exists under conditions of spraying in the field. The problem, therefore, is to determine the minimum quantity of solution needed for satisfactory coverage, and to relate the acid concentration to this amount so that under all conditions the entire top growth will be quickly killed.

If the plots in table 1 represent the normal variation under California conditions for stands completely covering the soil, then the average

square yard would have a cover weighing about 1.2 kilograms or 36 kilograms per square rod. In order to kill morning-glory tissue completely, the pH must be shifted from pH 5.87 to pH 4.00 (fig. 7). There would be required 36.2 minus 23.6 or 12.6 cc of 0.1 N sulfuric acid per 10 grams. This would be 126 cc of 1.0 N acid per kilogram and 36 $\times$ 126 $\div$ 1,000 or 4.5 liters per square rod of foliage. The average applica-

TABLE 1

SPRAY MEASUREMENTS ON MORNING-GLORY PLOTS

Plot No.	Fresh weight, grams	Sprayed weight, grams	Water applied, grams	Volume per square rod, gallons	Volume per acre, gallons
1	1,140	1,350	210	1.7	269
2	1,960	2,350	390	3.1	499
3	1,680	2,000	320	2.6	409
4	820	1,115	295	2.4	378
5	465	675	210	1.7	269
6	725	1,010	285	2.3	365
7	1,305	1,815	510	4.1	653
8	1,130	1,575	445	3.6	570
9	1,225	1,660	435	3.5	557
10	1,150	1,650	500	4.0	640
11	1,300	1,660	360	2.9	461
12	800	1,190	310	2.5	397
13	1,230	1,530	300	2.4	384
14	1,780	2,300	520	4.2	665
15	1,085	1,400	315	2.5	404
16	1,145	1,520	375	3.0	480
17	1,045	1,415	370	3.0	474
18	1,210	1,580	370	3.0	474
Total	21,275	27,795	6,520	.....	.....
Average	*1,182*	*1,544*	*362.2*	*2.9*	*464*

tion as indicated in table 1 would be 2.9 gallons per square rod. After thorough trials in the field, a rate of 3.0 gallons per square rod has been selected as a standard application in plot work; it seems to provide maximum coverage for all but exceedingly heavy stands. This would be 3.785 $\times$ 3 or 11.355 liters per square rod. The normality of acid required for killing the foliage of the average plot would therefore be 4.5 $\div$ 11.355 = 0.4 N; and in the ideal case where the acid can come directly in contact with all the tissue, the time required for killing would, according to figure 6, be 27.8 hours.

Similar calculations show the time required by acids of different normalities. With 0.5 N acid, for instance, there would be required 3.785 $\div$ 36 $\times$ (3 $\times$ 0.5 $\times$ 1,000) = 157 cc of 1.0 N acid per kilogram, or 15.7 cc of 0.1 N acid per 10 grams. Then from figure 7, 40 — (15.7 + 3.8) = 20.5 cc, which corresponds to pH 3.7. Checking back on figure 6, 13.9

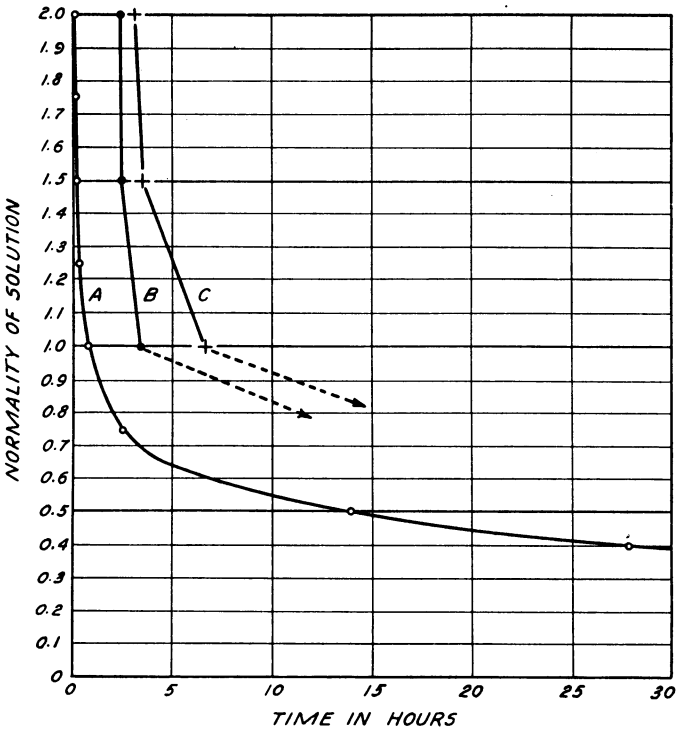

Fig. 12. The relation between normality of acid and killing time for morning-glory tissue in sulfuric acid.

