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## An investigation into the mode of action of alachlor.

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AN INVESTIGATION INTO THE MODE OF  
ACTION OF ALACHLOR

A Thesis Presented

By

James Russell Bates

Submitted to the Graduate School of the  
University of Massachusetts in partial fulfillment  
of the requirements for the degree of

MASTER OF SCIENCE

December 1975

Department of Plant and Soil Sciences

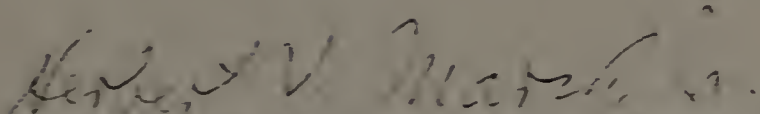
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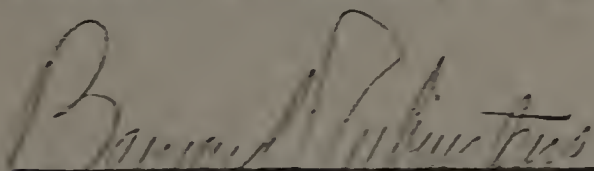
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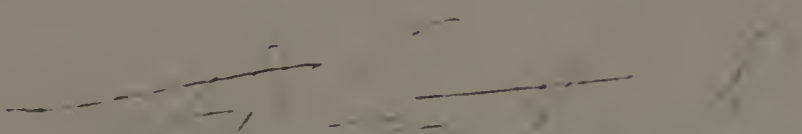
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## ABSTRACT

An Investigation Into The Mode of  
Action of Alachlor

(December 1975)

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Evidence is presented that 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide (alachlor) inhibits auxin-promoted elongation of Avena coleoptile sections and auxin-promoted hydrogen ion secretion from these sections. Using 0.1 mM alachlor, the onset of inhibition in both cases occurred between three and four hours after treatment. Further investigation revealed that 0.1 mM alachlor strongly inhibited auxin transport in Avena coleoptiles, which may explain alachlor's effect on growth and the growth related hydrogen ion secretion.

Morphological observations on the effects of alachlor on oat roots indicated a strong inhibition of growth of primary and lateral roots. Anatomical examination of the region of cellular expansion showed a pronounced inhibition of cortical cell enlargement. Examination of mitotic indices showed that alachlor may also interfere with cell division. A time course of increase in fresh and dry weights of oat seedlings showed an inhibition by alachlor of increase in fresh weight of roots preceding by a day a similar inhibition

in the shoots. Increases in dry weight were inhibited by alachlor in the roots only, no inhibition of increase in dry weight was noted in the shoots.

Alachlor at a concentration of 0.1 mM inhibited uptake of leucine, alpha-amino isobutyric acid, 3-O-methyl glucose and potassium but not calcium. Inhibition of leucine uptake was noted within 30 minutes. Examination of leucine incorporation into protein indicated that alachlor does not inhibit this process. In addition, no effect of alachlor on respiration or on membrane leakage could be detected.

Alachlor has been shown to severely inhibit the root and shoot growth of zero to 3-day old corn seedlings growing in sand when applied by subirrigation. The roots of 5-day old seedlings were inhibited by the same treatment, but shoots of the older seedlings showed no response. The coleoptile of corn is apparently insensitive to alachlor in that the herbicide neither inhibited elongation of coleoptile segments nor  $^{14}\text{C}$ -leucine uptake by this tissue. Leucine uptake by excised roots, however, was inhibited 40 to 67%.

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SECTION I

THE EFFECT OF ALACHLOR ON AUXIN PROMOTED  
GROWTH IN AVENA COLEOPTILES

### Abstract

Evidence is presented that 2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide (alachlor) inhibits auxin-promoted elongation of Avena coleoptile sections and auxin-promoted hydrogen ion secretion from these sections. Using 0.1 mM alachlor, the onset of inhibition in both cases occurred between three and four hours after treatment. Further investigation revealed that 0.1 mM alachlor strongly inhibited auxin transport in Avena coleoptiles, which may explain alachlor's effect on growth and the growth related hydrogen ion secretion.

