# EFFECTS OF CERTAIN INSECTICIDES AND INERT MATERIALS UPON THE TRANSPIRATION RATE OF BEAN PLANTS<sup>1</sup>

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(WITH SEVEN FIGURES)

# Introduction

The changes in physiological processes of plants brought about by the application of spray materials are not well understood, nor have they been investigated in detail. That such changes do occur is evidenced by both the beneficial and the harmful effects of these compounds quite apart from their value as fungicides or insecticides. The increased growth and greater yield of potatoes sprayed with Bordeaux, the shot-hole effect of copper fungicides on peach leaves, the russeting of fruit and burning of foliage by arsenicals all point to profound changes in the physiology of the sprayed tissues.

In an attempt to determine the basis for the relative "safeness" of certain arsenates as compared with others, investigations into the effects of both kinds of sprays on the physiological processes in plants have been made. The process most widely studied in this connection has been transpiration. Some writers have suggested that the phytocidal action of certain sprays might be attributed, at least in part, to the sudden increase in the rate of water loss from the sprayed plants. Thus it was thought that perhaps a so-called "safe" compound which did not cause the typical burning of sprayed leaves, was innocuous because it did not bring about the increase in water loss which resulted when the injurious sprays were applied to the leaves.

Not all investigators are agreed as to the effect of sprays on transpiration. Many have recorded results in which there was no increase, but a decrease, or no change at all in the rate of water loss. In fact, it has been suggested that the stimulatory effect of Bordeaux on potatoes may be attributed to a conservation of the water supply.

The earlier workers investigated the effect of Bordeaux on crop plants. In general, their results showed a decrease in the transpiration rate of the sprayed plants as compared with that of the controls. Many of the data, however, were not very quantitative.

Some time later, DUGGAR and COOLEY (3), in studies of cut plants in potometers, found that Bordeaux increased the transpiration rate of castor bean, squash, Phytolacca, potato, and Iresine. They also studied the rates

<sup>1</sup> Papers from the Department of Botany no. 406, the Ohio State University. This work was done in cooperation with the Sherwin-Williams Co., and the Ohio State University Research Foundation.

of water loss from potted plants of potato and tomato with the same results. The tomato plants did not show an increase in transpiration resulting from the application of calcium hydroxide, clay, aluminum hydroxide, calcium carbonate, or lime-sulphur 1-25, but the potato plants lost more water when sprayed with lime water, Bordeaux and lampblack, lampblack alone, lime-sulphur, and lime wash, than did the control plants. Lime-sulphur did not cause an increase in water loss.

MARTIN (8) reported that data from potometer experiments with leaves of radish, bean, swiss chard, Hibiscus, Clerodendron, Caladium, Datura, and castor bean, showed that spraying with Bordeaux brought about a rapid increase in the transpiration rate, which later tended to return to the original rate. In some experiments with potted plants of tomato, cabbage, pepper, eggplant, and soybean, the rate of water loss was increased after the application of Bordeaux although this increase was more pronounced for the cut leaves in potometers. The effect was greatest during the first 2-hour period following application, and showed a variation in the amount of effect with the species.

SHIVE and MARTIN (10), using standardized cobalt chloride paper, studied the effect of Bordeaux on the daytime transpiration rate of tomato plants in the field and found that there was a decided increase after spray-DUGGAR and BONNS (2), by weighing potted plants of potato, tomato, ing. marguerite, and tobacco, obtained data which indicated that the increase in the rate of water loss resulting from the application of Bordeaux mixture occurred almost entirely during the night. Potted plants of Cyperus escu*lentus* showed no increase. Leaves of castor bean in a potometer gave a continuously increased rate of transpiration, the increase not being confined to the night period. MARTIN and CLARK (9) experimented with potted potato plants grown in soils of three different moisture contents, and found that the greatest average increase in water loss occurred in the plants grown at the highest soil moisture content. WILSON and RUNNELS in several papers (12 to 19) report an increased transpiration rate of different species for plants sprayed with Bordeaux and other compounds. They also found that the greatest increase in water loss occurred at night. CHILDERS (1) found, on the contrary, no increase in the rate of water loss from potted tomato plants sprayed with Bordeaux. KRAUSCHE and GLEBERT (7), in a study of tomato plants treated with copper sprays, reported an increase in the transpiration rate, especially at night. They could find no visible change in the stomata after spraying.<sup>2</sup>

