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STUDIES ON CACODYLIC ACID:
I. EFFECT OF GIRDLING ON MOVEMENT IN *PHASEOLUS VULGARIS*

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Plant Physiology Division
PLANT SCIENCES LABORATORIES

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ABSTRACT

Experiments were conducted to study the absorption and translocation of three levels (2.5×10^{-4}, 5.0×10^{-4}, and 7.5×10^{-4} M) of root-applied cacodylic acid by girdled and nongirdled bean plants (*Phaseolus vulgaris* L. var. Black Valentine). Daily visual observations indicated that the effectiveness of root-applied cacodylic acid was not only reduced, but also delayed, by steam-girdling. Fresh and dry weight measurements of plants also demonstrated the reducing effect of girdling on absorption of root-applied cacodylic acid. Growth inhibition was greatest at the highest treatment level and decreased with lower concentrations on both girdled and nongirdled plants. However, when comparing percentage of growth inhibition, as evaluated by changes in plant weight, girdling caused a reduction in herbicide effectiveness at all levels compared with similarly treated nongirdled plants. Reduction in herbicide effectiveness caused by girdling decreased as concentration of the herbicide increased. A decrease in herbicide movement brought about by girdling was demonstrated when arsenic in plant tops and roots was analyzed. Roots of girdled and nongirdled plants contained equal amounts of total arsenic. A similar comparison between plant tops showed that a difference did exist and that girdling reduced acropetal movement of cacodylic acid. These data suggest that acropetal translocation of root-applied cacodylic acid occurs in both the xylem and phloem of bean plants and may be time- and concentration-dependent.

I. INTRODUCTION*

Cacodylic acid (dimethylarsenic acid) has been used as a contact herbicide for grasses since the late 1950's.[1,2] The movement of cacodylic acid has received little attention, because its main effect as a contact herbicide is exhibited in a very short period of time. More recently, with the extensive use of cacodylic acid, concern has been expressed as to the accumulation of the compound in soil and its movement into plant roots. Many investigators have shown that repeated applications of arsenicals to crops have resulted in an increase in the level of soil arsenic and the subsequent root absorption and translocation of the element to other plant parts.[3-7]

In order to study the absorption and translocation of root-applied cacodylic acid, an experiment was undertaken using girdled and nongirdled bean plants grown in solution culture. The objective of this study was to determine the amount of arsenic absorbed from root-applied cacodylic acid and to elucidate the method of translocation into apical plant parts.

II. MATERIALS AND METHODS

Bean plants (*Phaseolus vulgaris* L. var. Black Valentine) were germinated in sand, transferred to aerated 0.5X Hoagland's nutrient solution, and grown in a controlled-environment growth chamber. Uniform plants in which the first trifoliolate leaf had just opened were selected for use. Experiments were conducted under the following controlled environmental conditions: 25±2 C, 60±5% relative humidity, and a 16-hour photoperiod of 1,300 ft-c of illumination at plant-top level provided by a mixture of fluorescent and incandescent lights.

Plants selected on the basis of uniformity were divided into two equal groups. One-half of the plants were steam-girdled before treatment application and the remaining plants were not girdled. The girdle was made by rotating the plant several times not more than 2.5 cm from the direct point of steam emission. The girdle was positioned 2 to 3 cm below the cotyledonary node. Three days elapsed from girdling to plant selection, at which time uniform plants were chosen for treatment with cacodylic acid.

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Plants were treated by immersing the root systems in foil-covered pint jars containing 300 ml of the following treatment solutions: (i) 2.5 x 10^{-4} M cacodylic acid in 0.5X Hoagland's, (ii) 5.0 x 10^{-4} M cacodylic acid in 0.5X Hoagland's, (iii) 7.5 x 10^{-4} M cacodylic acid in 0.5X Hoagland's, and (iv) controls in 0.5X Hoagland's.

The experimental treatments constituted a split-plot design with five replications. The whole plots were designated girdled vs. nongirdled; the subplc.s were cacodylic acid treatments.