TABLE 2

TIME REQUIRED FOR KILLING MORNING-GLORY LEAF TISSUE WITH ACID OF DIFFERENT NORMALITIES

Normality	pH attained	Time required, hours
0.40	4.00	27.80
0.50	3.70	13.92
0.75	2.95	2.49
1.00	2.33	0.61
1.25	1.96	0.26
1.50	1.70	0.15
1.75	1.50	0.10
2.00	1.40	0.08

hours would be required for killing at pH 3.7. By this same method a series of calculations have been made indicating the time required for killing morning-glory tissue with different normalities of acid. These figures are given in table 2 and are represented by curve *A* in figure 12. Since the times recorded in table 2 are taken from figure 6, they apply only to the ideal case where an excess of acid is in intimate contact with thin leaf sections. From figure 10 curve *B* is drawn, which shows, in comparison with *A*, the retardation caused by the plant cuticle, and the absorption by buffers in the cell walls. Curve *C* comes from figure 11 and illustrates the part played by evaporation. High humidity in this case lengthens the time necessary for killing. Field observations often show that sprays applied on a dewy night act more slowly because the acid is not concentrated by evaporation. The broken lines in these curves represent simply the tendency of the action. Final killing in these cases was from secondary effects.

Complete killing in the dipping experiments or on sprayed plots with a low concentration of acid is difficult to obtain primarily because of uneven distribution. The solution accumulates in drops in certain regions and remains only as a very thin layer in others. The portions that retain the greater amount of the solution are completely killed, but those intervening are not and will die only when their water supply fails from disruption of the conducting system.

These studies show that for rapid and complete killing of the foliage, a certain minimum quantity of acid must be used. Under average field conditions in California 3 gallons of 1.0 N sulfuric acid per square rod has proved a satisfactory minimum. This agrees well with the data in table 1. Where foliage is exceedingly heavy, the amount might be increased considerably. Since the active hydrogen ions in the solution are taken up by the plant buffers and effectively removed from the field of action, obviously this minimum quantity cannot be reduced without some loss in effectiveness.

BUFFER EFFECT OF THE ARSENIC COMPOUNDS IN THE SOLUTION

Since the arsenious ions used in this spray solution can form undissociated weak acid molecules, their buffer action upon the mixture should be studied. The dye "gentian violet improved" of the Coleman & Bell Company was found to give color differences throughout the necessary range of acid concentration, and to be unimpaired by the addition of the arsenic solution. Since the color of this indicator in strong acid solution fades rapidly, check series of acid solutions were used in each experi-

ment, and the arsenic-acid mixtures were compared rapidly with these. The hydrogen-ion concentrations of the standard acid solutions were determined from figure 2; those of the mixtures by color comparison with the standards. Figure 13 presents the data from these tests. When the acid arsenical solution contains $\frac{M}{20}$ As_2O_3 or less and sulfuric acid 1.0 N or higher in concentration, the decrease in hydrogen ions appears negligible. According to tests already described,[5] this is the maximum concentration of arsenic needed. The buffering action of the arsenic is therefore not of primary importance in the action of this type of spray.

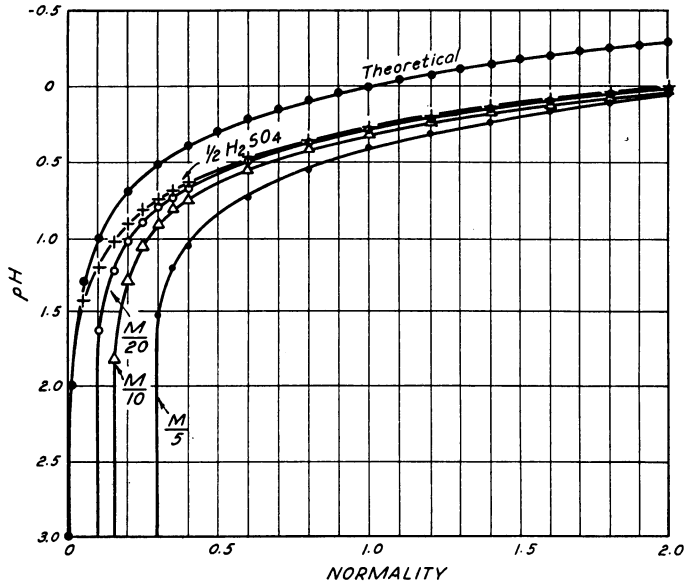

Fig. 13. Relation of pH to normality in acid arsenic solutions. M = Mols of As_2O_3 per liter.