Recently some experimentation has also been done on the effects of oil sprays on the transpiration of sprayed plants, from which data it is apparent

<sup>2</sup> See also HORSFALL, JAMES G., and HARRISON, A. L. Effect of Bordeaux mixture and its various elements on transpiration. Jour. Agr. Res. 58: 423-443. 1939, for additional data published since the completion of this paper. that the application of oils brings about a reduction in the rate of water loss (5, 6). Very little work has been done on calcium arsenate in this connection.

# Methods

Two different methods of determining the rate of water loss before and after spraying were used. For both of these methods, the test plants were grown in the same way and used when at the same stage of develop-The cranberry bean seeds were selected for uniformity in size, the ment. average sized seeds being chosen; the large or small beans were discarded. They were soaked for six hours, drained, allowed to germinate in a moist atmosphere, and then planted in sand in paraffined trays watered with a cotton wick dipping into an attached water jar (11). All of the plants to be used indoors were kept in a culture room until used, while those to be used in the greenhouse were moved into it when the hypocotyls were about ten centimeters long. By indoors is meant a basement culture room kept at a constant temperature, 24° to 26° C., and a constant relative humidity of 50 per cent. Light was supplied by several 100-watt lights for 19 hours each day. The room was aerated with fans, but free from drafts, being divided into compartments which deflected the air currents. This room was an ideal place for experimentation with potometers. The plants were used for experimental purposes as soon as the first two leaves were fully expanded. The terminal bud was removed to prevent further growth of the plants, since they became very weak and spindly under the artificial light. In order to keep the experimental conditions uniform, this procedure was followed with the greenhouse plants as well.

### POTOMETER DETERMINATIONS

While doubts may be raised as to the quantitative value of data derived from studies of water absorption by cut plants in potometers, the fact remains that this method affords a means of determining rapid changes in the rate of water intake. With mature plants over short periods of time these changes in the rate of absorption should reflect rather accurately the rate of water loss from the leaves. That the transpiration rate of cut shoots differs quantitatively from that of intact plants cannot be denied, but experiments on different samples under the same environmental conditions should yield comparative data of value.

Four potometers were arranged under a light in a draft-free compartment in the constant temperature culture room. The bean stems bearing two well expanded unifoliate leaves were cut under water, inserted into holes in a rubber stopper which was quickly sealed into the potometer; two stems were used for each potometer. The different samples were allowed to stand for an hour. At the end of this period readings were made every five (or in some cases, ten) minutes for an hour; the test materials were then applied and as soon as the leaves were dry, readings were again made for several hours. A duplicate series was run the next day, using fresh plants and changing the order of the potometers to obviate any effects of variations in the tubes or differences in the light conditions. Some tests with an atmometer, substituted for the bean shoots, showed that fluctuations caused by slight changes in the environmental factors were negligible. The amount of water loss from the atmometer was quite constant, showing none of the fluctuations characteristic of the curves which show the water absorption by bean stems.

Preliminary experimentation showed that variation in the method of applying the spray materials made no important differences in the order of the results obtained. A standard procedure was adopted which consisted of wetting the under surfaces of all of the leaves with distilled water from an atomizer, dusting the wetted areas with the materials to be tested, and then rewetting the dusted surfaces until droplets of a suspension This method of application brought about the least injury with formed. unsafe materials and eliminated any possible differences due to light effects In all of the experiments, one potometer conon the whitened surfaces. tained check plants sprayed with water only, one contained plants treated with ordinary commercial calcium arsenate, and the two remaining were used to test various other materials. The materials tested were: zincsafened, basic, and ordinary commercial calcium arsenates, lead arsenate, a solution of dicalcium arsenate, Bordeaux, copper sulphate, lime, and Ban-The ordinary commercial calcium arsenate gave a test for croft clay. 5 per cent, water soluble arsenic when 2 grams in a liter of water were in equilibrium with the carbon dioxide of the air, and caused 100 per cent. injury on bean leaves kept wet for 2 hours after spraying. The basic calcium arsenate under the same conditions yielded 2.5 per cent. water soluble arsenic and brought about only moderate injury on sprayed bean leaves kept wet for two days, while the zinc-safened material showed 0.7 per cent. water soluble arsenic, and caused only slight injury after the sprayed leaves had been kept wet for two days after spraying.