Daily observations of plant conditions were recorded. Plants were rated according to the severity of their symptoms into the following categories: slight, moderate, or severe. Plants that were rated slight showed wilting and necrotic flecks on their primary leaves. Moderate symptoms were those in plants that exhibited wilting and necrotic flecks throughout the entire plant. Those plants that were more than one-half defoliated were placed into the severe group. Photographs were taken when appropriate.

Seven days after treatment, plants were harvested and fresh weights were recorded. All plants were divided into two fractions. Plant "tops" consisted of the entire plant portions above the girdle in the case of girdled plants or above the cotyledonary node for nongirdled plants. Plant "roots" included all plant portions below the girdle for girdled plants or below the cotyledonary node for nongirdled plants. After a minimum of 48 hours at 95 C, dry weights were recorded.

After being dried, the plant material was ground in a micro-Wiley mill, using a 20-mesh stainless steel screen. Because no analytical method is presently available for the direct determination of cacodylic acid in plant tissue, the amount of herbicide was analyzed indirectly by a standard wet-chemical analysis for arsenic. Standard nitric-perchloric-sulfuric acid wet ash digestions were made and arsenic was determined with a modified Gutzeit generator and the silver diethyldithiocarbamate method.[5]

III. RESULTS AND DISCUSSION

Forty hours after treatment, nongirdled plants that received the highest treatment level (7.5×10^{-4} M) began to show signs of cacodylic acid injury. All plants were severely wilted. No other plants, either girdled or nongirdled, showed any signs of cacodylic acid injury at this time. Three days after treatment, 40% of the nongirdled plants showed symptoms of cacodylic acid injury, while less than 10% of the similarly treated girdled plants were affected. The most severe symptoms were exhibited by those plants that received the highest level of cacodylic acid; slight to moderate symptoms appeared on those receiving less herbicide (Fig. 1). At the same time, several girdled plants at the highest treatment level began to show slight signs of herbicide injury. Four days after treatment, symptoms of cacodylic acid injury appeared on plants in every treatment group. However, the most severe symptoms appeared on nongirdled plants, while only slight to moderate symptoms appeared on similarly treated girdled plants (Fig. 2). Five days after treatment, nongirdled plants were severely affected by the herbicide, while the severity of symptoms in girdled plants was not as great. As the concentration of root-applied cacodylic acid on girdled plants decreased, the delay in the time of appearance and severity of symptoms increased. Seven days after treatment, all nongirdled plants at the highest and second treatment levels (7.5×10^{-4} and 5.0×10^{-4} M) had died; only one girdled plant at the highest treatment level had died, although all others were severely affected.

Both fresh and dry weight measurements indicate that the effectiveness of root-applied cacodylic acid is reduced when treated plants are girdled (Tables 1 and 2). Although girdling per se markedly reduced plant growth, as shown by weight differences between girdled and nongirdled control plants, it is evident that girdling significantly reduced the percentage of growth inhibition caused by cacodylic acid. Using reduction in fresh weight as the criterion of injury, girdling caused an approximate one-half reduction in herbicide effect at the intermediate level of cacodylic acid (Table 1). These data suggest that acropetal cacodylic acid transport may be due in part to phloem movement.

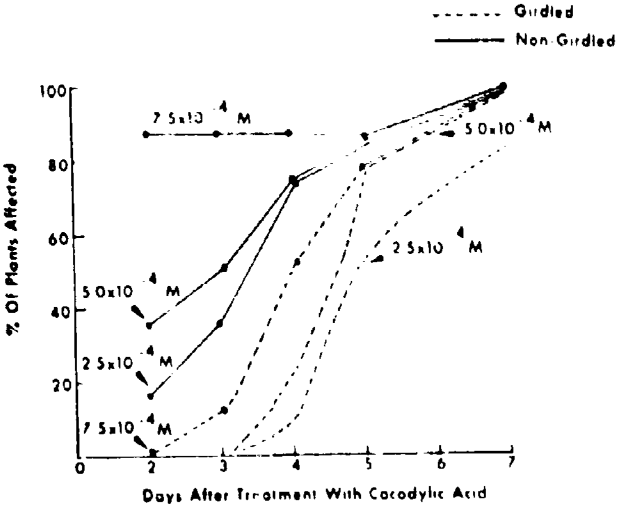

FIGURE 1. Sequential Development of Root-Applied Cacodylic Acid Symptoms on Girdled and Nongirdled Black Valentine Bean Plants.

