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The Production of Injury to Beans Leaves by Certain Arsenicals

THE PRODUCTION OF INJURY TO BEAN LEAVES BY CERTAIN ARSENICALS

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The Production of Injury to Bean Leaves by Certain Arsenicals

It has long since been proved that arsenical sprays injure the foliage leaves of many plants of economic importance. (5) The following pages contain the results obtained by experiments with sodium arsenite, calcium hydrogen arsenate and tri-calcium arsenate, applied to bean leaves. The object was to investigate, to some extent at least, the degree and kinds of injury produced by these three sprays, and the conditions governing its occurrence.

The writer is greatly indebted to Doctor W. A. Ruth for his kind advice and valuable suggestions, and wishes to express his hearty appreciation for his help and kindness.

I Materials

There are several methods by which sodium arsenite, Na_3AsO_3, can be prepared. The following method was used because of its simplicity. Twenty-five grams of pure anhydrous sodium carbonate, Na_2CO_3, were dissolved in 250 cc. of water in a 500 cc. measuring flask, and to it was added 4.95 grams of Baker and Adamson's C.P. arsenic trioxide, As_2O_3, and it was heated on the water bath until all of the arsenic trioxide was dissolved. It was then

cooled to room temperature. The resulting solution was alkaline because of the large excess of sodium carbonate used. Therefore, it was made neutral to litmus with hydrochloric acid, HCl, so that the effect, when used as a spray, would not be complicated by strong alkalinity.

Robinson's (8) procedure was followed for the preparation of calcium hydrogen arsenate, $CaHAsO_4$. He has shown that the salt is formed by the reaction between calcium chloride, $CaCl_2$, and sodium hydrogen arsenate, Na_2HAsO_4, according to the following equation:

$CaCl_2$ plus Na_2HAsO_4 equals $CaHAsO_4$ plus 2 NaCl. Solutions of calcium chloride and sodium hydrogen arsenate were prepared by dissolving 15 grams and 18.6 grams of each respectively. This gives an excess of calcium chloride over the amount required in the equation above. The solutions were slightly acidified with hydrochloric acid. After filtering, they were diluted to 250 cc. each. Then the cold solution of calcium chloride was slowly added to the cold solution of sodium hydrogen arsenate with constant stirring. A white precipitate formed which settled slowly. The clear, supernatant liquid was decanted and the precipitate washed further by decantation several times. Finally, it was brought upon the Buchner funnel and washed there with hot distilled water several times. It was then dried at room temperature. The dried salt was a white powder.

The method given by Robinson (8) for the preparation of tri-calcium arsenate, $Ca_3(AsO_4)_2$ from calcium chloride, sodium hydrogen arsenate and sodium hydroxide was tried without success,

because of the formation of a colloidal precipitate under many varying conditions of concentration and temperatures. It was found that a powdery precipitate was formed when lime water was substituted for calcium chloride. Calcium hydroxide, $Ca(OH)_2$, was prepared by slaking 20 grams of lime with a little water, diluting to 4000 cc. and filtering after standing for half an hour. Merck's C.P. sodium hydrogen arsenate was dissolved in water and just enough of a solution of sodium hydroxide, NaOH, was added to make it neutral to litmus. It was then filtered to get a clear solution. The lime water was slowly added to the clear solution of sodium arsenate with constant stirring. Immediately a voluminous precipitate was formed which remained in suspension. Upon heating to 90 degrees C. the precipitate settled very rapidly. The supernatant liquid was decanted and the precipitate was further washed several times by decantation with hot distilled water to get it free from sodium hydroxide (litmus). The precipitate was dried at room temperature and a white, apparently amorphous, salt was obtained.

Bush Bean (Phaseolus vulgaris L.) seed of the variety Extra Early Refugee was used. This was obtained in March, 1920, from Vaughan's Seed Store, Chicago, Illinois. The seeds were planted in rows, four rows in a flat and six seeds in a row. The seeds were planted on the twenty-sixth of March. The flats were watered whenever it was necessary.

The flats used were fifteen by twenty-two by three and one-half inches, inside dimensions. The soil used in the flats was prepared by thoroly mixing together four parts of ordinary garden

loam soil, two parts of well-rotted barnyard manure, and one part of sand.

[illegible]

[illegible]

Figure I. Arrangement of Treatments and Checks

Sodium arsenite spray

1

C	C
A	B
U.M.	U.M.

2

C	C
A	B
U.E.	U.E.

3

C	C
A	B
L.M.	L.M.

4

C	C
A	B
L.E.	L.E.

Calcium hydrogen arsenate spray

1

C	C
A	B
U.M.	U.M.

2

C	C
A	B
U.E.	U.E.

3

C	C
A	B
L.M.	L.M.

4

C	C
A	B
L.E.	L.E.