Two checks on the changes in the rate of water absorption were possible. One was the rate of absorption of all of the plants before treatment with the test materials, the other was the rate of absorption of the check plants throughout the experiment. The importance of carrying out potometer experiments under as constant conditions as possible is quite apparent. For determinations over short periods of time, such as 5- or 10-minute intervals, air currents can cause a considerable error, which may not become apparent if the check samples are not touched by the moving air. Hence for such measurements, the environment outdoors, or even in the greenhouse, was too variable from moment to moment to allow accurate readings to be made.

## EXPERIMENTS WITH POTTED PLANTS

In order to obtain data showing the effects of spray materials over a longer period of time, some experiments were set up using bean plants grown in sand in paraffined trays fitted with bottles which furnished a constant water supply to the sand through a cotton wick (11). The water in the bottles was replenished as often as the level became low. At times there was a change of five centimeters in the water level. The roots appeared

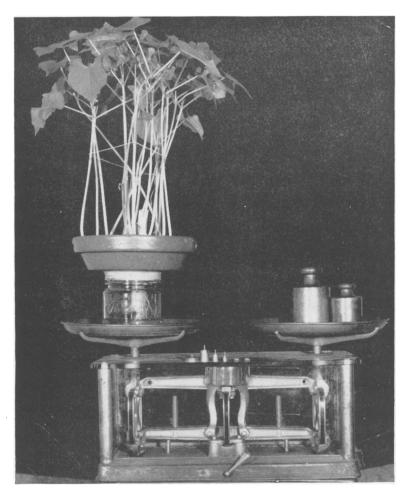


FIG. 1. Photograph of one of the trays of bean plants used in the weighing experiments to determine the amount of water lost by the plants.

healthy and apparently were not injured by the rather poor aeration of the sand. In the indoor culture room, the plants remained in good condition for from 2 to 3 weeks when the terminal bud was removed regularly. Figure 1 shows the tray with the attached water supply as the samples were weighed to determine the amount of water loss.

When the plants had fully matured, unifoliate leaves, the stand was thinned to 14 plants per tray, the sand covered with a paraffin-vaseline sealing compound, and the various trays arranged indoors or in the greenhouse. A control, set up in the same way but without plants, lost no weight over a period of several days. Two or three trays were used for each material tested, and, in addition, the experiment was repeated at least twice, each time with slight variations in procedure but showing no significant differences in the order of the results. The trays, including the jars of water, were weighed twice daily just after dark at night, and just before daylight in the morning. In the winter months, this was about 6:00 A.M. and 6:00 These data gave an indication of the effects of periods of light and P.M. darkness upon the transpiration rate of the greenhouse plants. A duplicate series was also run indoors and weighed at the same time; the plants had a longer light period indoors since the lights were on 19 hours a day.

The plants were weighed morning and evening for three or four days, before treatment with the spray materials, in order to get the relative rates of the various samples. The different trays were selected so that the samples used for each compound were assorted with one tray of plants showing a high transpiration rate, one an average rate, and one a low rate. An attempt was made to distribute the different samples at random, and to arrange them so that one of each set was surrounded by plants and the other two were at opposite edges of the table. A comparison of the rates of water loss from samples at different positions on the table did not reveal any correlation between the amount of transpiration and the position of the tray. As a precautionary measure, however, the random distribution was used.