FIGURE 2. Effect of Girdling on Severity of Cacodylic Acid Symptoms. All plants have been treated with 2.5×10^{-4} M concentrations of root-applied cacodylic acid. Note effect of the girdle in reducing the severity of cacodylic acid injury.

TABLE 1. COMPARISON OF EFFECT OF ROOT-APPLIED CACODYLIC ACID ON FRESH WEIGHT OF PLANT TOPS OF GIRDLED[a] AND NONGIRDLED PLANTS

Treatment	Nongirdled		Girdled		Decrease in Cacodylic Acid Effect due to Girdling, %
	Fresh Weight,[b] g	Inhib., %	Fresh Weight,[b] g	Inhib., %	
0	21.7	0	7.3	0	-
2.5×10^{-4} M	9.6	56.0	5.8	20.9	62.7[c]
5.0×10^{-4} M	4.3	80.2	4.1	43.9	45.3
7.5×10^{-4} M	3.0	86.3	3.4	53.2	38.4

a. Black Valentine beans were 7 days old when girdled and 10 days old when placed in the treatment solution.

b. Values are means of five replications.

c. [(56.0 - 20.9) ÷ 56.0] x 100. Other percentages in this column determined similarly.

TABLE 2. COMPARISON OF EFFECT OF ROOT-APPLIED CACODYLIC ACID ON DRY WEIGHT OF PLANT TOPS OF GIRDLED[a] AND NONGIRDLED PLANTS

Treatment	Nongirdled		Girdled		Decrease in Cacodylic Acid Effect due to Girdling, %
	Dry Weight,[b] g	Inhib., %	Dry Weight,[b] g	Inhib., %	
0	2.71	0	1.42	0	-
2.5×10^{-4} M	1.34	50.5	1.14	19.5	61.3[c]
5.0×10^{-4} M	0.96	64.7	1.02	28.1	56.5
7.5×10^{-4} M	0.90	66.8	0.93	35.0	47.7

a. Black Valentine beans were 7 days old when girdled and 10 days old when placed in treatment solution.

b. Values are means of five replications.

c. [(50.50 - 19.54) ÷ 50.50] x 100. Other percentages in this column determined similarly.

It is tempting to speculate that a binary path exists for the translocation of root-applied cacodylic acid and that when one of these pathways is blocked, as by girdling, a reduction in effect results. This hypothesis is further substantiated by growth measurements, because a reduction in the percentage decrease in cacodylic acid effect due to girdling occurred with an increase in the level of root-applied material (Tables 1 and 2). A binary movement theory is supported by these data, since the injury of the phloem would be more severe at the higher treatment levels and hence its capability for active transport would be diminished. Thus, blockage of the phloem by girdling would be expected to have less effect on acropetal movement at high herbicide concentrations; this is, in fact, what is reflected by the values for the decrease in effect due to girdling for both fresh and dry weight.

The fact that plants absorb arsenic from the media surrounding the roots, and further transport it acropetally, is readily evident from the tissue analyses (Fig. 3 and 4). Figure 4 shows, as has been shown by several other investigators,[3,7] that when arsenic is available in the rhizosphere, the roots contain the greatest amount of the element, with smaller amounts occurring in the apical portions of the plant.