### Introduction

Alachlor inhibition of the growth of Avena seedlings has been previously reported (3). More recently reported is the inhibition of auxin-induced and acid-induced elongation of Avena coleoptile sections by alachlor (1). Hydrogen ion secretion in response to auxin has been shown in sunflower hypocotyls (5) and in oat coleoptiles (4, 6). These observations support the concept that hydrogen ions act as a second messenger in auxin-induced cell elongation. It will be shown here that alachlor interferes with the auxin-promoted hydrogen ion secretion, that this interference is probably brought about by the inhibition of auxin transport, and that it may explain in part the

alachlor inhibition of growth.

### Materials and Methods

Plant material: In all experiments the plant material consisted of coleoptile tissue of Avena sativa var. orbit. The Avena seedlings were grown on vermiculite in plastic boxes. The boxes were kept at room temperature in the dark for four days at which time the seedlings were harvested. All experiments were carried out under incandescent room lighting.

Growth tests: Sections (7.6 mm) of isolated coleoptiles were cut approximately 3mm from the tip. Sections were then placed in plastic dishes containing 10 mM potassium phosphate buffer (pH 6.5) with and without 0.1 mM alachlor. Growth measurements were taken periodically using a dissection microscope and a lens micrometer.

H<sup>+</sup> secretion assay: Isolated coleoptile sections (10 mm) were cut approximately 3 mm from the tip. The cuticle and adhering outer epidermal cell wall was stripped off using fine forceps according to the procedure of Cleland (4). No visible damage to cortical cells was noted under microscopic examination. The sections were floated in 1 mM potassium phosphate (pH 5.9) for 30 minutes and then 15 each were placed into 25 ml beakers which contained 3 ml of 1 mM potassium phosphate (pH 5.9) with and without 0.1 mM alachlor. Small magnetic spin bars were placed in

the beakers for agitation. Hydrogen ion secretion was monitored using a combination pH electrode and an expanded scale Beckman pH meter.

Auxin transport assay: Sections (3 mm) of isolated coleoptiles were cut approximately 5 mm from the tip and floated for 3 hours in distilled water containing 10 mM potassium phosphate (pH 6.5) with and without 0.1 mM alachlor. Sections, in groups of four each, were placed upright on 1% agar blocks. A second agar block containing 20  $\mu$ M  $^{14}$ C-1-IAA (51 Ci/mM) was then placed on top of the sections for one hour. Receiver blocks were collected after a one hour transport period, placed in scintillation fluid (Beckman Ready-Solv solution VI) and counted on a Beckman scintillation counter.

### Results and Discussion

The results shown in Figure I concur with those of Bardzik (1). Using 0.1 mM alachlor there is an inhibition of the auxin-promoted elongation which is first apparent at three to four hours after treatment. Inhibition is almost complete by four hours. When the concentration of alachlor is doubled the lag time prior to inhibition is cut in half, indicating that a threshold concentration of alachlor within the tissue is necessary for onset of inhibition.

Results shown in Figure 2 demonstrate the auxin-promoted hydrogen ion secretion reported for Avena