After treatment with the test materials, the plants were weighed for ten days or two weeks afterwards depending upon the condition of the plants. There was some increase in leaf areas of the unifoliate leaves under greenhouse conditions but it appeared to be approximately equal for all of the samples. In one series, the terminal bud was allowed to grow, with the result that the plants differed considerably in amount of growth; the ones sprayed with zinc-safened calcium arsenate showed the greatest growth and made such a difference in leaf areas that the transpiration rates of the different samples could not be compared. A certain amount of injury with the ordinary commercial calcium arsenate was unavoidable, but was kept at 5 per cent. or less. The materials were applied as dusts to the wetted undersurfaces of the leaves as in the potometer experiments. The compounds tested were: zinc-safened, basic, and ordinary commercial calcium arsenates, a solution of dicalcium arsenate, copper sulphate, lime, silica,<sup>3</sup> and Bancroft clay.

Some tests using cobalt chloride paper on bean plants in the field were set up to determine the time when the night changes in water loss occurred. The data, however, were difficult to interpret and yielded no significant

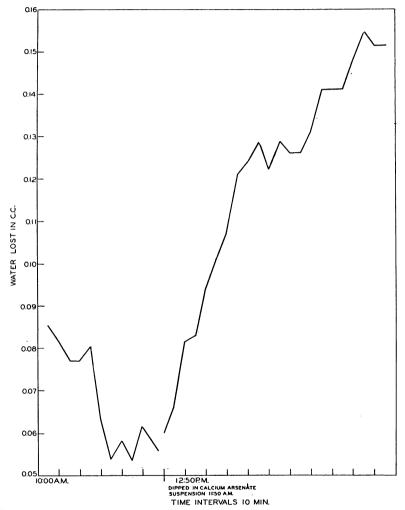


FIG. 2. Potometer curve of the rate of water absorption by bean shoots before and after treatment with ordinary commercial calcium arsenate.

<sup>3</sup> The words silica and silicon dioxide are used interchangeably, both referring to the pure compound silicon dioxide in powdered form.

information. Several errors were possible. One was the effect on the cobalt chloride paper of the material dusted on the leaves, and another was the error introduced by the presence of condensed moisture on the leaves at night. In addition, the color change was difficult to determine with artificial light. Since the data from the other methods showed such uniformity of results, it was felt that this method was in error rather than that the other data were invalidated.

# Results

Figure 2 shows the immediate increase in the rate of water absorption of the bean shoots after treatment with ordinary commercial calcium arsenate. This result was characteristic of all the materials tested. Whether the effect was to increase water absorption or to decrease it (as in the case of copper sulphate), the change in rate was apparent as soon as the leaves had dried. The results shown in figure 3 indicate that the zinc-safened

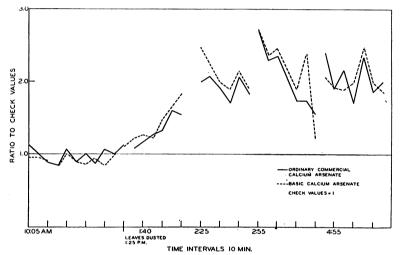


FIG. 3. Potometer curve of the rate of water absorption by bean shoots before and after treatment with ordinary commercial and basic calcium arsenates.

calcium arsenate brought about nearly the same amount of increase in water absorption as did the ordinary commercial material when both are compared with controls sprayed with water. For the preceding graph and for those that follow, the check values were reduced to unity in order to reduce the number of graphs per figure and to show the results more clearly. For these calculations, the average number of grams of water lost by the check samples was divided into the average number of grams of water lost by the samples treated with any one material for that same period of time. In this way a correction was made for weather changes such as rain following sunshine, as well as for the physiological changes taking place in the plants with the passage of time. The check value, therefore, became a straight line. The deviations from the line by the other curves should indicate the relative amount of change brought about by the application of the test materials.