Tri-calcium arsenate spray

1

C	C
A	B
U.M.	U.M.

2

C	C
A	B
U.E.	U.E.

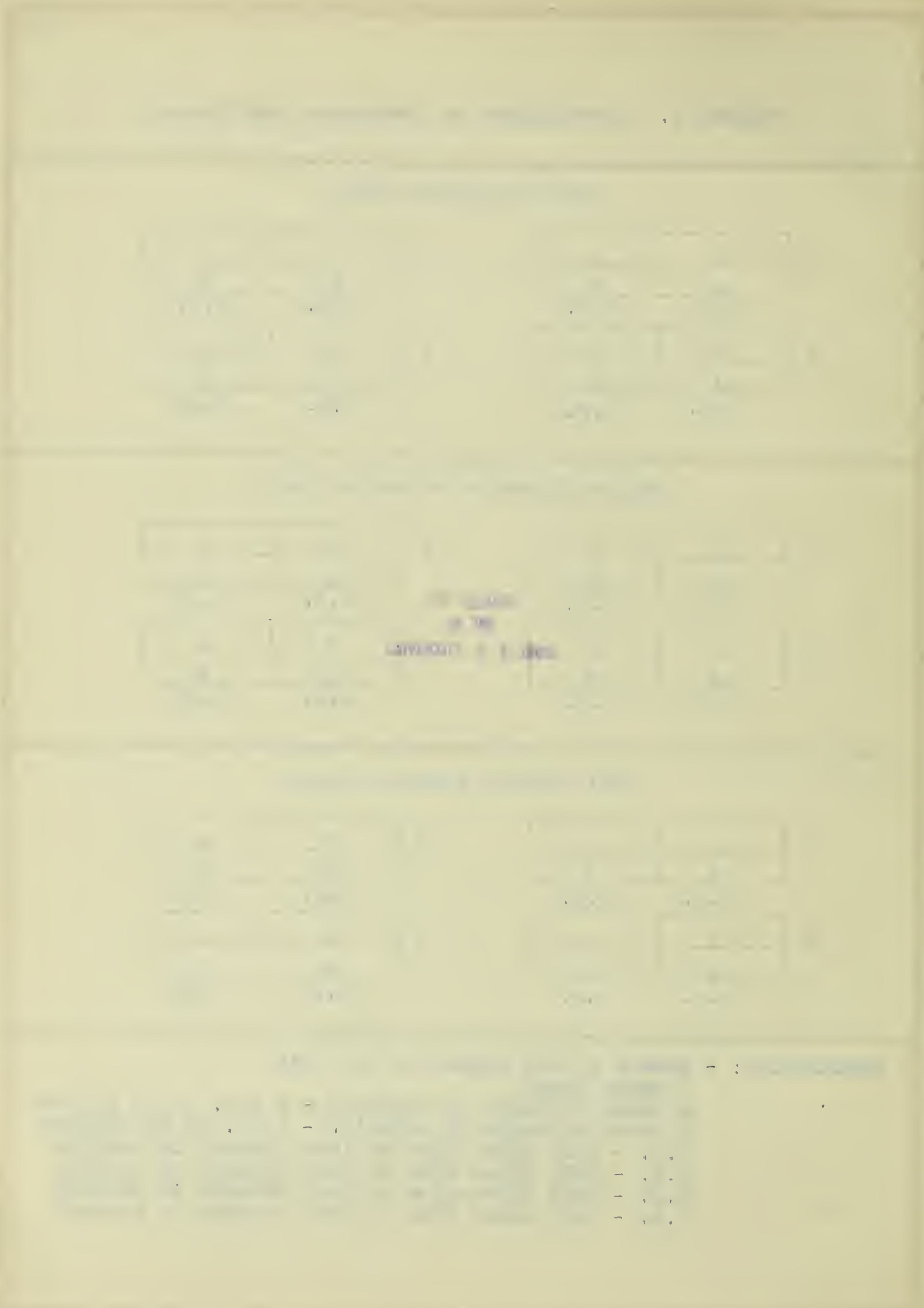
3

C	C
A	B
L.M.	L.M.

4

C	C
A	B
L.E.	L.E.

Explanation: - Number is the number of the flat
C means Check
A means strength of solution - 2 lbs. to 50 gallons
B means strength of solution - 1 lb. to 50 gallons
U.M. - only upper side of leaf sprayed in morning
U.E. - only upper side of leaf sprayed in evening
L.M. - only lower side of leaf sprayed in morning
L.E. - only lower side of leaf sprayed in evening



The young plants began to appear above the ground by the thirty-first of March. At the end of one week about 75 percent of the seed was up. No plants were observed to come up after this date.