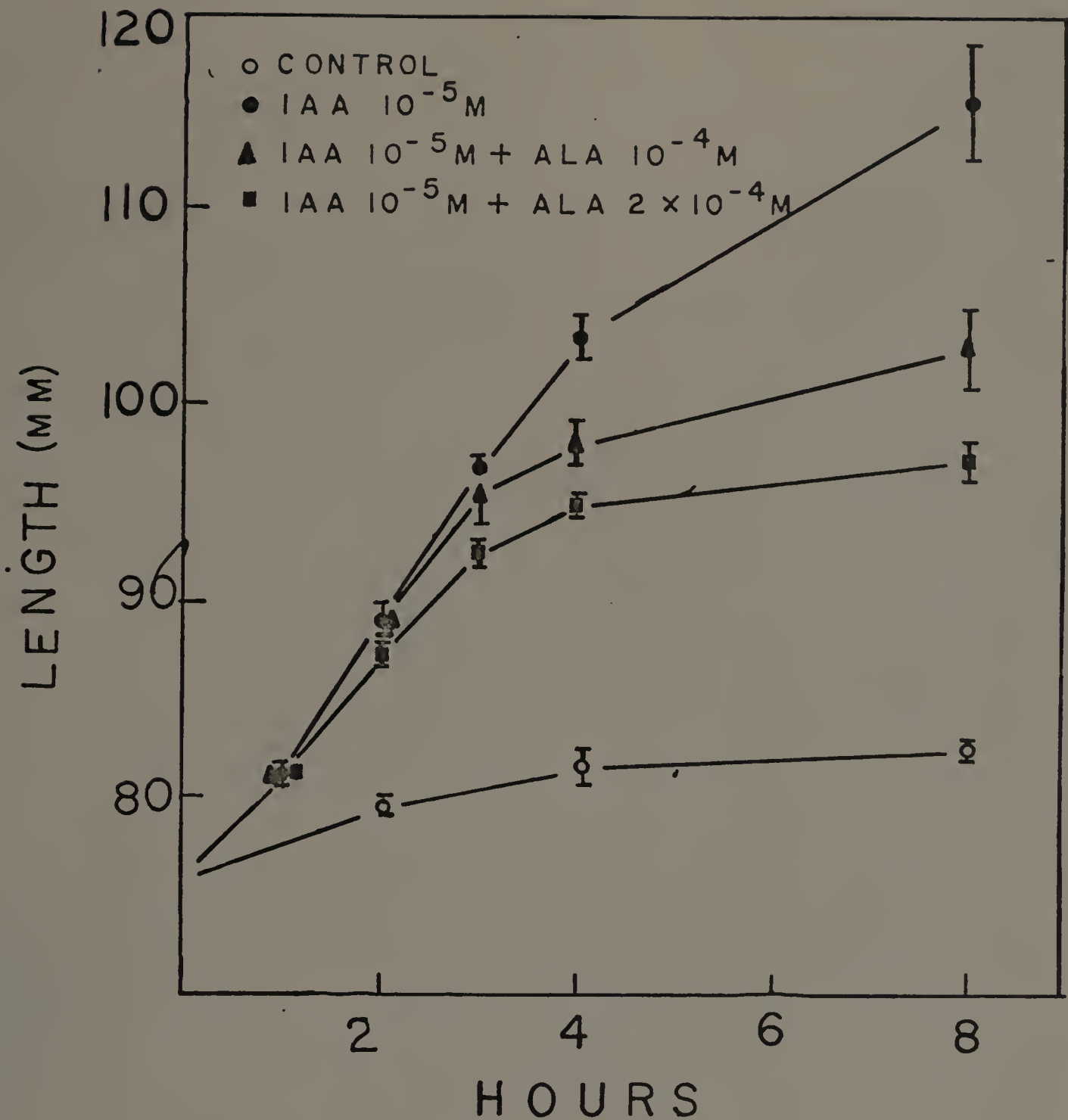


Figure 1. Time course of auxin-promoted elongation in oat coleoptiles with and without alachlor.

coleoptiles. The addition of 0.1 mM alachlor is shown to block the secretion of hydrogen ions. A lag before hydrogen ion secretion due to the auxin treatment becomes apparent has been noted by others (4, 6). Additional tests showed that peeled coleoptiles do grow in response to auxin (data not shown). If the concept (4, 6) that these secreted hydrogen