Other materials which brought about increases in the rate of water absorption as measured by potometers were lead arsenate, Bordeaux, hydrated lime, and Bancroft clay. The application of copper sulphate under the same conditions resulted in a decrease in the rate. During the course of

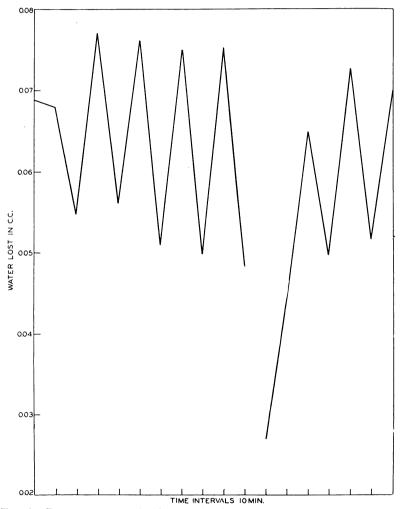


FIG. 4. Potometer curve showing rhythm in the rate of water absorption by two shoots of the check plants.

experimentation with cut plants in potometers, some curves of water absorption were obtained which showed unusual rhythmic fluctuations (fig. 4). Tests with an atmometer in place of the bean stems showed that these variations were not a result of changing environmental conditions, such as air currents, nor of physical forces involved in the movement of a bubble down the tube, for the atmometer curves showed no such variations. Apparently

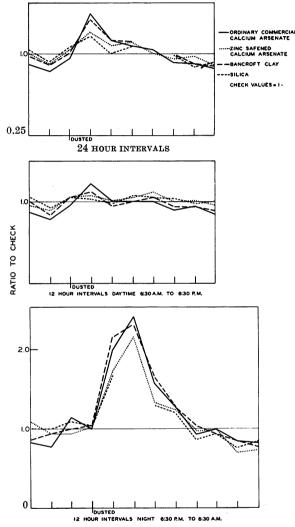


FIG. 5. Relative amounts of water lost from check plants and plants treated with certain materials applied as dusts to the wetted leaves. The test samples were potted bean plants in the greenhouse.

the fluctuations were brought about by physiological changes within the plants,—perhaps a lag of water absorption by the stem behind the water loss from the leaves. Sometimes the data from two samples run on the same day would show these variations, with one curve showing maximum where the other showed minimum.

Figure 5 and table I show the comparative effects of the zinc-safened and ordinary calcium arsenates, Bancroft clay, and silica, on the amount of water loss from potted bean plants in the greenhouse. The table is included to show the absolute amount of water lost by the different samples. The amounts of loss for the 24-hr. period and the 12-hr. periods of daylight and darkness show that the greater change occurs during the night period. These results are in accord with those of DUGGAR and COOLEY (4), and Wilson and Runnels (12). The total amount of water lost by the treated plants for the 24-hr. period was not much greater than that lost from the check plants (for the greenhouse samples), which fact may account for some of the discrepancies in the literature. The 24-hr. measurements might not reveal the great increase in transpiration of the sprayed plants at night as compared with the checks, since the total number of grams of water lost by any of the samples at night is usually so much less than the amount lost during the daytime that the changes might not be apparent.

The curves also show that such materials as silica and Bancroft clay can bring about increases as great as those resulting from the application of ordinary spray materials. To ascertain whether or not these results might be attributed to some chemical contamination of the silica and Bancroft clay, another series was run, using these materials washed until chemical tests showed scarcely a trace of any other materials present. The results were the same for the second series. After several days the rates of all of the treated plants began to approach the original rates of water loss.

The curves (fig. 6) and data in table II show the effects of the same materials tested on a similar series of plants kept indoors in the culture The high rate at one time before treatment is apparently caused room. by an error in recording the loss in weight of the check plants. Here there is no pronounced difference between the daytime and the night losses. These plants had only 5 hours of darkness, being illuminated for 19 hours every day with electric lights. These curves show a separation into pairs, the ordinary commercial calcium arsenate and the Bancroft clay bringing about approximately the same increases, and the zinc-safened calcium arsenate and the silica showing somewhat similar effects. Here the application of a zincsafened material did not bring about as great an increase in the transpiration rate as did the ordinary commercial calcium arsenate. The effect of the latter, however, may be somewhat exaggerated, because the plants in these samples showed as high as 10 or 20 per cent. injury. What the water