The analyses also indicate that girdled plants translocate less root-applied cacodylic acid than nongirdled bean plants. An increase in arsenic from 169 μg to 377 μg/g dry weight in plants tops was recorded in nongirdled plants when the level of applied cacodylic acid increased from 2.5×10^{-4} to 7.5×10^{-4} M. Similar treatments on girdled plants resulted in an increase from only 79 μg to 222 μg/g dry weight. It is not immediately clear whether this decrease in apical arsenic accumulation is due entirely to phloem removal or to reduction in transpiration resulting from a decrease in plant size because of the girdling process. Using the arsenic content values for the lowest cacodylic acid treatment (2.5×10^{-4} M) between nongirdled and girdled plant tops (169 and 79 μg/g, respectively) it was possible to calculate the decrease in acropetal arsenic transport attributable to girdling. This calculated value is 53%, which agrees very well with the 50% hypothetical reduction that would be expected from phloem removal in a binary system with each component transporting one-half of the herbicide. If a similar comparison is made between arsenic content in girdled and nongirdled plants at the high level of cacodylic acid treatment, the decrease from 377 to 222 μg/g is 41%. This value is in good agreement with the 38.4% decrease in effect due to girdling calculated from reductions in fresh weight (Table 1) and implies that there is a direct correlation between the diminished cacodylic acid effect on plant weight from girdling and the actual arsenic content in the plant tissue.