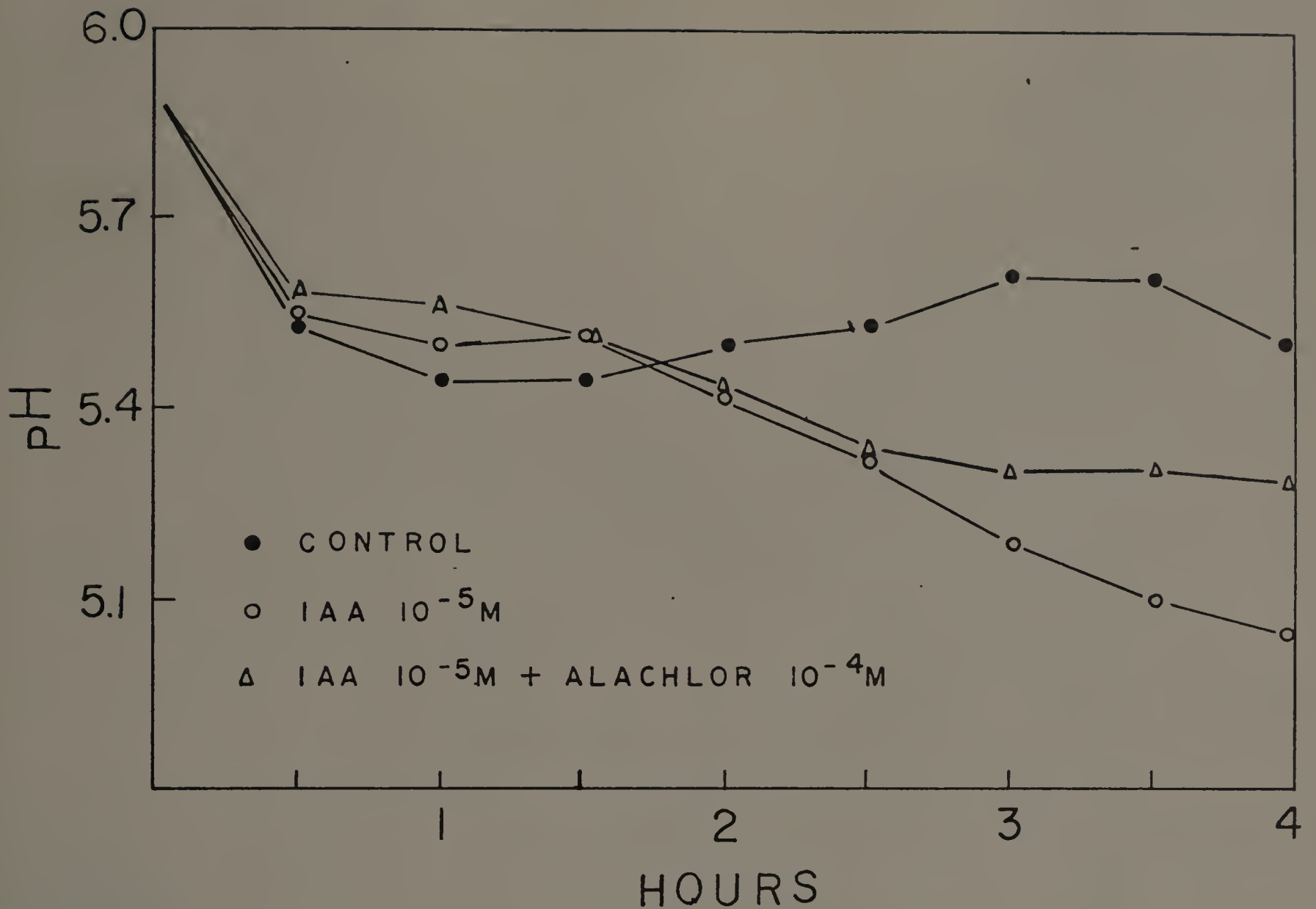


Figure 2. Time course of auxin-promoted  $H^+$  secretion with and without 0.1 mM alachlor. .

ions act as a messenger for auxin by acting as a cell wall loosening agent is valid, than this could explain the alachlor inhibition of auxin-promoted growth shown here. The time of onset of inhibition for hydrogen ion secretion is similar to the time of onset of alachlor-induced inhibition of auxin-promoted growth. This indicates that the inhibition of growth may be closely tied to and result from the inhibition of hydrogen ion secretion.