CORROSIVE ACTION OF THE ACID REDUCED BY ARSENIOUS IONS

The arsenious ions in the solution do have one important function besides the killing of plant tissues. Sulfuric acid is notably corrosive to metal equipment. Although the Arizona workers used oil and grease to eliminate this trouble, their method is not entirely satisfactory; and losses of machinery largely explain the limited use of this effective chemical in weed control. The addition of a little sodium arsenite greatly checks this corrosive action. Table 3 presents some data on the effect of acid solutions, with and without arsenious ions, upon iron. Small pieces of iron rod were immersed in acid solutions with and without the addi-

tion of sodium arsenite. Obviously even the smallest addition of this chemical almost stopped the action, as has been noted when the acid arsenical solution was used in commercial spray equipment. The solu-

TABLE 3

EFFECT OF SULFURIC ACID AND SULFURIC ACID–SODIUM ARSENITE MIXTURES ON IRON*

(Weight in grams)

Solutions		Initial weight, Feb. 14	Weight on Feb. 19	Weight on Feb. 24	Weight on March 1	Weight on March 6	Weight on March 11	Weight on March 16
Acid normality	As_2O_3 concentration							
1.5	$\frac{M}{20}$	1.0408	1.0380	1.0350	1.0324	1.0295	1.0272	1.0244
1.5	$\frac{M}{40}$	1.0580	1.0550	1.0516	1.0492	1.0461	1.0444	1.0417
1.5	$\frac{M}{80}$	1.0983	1.0950	1.0912	1.0890	1.0860	1.0842	1.0818
1.5	....	1.0453	0.5914	0.1504	0.0130	.....	.....	.....
1.0	$\frac{M}{20}$	1.0513	1.0480	1.0442	1.0420	1.0394	1.0370	1.0342
1.0	$\frac{M}{40}$	1.0872	1.0836	1.0804	1.0788	1.0758	1.0740	1.0713
1.0	$\frac{M}{80}$	1.0346	1.0310	1.0274	1.0248	1.0218	1.0197	1.0168
1.0	....	1.0796	0.7026	0.2426	0.0400	.....	.....	.....
0.5	$\frac{M}{20}$	1.1016	1.0982	1.0940	1.0910	1.0876	1.0846	1.0815
0.5	$\frac{M}{40}$	1.0216	1.0172	1.0136	1.0110	1.0079	1.0053	1.0017
0.5	$\frac{M}{80}$	1.0022	0.9978	0.9932	0.9900	0.9870	0.9841	0.9807
0.5	....	1.0874	0.8142	0.4930	0.2834	0.1435	0.0736	.....

* Iron rods immersed in solutions.

tion may be used in large quantities with little or no injury to iron, bronze, or brass equipment. It will, however, rapidly etch and dissolve the porcelain lining from the cylinders of a spray pump and ruin it for orchard spraying. All pumps used should have bronze-lined cylinders.

DISCUSSION

The most obvious effect upon living protoplasm of solutions of high hydrogen-ion concentration is killing, evidenced by cessation of streaming, by swelling, and by complete loss of the property of semipermeability. Sulfuric acid is incorporated in the acid arsenical solution under consideration for the express purpose of killing the protoplasm and rendering it permeable.

The experiments described indicate that the killing action is directly related to the hydrogen-ion concentration of the solution. The temperature coefficient suggests that the reaction is preponderantly chemical. The principal retarding factors are diffusion through the cell walls and reaction with cell wall buffers.

Although the diffusion distance may be slightly increased by swelling of the walls with concentrated acid, the rate of diffusion is enhanced by the greater concentration gradient. Apparently, therefore, the most effective means of lowering the time of penetration is to increase the acid concentration. Likewise, the amount of acid available for the killing reaction after reduction by cell wall buffers depends, since the total volume applied is limited, upon the concentration. The rate of killing and arsenic penetration is therefore determined primarily by the concentration of the acid in the spray solution.

For mechanical and economic reasons, however, the acid concentration is limited. Experiments previously cited[5] indicate that an acid concentration of 1.0 N is optimum and that 1.5 N is of maximum effectiveness under ordinary conditions. The selection of the proper concentration under any particular set of conditions, however, depends upon several factors; and, though the values given may be generally applicable, even higher concentrations may be advised under special circumstances.