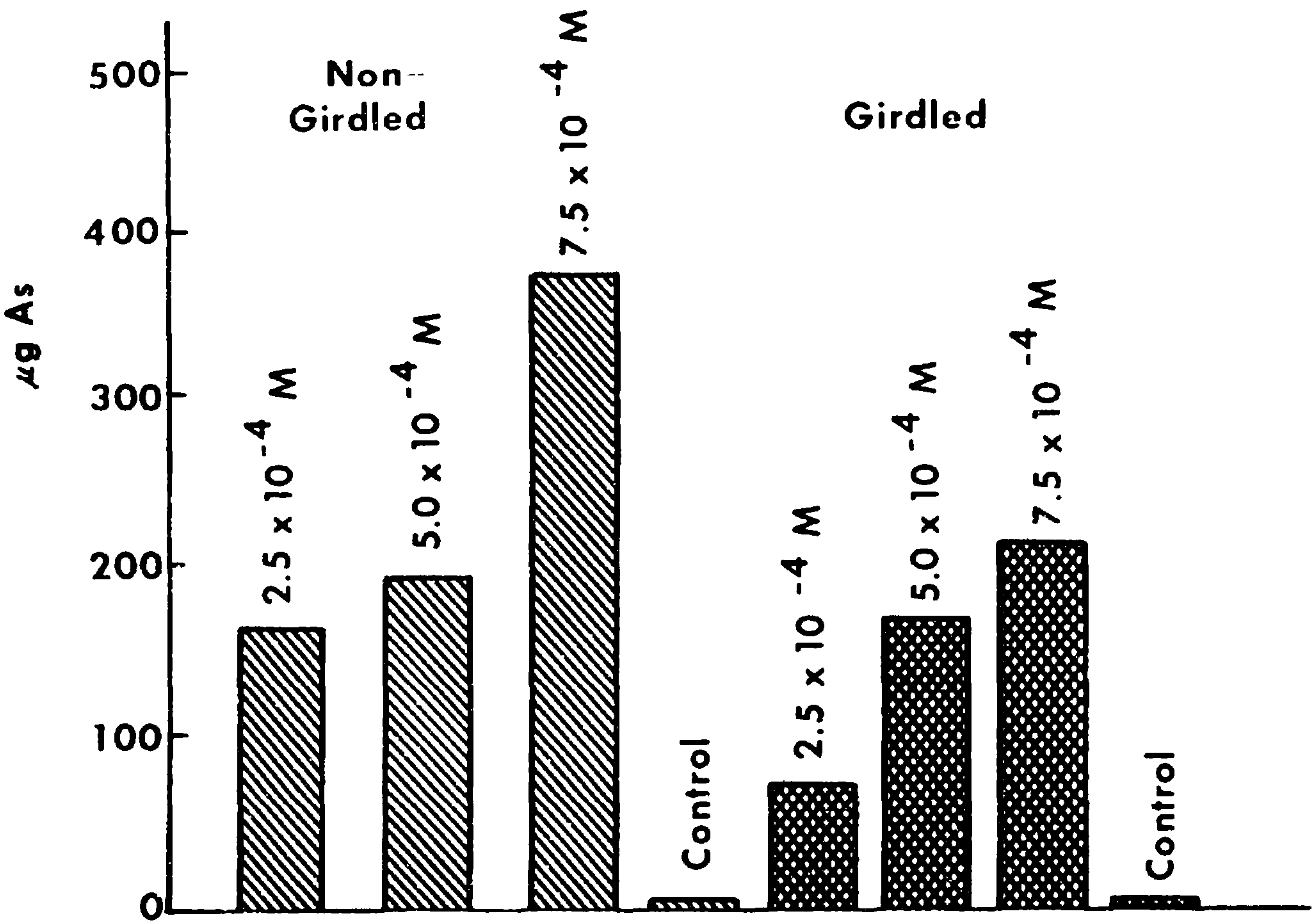

FIGURE 3. Arsenic Content of Tops of Black Valentine Beans 7 Days after Root Treatment by Addition of Cacodylic Acid to Nutrient Culture Solution.