Additional experiments were conducted to determine if

TABLE I

IAA Transport by Avena  
Coleoptile Sections

<u>TREATMENT</u>	<u>I</u>	<u>II</u>	<u>III</u>
Control (3 hr)	152.7 <u>+14</u>	2226 <u>+598</u>	418 <u>+86</u>
Ala $10^{-4}$ M (3 hr)	76.6 <u>+14</u>	2123 <u>+214</u>	274 <u>+49</u>

I - cpm/receiving block  
 II - cpm/apical 1.5 mm/4 sect.  
 III - cpm/basal 1.5 mm/4 sect.

auxin movement was being affected. Table I shows the effects of 0.1 mM alachlor on auxin transport in Avena coleoptile sections. The inhibition of auxin transport by alachlor is probably a membrane related event. Bardzik (1) reported alachlor inhibition of chloride ion uptake in Avena coleoptiles. Recent reports of alachlor inhibition of uptake of a broad range of solutes in Avena root sections (2) indicate that the inhibition of auxin transport by alachlor may be due to inhibited membrane transport systems. This inhibition of solute uptake, by effecting osmotic pressure of the cells, could result in a limited ability for water uptake thereby restricting growth and explaining the apparent inhibition of acid-induced growth.

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SECTION II

MORPHOLOGICAL AND ANATOMICAL EFFECTS  
OF ALACHLOR ON OAT ROOTS

### Abstract

Morphological observations on the effects of the herbicide, 2-chlor-2',6'-diethyl-N-(methoxymethyl)acetanilide (alachlor), on oat roots (Avena sativa cv. Orbit) indicated a strong inhibition of growth of primary and lateral roots. Anatomical examination of the region of cellular expansion showed a pronounced inhibition of cortical cell enlargement. Examination of mitotic indices showed that alachlor may also interfere with cell division.

### Introduction

Previous investigations of the herbicidal activity of alachlor by Eshel (7) and Chang (6) showed that its application at the seedling stage caused noticeable inhibition of root growth. Initial examination of the roots confirmed the growth inhibition and also revealed a region of apparent nonenlargement of the cortex. In addition, the absence of lateral roots was apparent. Examination of the effect of alachlor on mitosis indicated possible interference with the mitotic processes.

Although probable modes of action of some chloroactamide herbicides have been reported (10), no primary mode of action has yet been determined for alachlor. The investigations carried out in this study were done to determine the anatomical and morphological effects of

alachlor on seedlings.

### Materials and Methods

Oat seeds were sown in 6.5 cm diameter plastic pots filled with quartz sand. The pots were saturated by a sub-irrigation application of distilled water and placed in a controlled environment chamber with a 30 C-light period and a 21 C-dark period. Day length was 14 hours at a light intensity of 7000 Lux. Five days after imbibition, all pots were thinned to 4 plants approximately 3 cm in height. Treatments consisted of a daily subirrigation application of Hoagland nutrient solution (9) (half-strength) containing  $10^{-4}$ M alachlor,  $10^{-6}$ M alachlor, or no alachlor. Random pots were selected on a daily basis for visual and cytological examination of root growth inhibition.

Material for cytological study was fixed in ethanol, acetic acid, formalin and water (14:1:1:4, v/v/v/v) and passed through a series of N-butanol and xylene, then embedded in tissue-mat (m.p. 56 to 58°C). Material was sectioned at 5 $\mu$  and stained with toluidine blue-0 before removal of paraffin matrix according to McGrath (12).