Evaporation is another important factor in the action of this type of spray. From the standpoint of the translocation mechanism, low evaporation resulting in a maximum available volume would be most effective. High evaporation, however, concentrates the solution, increasing penetration rate. Probably an intermediate value would be most practical. According to field observations, killing of the foliage should be evident within a half hour after the application and should be fairly complete after two hours. Translocation, though often very rapid,[4] is enhanced by uniform and thorough penetration. Only under limited conditions is the resultant of these several reactions most favorable for rapid and complete killing of the foliage and for thorough distribution of arsenic within the root system.

The exact relations between temperature, humidity, air velocity, and concentration of the spray solution are yet to be worked out. Eventually, it is hoped these will be known so accurately that the effectiveness of a treatment may be predicted. In the meantime, these variables must be appraised by the operator and properly considered in mixing and applying the spray solution. There is a wide latitude for adjustment of the acid concentration to fit conditions and only when this technique is mastered will the optimum results be obtained.

The acid arsenical solution discussed in these pages stands alone among the weed sprays that may be prepared from commercial chemicals generally available. Its particular value depends upon its ability to penetrate foliage tissues rapidly and, by utilizing the peculiar mechanical situation that develops within the plant, to be carried deep into the roots. The sulfuric acid in the solution is of fundamental importance in the process.

For several reasons sulfuric acid is adapted for its use in this method. It is nonvolatile at field temperatures, hygroscopic, nonoxidizing, inexpensive, and readily available. Arsenic trioxide is also cheap and convenient. At present market prices the spray solution can be made for as little as one cent per gallon and in large quantities should cost even less.

Although arsenic acid has been used as a herbicide,[7, 9] laboratory and field trials have shown its inferiority to the trivalent form. It is only one-half as toxic in equimolecular concentration, more difficult to handle under field conditions, and more expensive. Although it forms an acid solution at the concentration used for application, and although the commercial product often contains a contamination of nitric acid, the total acid content at field strength (for the arsenic) is not optimum. It has not compared favorably with the trivalent form in field-plot tests.

Two years of plot tests and field trials indicate that the simple formula previously described[5] leaves little room for improvement. Future experimentation will be directed toward improving the methods of application.

SUMMARY

In *Elodea* cells killed with strong sulfuric acid, protoplasmic streaming slows and ceases; the protoplasm becomes viscous, somewhat swelled, and completely permeable; and the chlorophyll changes to a light yellowish green. The chemical reaction resulting in death of the cell takes place very rapidly in strong acid.

There exists a straight-line relation between the time rate of killing plant cells and the hydrogen-ion concentration of the solution bathing them.

Ground foliage of morning-glory has a fairly large buffering capacity. It required 12.5 cc of 0.1 N HCl to shift 10 grams of this material from the initial pH of 5.8 to pH 4.0.

The cuticles of many leaves are fairly permeable to water vapor and molecularly dissolved solutes. Sulfuric acid readily diffused into morning-glory leaves. The stomata take little or no part in the penetration of acid sprays.

Morning-glory leaves immersed in sulfuric acid solutions of 1.0, 1.5, and 2.0 normal concentrations were killed in about the same time at

100° F. Leaves in 0.5 N acid were killed much more slowly. Lower temperatures also decreased the rate of killing.

Morning-glory shoots dipped in similar acid solutions and allowed to stand with their cut ends in tap water were killed at considerably lower rates. Those dipped in 0.5 N acid were injured somewhat less rapidly than the comparable immersed shoots and were never completely killed, because of the uneven distribution of the acid. Maintaining a saturated atmosphere around these shoots lowered the killing rate, especially for the lower acid concentrations.

Under ideal conditions of contact between spray solution and the plant, application of 3 gallons of 0.5 N acid per square rod would result in an average pH of 3.7 in the tissue, and killing would require 13.9 hours. For 1.0 N acid the pII would be 2.33 and the time about 35 minutes.

Under actual field conditions, application of 0.5 N acid at a rate of 3 gallons per square rod seldom completely kills the foliage of morning-glory. Stems and basal leaves remain spotted or uninjured.

At the recommended concentrations of acid and arsenic the latter has little buffering action upon the spray solution. It practically eliminates, however, the corrosive action of the acid upon iron equipment.

The acid arsenical solution etches and slowly dissolves porcelain. Spray pumps should be equipped with brass or bronze-lined cylinders.

There remains to be studied the relation between humidity, temperature, and air velocity as affecting evaporation after the spray is applied. An accurate determination of the effects of these should increase the probability of successful use of this method.

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