			WAI	WATER LOSS IN		24 HOURS (AVERAGE OF	TERAGE C	JF TWO TRAYS)	RAYS)					
SAMPLE	JAN. 7	JAN. 8	JAN. 9	JAN. 10	JAN. 11+	JAN. 12	JAN. 13	JAN. 14	JAN. 15	JAN. 16	JAN. 17	JAN. 18	JAN. 19	JAN. 20
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Crdinary commercial calcium arsenate		65	80	58	!	66	16	92	98	84	105	93	79	104
zinc-safened calcium arsenate		99	83	58		79	82	84	95	81	102	87	72	83
Bancroft clay	1	76	90	63		86	87	94	104	88	102	93	83	101
Silicon dioxide Check		72	$\begin{array}{c} 92 \\ 101 \end{array}$	65 61		82 66	86 79	00 co 00 co	97 91	06 88	101 110	$95 \\ 102$	88 86	80 90
			WATER	TOSS DU	WATER LOSS DURING THE	PERIOD	6:45	A.M. TO 6:	6: 45 P.M.					
Ordinary commercial calcium arsenate		54	70	48		55	74	69	62	63	65	67	53	61
arsenate calculu		56	72	48		46	68	65	78	62	65	67	50	58
Bancroft clay		62 62	78 81	53		43 48	71 73	202	81 80	66 71	65 65	202	59 78	58
Check Check		61	88	20		46	72	89	76	99	66	73	56	57
			WATER	LOSS DU	WATER LOSS DURING THE PERIOD 6:	PERIOD	45	P.M. TO 6:	6:45 A.M.					
Ordinary commercial calcium arsenate	11	10	10	10	44	17	23	19	21	41	26	26	43	46
arsenate carcium	10	II	10	10	33	14	19	17	19	37	20	22	25	29
Bancroft clay Silioon diavida	14	11	91	11	43 34	16	24	23	52 0	37	5 53	24 24	43	41 73
Check	11	13		10	50	21-	12	12	38	44	68 68	308	33	20

20 were treated with the test materials. † The plants were treated with the test materials during the afternoon.

TABLE I

# PLANT PHYSIOLOGY

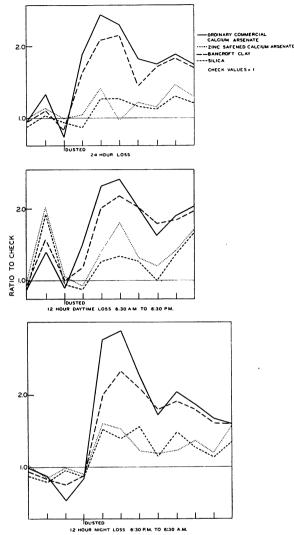


FIG. 6. Relative amounts of water lost from check plants and plants treated with certain materials applied as dusts to the wetted leaves. The test samples were potted bean plants in the indoor culture room.

loss from such injured areas may be has not been determined. For these plants the increased rates of water loss do not tend to return to the original rates.

A comparison was then made of the effects of washed Bancroft clay and silica suspended in water and in a solution of dicalcium arsenate containing one-half per cent. water-soluble arsenic. The leaves were dipped