Because of the difficulty of preserving cortical and epidermal tissue in paraffin, a condition not uncommon to roots (4), additional material was fixed in acrolein and embedded in glycol methacrylate. Material was sectioned

at 2 $\mu$  and stained with toluidine blue-0 according to the procedure of Fedder and O'Brien (8).

Treated and untreated root tips were hydrolyzed in 1N HCl for 7 minutes at 60°C. Root tips were then stained with Feulgen's Reagent and mascerated on a microscope slide for determination of mitotic indices.

### Results and Discussion

The most noticeable effect of alachlor on the oat root system was that of inhibition of growth (Figure 1). Inhibition of root growth by 10<sup>-4</sup>M alachlor was first apparent at about 3 days after treatment and became more pronounced with time. Inhibition of root growth also occurred with 10<sup>-6</sup>M alachlor, but onset was not as rapid and the degree of inhibition was not as severe. The absence of lateral roots in treated seedlings is shown in Figure 2. In addition to this, a region (5-10 mm from the root tip) of nonenlargement of cortical cells was noted. Root inhibition by alachlor, although previously reported (6, 7) was not characterized in respect to any of these differences.

Alachlor had no effect on the formation of lateral root primordia, however, the number of laterals per root was not examined. The treated primordia failed to develop further and did not elongate and rupture the cortex and epidermis (Figure 3) as normally occurs. These inhibited



Fig. 1. seedling root system of control (right) and  $10^{-4}$ M alachlor treated plants (left) 5 days after treatment.



Fig. 2. Alachlor ( $10^{-4}$ M) treated root system 5 days after treatment showing lack of lateral roots and zone of nonenlargement (arrow).

primordia were shown to be anatomically normal in positioning (forming opposite xylem poles), in organization and with a small root cap.

Examination of the terminal region of both treated and untreated roots showed no difference in root cap structure or, in cross section, in the differentiation of the primary root tissues.