II Experimental

Arrangement of treatments and checks was made in the manner shown in Figure I. The entire number of flats used was twelve. These were divided into three sets of four flats each. One set was for each of the three materials. Each flat was divided into four sections. Plants in two sections on one side and adjacent to each other were used for checks; and those in the remaining two sections of the same flat were used for spraying. The purpose in doing this was to insure uniform conditions of temperature, light and moisture to both the checks and the treatments.

Suspensions of the arsenates were prepared beforehand and were allowed to stand for three days before the first application. Only the supernatant liquid from the calcium arsenates was used in spraying. Suspensions were made of two different concentrations. One was made by suspending the material at the rate of two pounds to fifty gallons and the other was made similarly with one pound to fifty gallons. One pound to fifty gallons is the standard spray in general use. Sprays were applied with a DeVilbiss atomizer.

Spraying was done in the mornings and evenings to see whether the differing external conditions of light, heat and humidity

taken as a whole, caused variations in effect.

Upper and lower surfaces of the leaves were sprayed in order to determine which of the two surfaces was more susceptible to arsenical injury under the same conditions of temperature, light and moisture.

Dark green leaves and pale green leaves were sprayed and dusted with powders of calcium hydrogen arsenate and tri-calcium arsenate. The purpose in doing this was to see whether the leaves having more chlorophyll were more or less susceptible to the arsenical injury than those having less chlorophyll. It has been proved conclusively that dark green leaves contain much more chlorophyll than pale green leaves (4).

Upper and lower surfaces of leaves were dusted with powders of calcium hydrogen arsenate and tri-calcium arsenate, the leaves being kept dry. The object of doing this was to ascertain whether water was necessary for the production of injury. It is presumed that only arsenicals in solution cause injury (8) and by certain investigators that the carbon dioxide and water given off in respiration is a factor in the production of injury.

The first application was made on the sixth of April when the leaves were about five to seven cc. long. This was followed by two more applications on the same leaves, one on the seventh and the other on the eighth of April. The sodium arsenite and calcium hydrogen arsenate sprayings were discontinued after the third application because of the fact that the leaves sprayed with the former were totally destroyed by this time and those sprayed with the

latter had 40 percent of their surfaces injured. Spraying with tri-calcium arsenate was continued for five days. At the end of this period very little of the foliage was injured.

III Results

1. Leaves sprayed with sodium arsenite were completely destroyed by three applications. No difference in the severity and extent of injury was noticed between the leaves which were sprayed on their upper and lower surfaces, or between those sprayed in the evening and those sprayed in the morning.

2. Three applications of calcium hydrogen arsenite spray injured, on the average, 40 percent of the leaf surface.

3. No leaves sprayed with tri-calcium arsenate were injured as a result of five applications. After the fifth spray only a few spots were noticed, most of them being on leaves sprayed on the lower surface.

4. Leaves which were sprayed on the under surface were injured more than those sprayed on the upper surface.

5. Dark green leaves were less injured than pale green leaves.

6. A very little injury was produced by dusting of calcium hydrogen arsenate on lower and upper surfaces of leaves.

7. No effect whatsoever was noticed from dusting with tri-calcium arsenate.

8. The leaves which were sprayed in the mornings and

those which were sprayed in the evening showed no difference in the severity and intensity of injury produced.

Table I. - Comparative Injury [1] Produced by Applications of Calcium Hydrogen Arsenate and Tri-Calcium Arsenate on Upper and Lower Sides of Leaves

[1] Estimation of injury was made by taking the whole leaf surface as 100 and the injury is expressed here in percentage. For example, if the injury is stated to be 10, it means that 10 percent of the foliage surface was destroyed.

Spray material used	Strength of Spray solution	Injury produced by spraying on upper surface	Injury produced by spraying on lower surface	Average percent of injury	Number of applications
$CaHAsO_4$	2# to 50 gall.	30	50	40	3
$CaHAsO_4$	1# to 50 "	20	40	30	3
$Ca_3(AsO_4)2$	2# to 50 "	.01	.1	.055	5
$Ca_3(AsO_4)2$	1# to 50 "	.001	.005	.003	5

The table given above shows that calcium hydrogen arsenate produced very much more injury than tri-calcium arsenate; and that the injury to leaves which were sprayed on the lower side is considerably more than the injury to leaves which were sprayed on the upper surface. In other words, the lower surface of bean leaves is more susceptible to calcium arsenate spray injury than the upper surface of bean leaves.

IV Character of Injury

Injury appears to occur anywhere on the leaf. The injured tissue before it dried, was lighter by reflected, but darker by transmitted, light. It soon dried and the color of the dried tissue when held against the light still was dark. Freehand microscopic sections of freshly injured tissue were made by cutting in elder pith. Under the microscope the injured tissue appeared black. Upon closer examination it was seen that the chloroplasts were dissociated. The section on the slide was heated with a drop of water to see whether the dark color was due to the presence of air, but the dark pigment still remained. It was then heated with 95 percent alcohol to see whether the dark pigment resembled chlorophyll in being soluble in it. But the dark pigment still persisted as before and the alcohol did not become green. Therefore, it appears that a new dark pigment was produced at the expense of the chlorophyll.