			WATI	WATER LOSS	R	24 HOURS (AVERAGE	ERAGE OF	TWO TRAYS)	60100					
SAMPLE	JAN. 7	JAN. 8	JAN. 9	JAN. 10	JAN.† 11	JAN. 12	JAN. 13	JAN. 14	JAN. 15	JAN. 16	JAN. 17	JAN. 18	JAN. 19	JAN. 20
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Crdinary commercial calcium arsenate		21	22	13		47	44	39	34	31	30	29	28	35
Line-sarened calcium arsenate Bancroft clay Silicon dioxide		222822	19 27 21 21	17 14 20		$29 \\ 25 \\ 25 \\ 29 \\ 29 \\ 29 \\ 29 \\ 29 \\ $	26 18 23 8 23 8 23 8 23 8 20 8 20 8 20 8 20	20 37 21	22 35 18 18	19 31 18 18	21 29 17	25 31 19	$234 \\ 244 $	$\begin{array}{c} 18\\36\\24\\21\end{array}$
			WATER	LOSS DI	WATER LOSS DUIRNG THE PERIOD 6	PERIOD	: 45	A.M. TO 6:	45 P.M.		_			
Ordinary commercial calcium arsenate		12	13	œ		52	22	20	18	15	14	15	14	17
zine-sarenea calcium arsenate Bancroft clay		12	10	<b>00 I</b> ~		14 28	13 19	10	11 18	$10\\16$	10 14	$12 \\ 16$	$10\\18$	10 22
Silicon dioxide		11	9 11	8 [3		14 16	12	11 9	11 9	9 10	10 8	6	$\frac{12}{14}$	13
		-	WATER	LOSS DU	WATER LOSS DURING THE PERIOD	PERIOD	6:45	P.M. TO 6:	45 A.M.					
Ordinary commercial calcium arsenate	6	6	5	7	25	22	19	16	16	16	14	14	18	14
bine-sarened carcium arsenate Silicon dioxide	120112	0.6186	0, 1 0 0	8 8 E s	15 14 18 10	11 119 119	10 118 10 8	10 11 10	11 11 11 12	112	10 110	13 12 12 12 13	111111111111111111111111111111111111111	10 12 12 12

TABLE II

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# PLANT PHYSIOLOGY

in a 2-100 suspension. The silica was further separated into coarse and fine particles by allowing it to settle in water. An attempt was made to separate out the particles smaller than the stomatal aperture of the bean leaf from those larger. It is apparent from the curves shown in figure 7

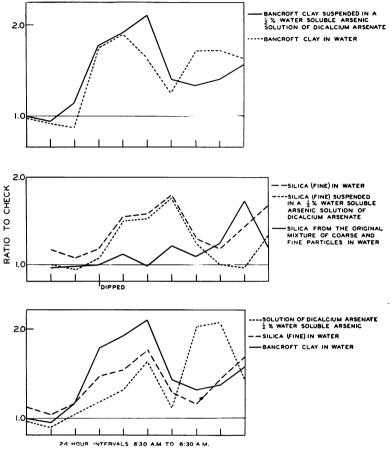


FIG. 7. Relative amounts of water lost from check plants and plants dipped in suspensions of the test materials. The samples were potted bean plants in the indoor culture room. Check value = 1.

that particle size affects the amount of change in the rate of water loss. It is also obvious that chemically inert materials can bring about quite as large increases in the transpiration rate as physiologically active materials such as dicalcium arsenate.

### Discussion

From the above results, several interesting conclusions may be drawn. In the first place, it is apparent that the relative safety of one calcium arsenate over another is not correlated with its effect on water loss. Second, chemically inert and innocuous materials such as Bancroft clay and silica, as well as such a physically inert material as soluble dicalcium arsenate, can both bring about very significant increases in the transpiration rate. Particle size is a factor affecting the amount of increase. Third, for greenhouse plants, the greatest relative increase in water loss of the treated plants occurs at night.

The question then arises as to the nature of the action of these materials which bring about such changes in the transpiration rate. Very few explanations have been offered in the literature. Early workers suggested the effects of the white coating on light absorption, leaf temperatures, or photosynthesis. These factors could not have entered into the problem here because the test materials, with one exception, were always applied only to the undersurfaces of the leaves. DUGGAR and BONNS (2) suggested the influence of surface films on water loss from the stomata and hydathodes since they also found a decrease in the effect with time. KRAUSCHE and GILBERT (7) thought there must be an effect on the cuticular transpiration.

It is difficult to see what changes in cuticular transpiration could take place after the application of chemically inert materials in the form of dusts. It is hardly possible that compounds like chemically pure silicon dioxide could change the permeability of the epidermal cells to such an extent that more water could pass through the walls, especially when they are applied in such a manner as to prevent injury to the leaf. Since differences in particle size brought about significant differences in the amount of change in the rate of water loss, it appears that perhaps an effect on the stomata might explain the facts more easily.