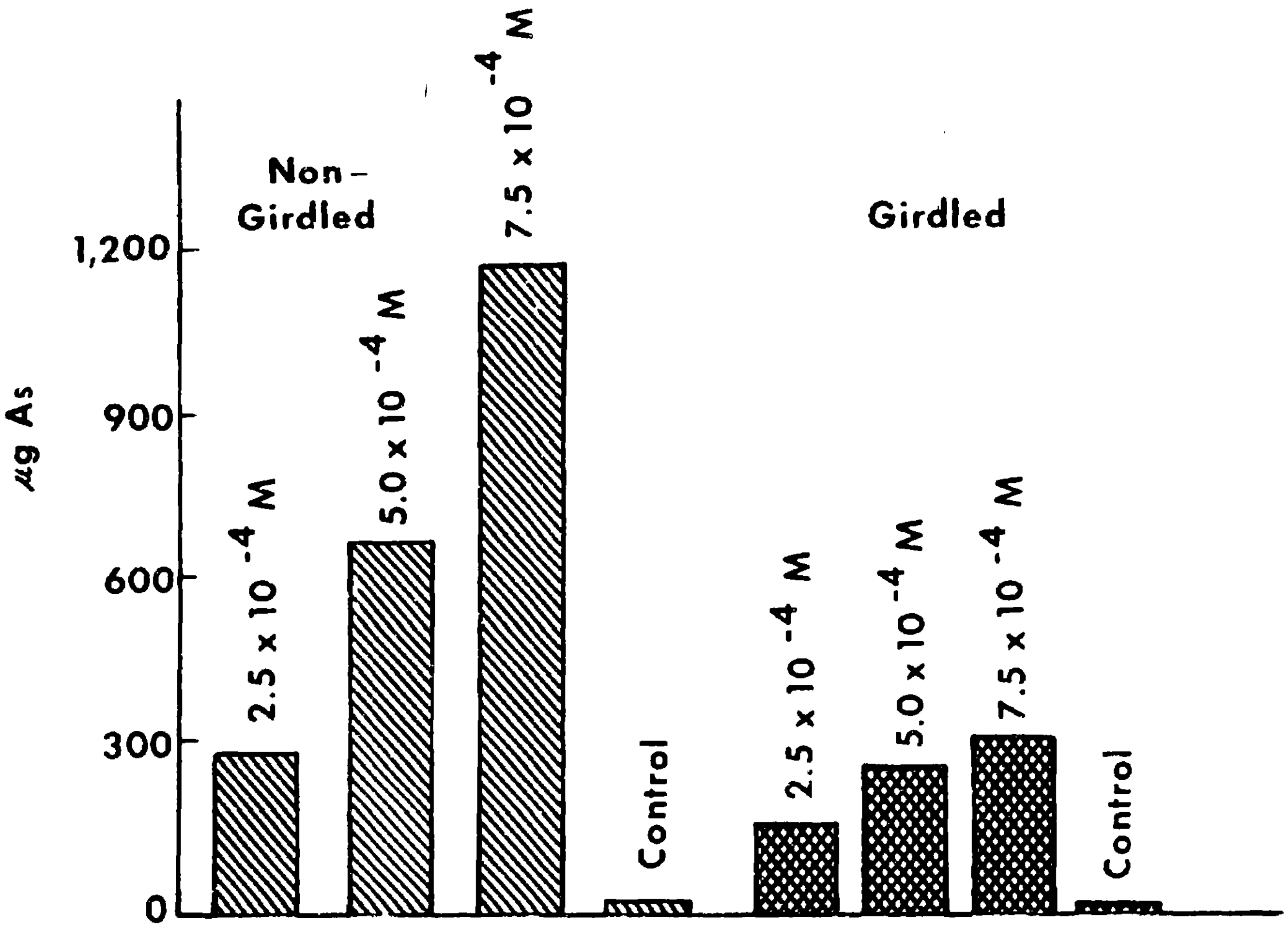

FIGURE 4. Arsenic Content of Roots of Black Valentine Beans 7 Days after Root Treatment by Addition of Cacodylic Acid to Nutrient Culture Solution.

When similar calculations are made for total or absolute arsenic content (microgram per gram times dry weight), one can logically anticipate that the above correlation between the decreased effectiveness of cacodylic acid (as measured by plant weight) and decreased arsenic content effected by girdling might not exist, because the absolute arsenic content values are biased by the reduction in plant growth from the girdling process, whether or not a herbicidal effect exists. However, it was found that for the high level of cacodylic acid treatment, the decrease in arsenic content of the plant tops (339 μg to 206 μg) due to destruction of the phloem was 39%. This value is in excellent agreement with the 38.4% reduction in cacodylic acid effect due to girdling shown in Table 1. A similar calculation for the effect of low level of cacodylic acid treatment on total arsenic revealed a 60% decrease in arsenic content due to girdling. This value is essentially identical to the 62.7% reduction in effect shown in Table 1 and the 61.3% value shown in Table 2.

Acropetal translocation of cacodylic acid (as measured by arsenic content) is also a function of time and concentration, in that foliar symptoms of injury occurred on the girdled plants at the high treatment level 1 to 2 days prior to the development of symptoms at the lowest treatment level (Fig. 1). This observation is further substantiated by the arsenic content of the plant tops shown in Figure 3. When treated for a similar period of time, girdled plants require almost two times the amount of root-applied cacodylic acid to bring about foliar arsenic concentrations equal to those in nongirdled plants.

Fresh and dry weight measurements, arsenic content in micrograms per gram of tissue, total arsenic content per plant, and development of foliar symptoms support the hypothesis that acropetal translocation of root-applied cacodylic acid occurs in both the xylem and phloem of bean plants and may be time- and concentration-dependent.

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13. ABSTRACT

Experiments were conducted to study the absorption and translocation of three levels (2.5×10^{-4}, 5.0×10^{-4}, and 7.5×10^{-4} M) of root-applied cacodylic acid by girdled and nongirdled bean plants (*Phaseolus vulgaris* L. var. Black Valentine). Daily visual observations indicated that the effectiveness of root-applied cacodylic acid was not only reduced, but also delayed, by steam-girdling. Fresh and dry weight measurements of plants also demonstrated the reducing effect of girdling on absorption of root-applied cacodylic acid. Growth inhibition was greatest at the highest treatment level and decreased with lower concentrations on both girdled and nongirdled plants. However, when comparing percentage of growth inhibition, as evaluated by changes in plant weight, girdling caused a reduction in herbicide effectiveness at all levels compared with similarly treated nongirdled plants. Reduction in herbicide effectiveness caused by girdling decreased as concentration of the herbicide increased. A decrease in herbicide movement brought about by girdling was demonstrated when arsenic in plant tops and roots was analyzed. Roots of girdled and nongirdled plants contained equal amounts of total arsenic. A similar comparison between plant tops showed that a difference did exist and that girdling reduced acropetal movement of cacodylic acid. These data suggest that acropetal translocation of root-applied cacodylic acid occurs in both the xylem and phloem of bean plants and may be time- and concentration-dependent.

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