V Discussion

The results stated in the foregoing pages can now be discussed. The weather record [1] shows that the weather during the

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[1] The weather conditions:- April 6, fair and warm; April 7, fair and warm; April 8, fair and warm in the morning and at noon but cloudy and warm in the afternoon and evening.

first two days [1] when the sprays were being applied, was fair and

[1] The weather conditions during night times were different from those during day times; the conditions with respect to temperature and humidity, at least, were, however, more or less uniform in the greenhouse during night times.

warm. Change in the weather came in the afternoon of the third day, when it was warm and cloudy. The sodium arsenite and calcium hydrogen arsenate, put on during these three days only, produced injury independent in severity and extent of the weather. Injuries produced by the sprays applied in the mornings and in the evenings did not differ in any way as regarded severity and amount. Again, on the fourth and fifth days there was a sudden drop in the atmospheric temperature and it was cloudy. During these two days the tri-calcium arsenate produced a little injury, while it had produced no injury during the first three days when the weather was fair and warm. It seems probable that the difference is to be attributed to the slower drying of the spray during the cold, cloudy weather altho no notes were made of the rate at which the spots of spray dried under the two conditions of weather.

The results show that the lower surface of the bean leaf is more susceptible to arsenical injury than the upper. It has been proved that "all plants have the power of absorbing water thru their leaves" (6). Burgerstein (3) says that "in general, the plants in which the absorption of water by leaves has been shown reveal a wide range in organization, mode of life and systematic

position. Therefore, since no exception has been shown it may be assumed that all plants have the power of absorbing water directly thru their leaves". Discussing the means by which the leaves absorb water he further observes that "the water can reach the interior of the leaf thru the epidermal cells, thru the hairs, and thru the stomata. The lower epidermis absorbs more actively than the upper as investigations have thus far shown. As a rule three factors combine to cause the greater absorption by the lower side of the leaf: (a) The fainter cuticularization of the outer walls of the epidermal cells; (b) the greater abundance of hairs; and (c) the greater numbers of stomata" (1).[1] The bean leaves under the

[1] Haberlandt, (6) asserts that under no circumstances do stomata absorb water.

microscope showed more stomata and hairs on the lower surface than on the upper. It then appears that the greater susceptibility of the lower surface of the bean leaves is directly related to the presence of a greater number of stomata and hairs on it. It may be questioned, however, as to the possibility of absorbing arsenical solutions. This has been proved conclusively by Noussingault (2) who says "that not only leaves may thus take up water but also at the same time will take up saline substances in solution".

The greater susceptibility of pale green leaves to the arsenical injury may be indirectly associated with the fact that pale green leaves contain a smaller amount of chlorophyll than the

dark green leaves (4). The smaller the amount of the chlorophyll, the less is the activity of the chloroplasts and the protoplasts in general; and a change in the susceptibility of the protoplast may be anticipated. A difference in the structure of the tissues may also be a factor, e.g., of the epidermis, cuticle, and hairs.

Dusting with calcium hydrogen arsenate, the leaves being kept dry, produced very little injury. Gillette (5) found the same thing with arsenites. But a contrary view is shared by Patten and O'Mara (7) who say that most of the injury that occurs from arsenates is due to the dissolving of arsenates by the carbon dioxide and water liberated in respiration. All plants give off water and carbon dioxide at night in the process of respiration. The water thus given off is charged with the carbon dioxide and forms a thin film on the surface of the leaf. Patten and O'Mara have, indeed, proved that carbonated water dissolves arsenates much more than ordinary water, but this explanation, it seems from the author's experiments, does not hold. Tri-calcium arsenate, which is very slightly soluble in water and is much more soluble in carbonated water, produced no injury whatsoever when applied in the dry form to the bean leaves. Injury, therefore, must have been initiated immediately after the wet material is applied, while liquid water was still present. Neither does the evolution of carbon dioxide in respiration seem to be an important factor in the production of injury since no more injury occurred following applications made just before it became dark in the evening than followed early morning applications.

VI Conclusions

1. Sodium arsenite produces the most injury to bean leaves.

2. Tri-calcium arsenate produces the less injury to bean leaves than calcium hydrogen arsenate.

3. The lower surface of bean leaves is more susceptible to arsenate injury than the upper.

4. Injury is initiated immediately after the material is applied.

5. Injury depends upon liquid water, applied in this case with the arsenical.

6. Respired water is not enough to initiate injury.

7. The arsenate injury to bean leaves causes the dissociation of chloroplasts and the formation of a black pigment which is soluble in water and alcohol.

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