It is significant that the greatest difference in the amount of water loss between the treated plants and the check plants in the greenhouse should occur at night. Since cuticular transpiration presumably continues at all times during the 24-hr. period, and is not influenced by the presence or absence of light as is stomatal transpiration, any differences in this rate should be apparent in the daytime as well as the night. It should also show up as a greater increase for the 24-hr. period as well. From these data, this The davtime, and even at times the 24-hr. increase in rate. is not the case. is almost negligible for the greenhouse plants. Even though the amounts of water lost at night are small, it seems that there should be more of a difference than there is in the daytime rate since the increases would be cumulative for the entire 24 hours. On the other hand, if one postulates that the effect is on stomatal transpiration, it is easy to account for the fact that the increase is always greater at night and almost negligible during the day, when the stomata would be open anyway.

Examination of the dusted leaves with a microscope showed particles

partially blocking the stomata. This observation, coupled with the fact that the smaller sized particles brought about a greater increase in the transpiration rate, indicates that the blocking of stomatal openings by these particles might prevent complete closure of the stomata. Since not all of the stomata would be blocked, necessarily, the rate of water loss would not be expected to approach the daytime rate; it would, however, be greater than the cuticular loss alone. That the greenhouse plants outgrow, so to speak, the effect of these materials, seems to be a result of an increase in area of the sprayed leaves, maturation of undeveloped stomata, and a gradual loss of the spray particles. Thus transpiring areas unaffected by previous treatment are acquired by the plant.

When indoor plants were used in the same experiment, there was neither the great change in the night rate of transpiration, nor the gradual return of the increased rates to the original values. The first effect appears to be related to the day-night rhythm of transpiration effective in the greenhouse, and the second to the increase in area of the spraved leaves which does not take place indoors under artificial light. Under these conditions, the accelerating effect of the inert materials (Bancroft clay and silica) upon the rate of water loss is still more difficult to explain, even when taken into consideration as effect on the stomata. Since there are no data available on the condition of the stomata in plants grown under these conditions, and one does not know whether or not the artificial light can affect the opening and closing of the stomatal apertures, it is impossible to speculate as to what the effect may be until more data are obtained. As in the case of the greenhouse plants, it is difficult to account for the effect of chemically inert materials on the amount of water passing through the cuticle.

That there is a chemical, as well as a mechanical effect, is evidenced by the fact that a solution of dicalcium arsenate will also bring about increases in the rate of water loss of the treated plants. There are two possible explanations of this result. Either there is a chemical effect on the cell walls, rendering them more permeable and thus increasing cuticular transpiration, or there is an effect on the guard cells of the stomata which causes the stomata to remain open longer. It is known that the guard cells differ from the other epidermal cells in physiological activity. The fact that certain workers have reported a greater increase in transpiration when the leaves were sprayed on the lower surfaces as compared with leaves sprayed on the upper surfaces, also indicates that the action of these chemically active materials may be on the stomatal responses rather than on cuticular permeability, since the plants studied had more stomata on the lower epidermis than on the upper. An investigation into the condition of the stomata of the sprayed and unsprayed plants at all times of the night and day will be necessary before an explanation of these effects of sprays and other materials can be based on direct evidence rather than inferred from experimental data.

## Summary

Zinc-safened, basic, and ordinary commercial calcium arsenates, chemically pure Bancroft clay and silica, and a solution of dicalcium arsenate all brought about significant increases in the transpiration rate of bean plants, as determined by both a study of cut shoots in potometers and the loss in weight of potted plants.

The application of zinc-safened and basic calcium arsenates brought about as great an increase in the rate of water loss of the treated plants as did the ordinary commercial material. In this case the phytocidal action of a compound is not correlated with its capacity to increase the transpiration rate of the injured plants. If injury becomes apparent, however, as burned areas on the leaves, these plants will show a higher rate of water loss than the uninjured plants.

For greenhouse plants, the increased rate of water loss was most apparent at night when the treated plants were compared with controls. This was not the case for the plants kept in the indoor culture room.

Chemically inert Bancroft clay and silica, as well as physically inert dicalcium arsenate in solution, brought about equally large increases in the transpiration rate of the treated plants as compared with controls.

An attempt is made to explain some of the results on the basis of changes in stomatal action brought about by the application of the different materials studied.

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