Microscopic comparison of the region of nonenlargement

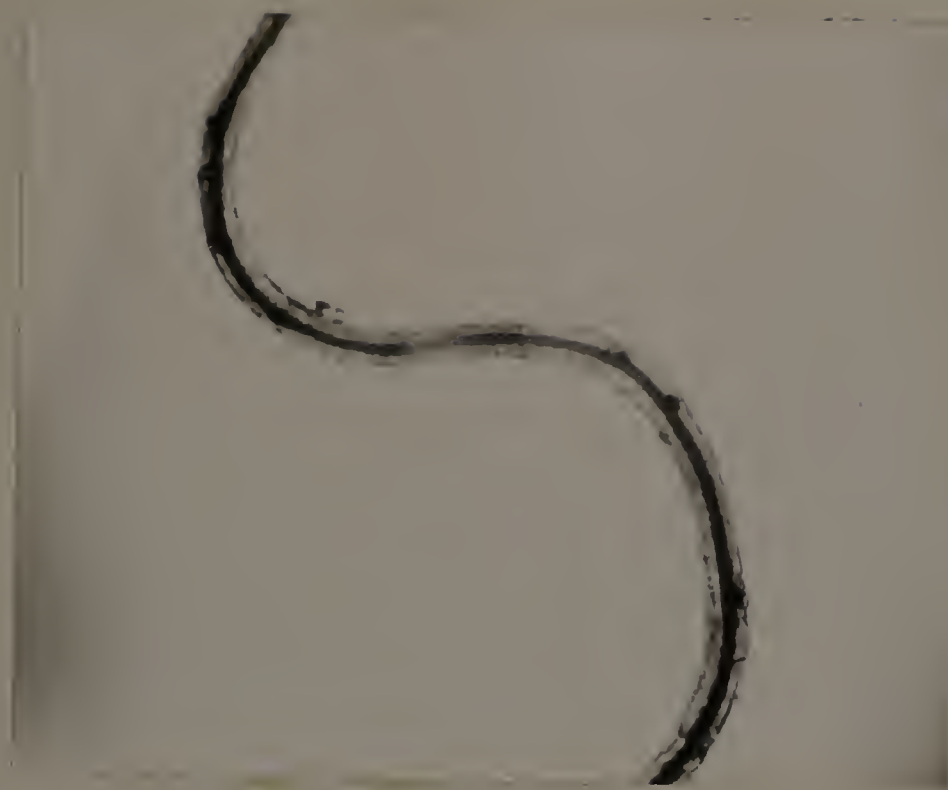


Fig. 3. Alachlor ( $10^{-4}$  M) treated root section showing formation of laterals still confined within root cortex tissue.

of cortical cells was made of the same region from treated and untreated roots (Figures 4A and 4B). Untreated roots appeared as expected, with the early loss of the epidermal layer and the conspicuous thickening of cortical cells below the original epidermis as has been reported by Avery (1). The most notable differences are in the persistence of the epidermal layer in treated roots and the enormous increase in size of cortex cells of untreated plants in comparison to treated plants. At later points in development the size of cortex cells in untreated plants is many times greater than shown in Figure 4A. Bardzik (2) has shown alachlor inhibition of cell enlargement with other tissues. He reported the lack of cell enlargement in oat

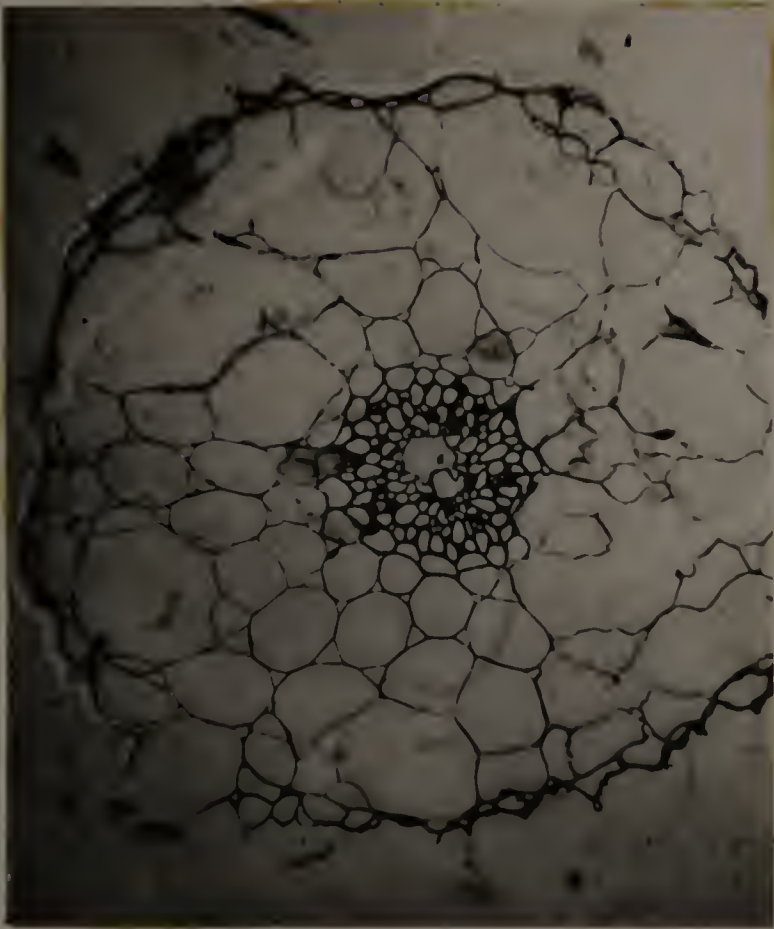


Fig. 4A. Cross section of control root showing greatly enlarged cortical cells and ruptured epidermis X100.

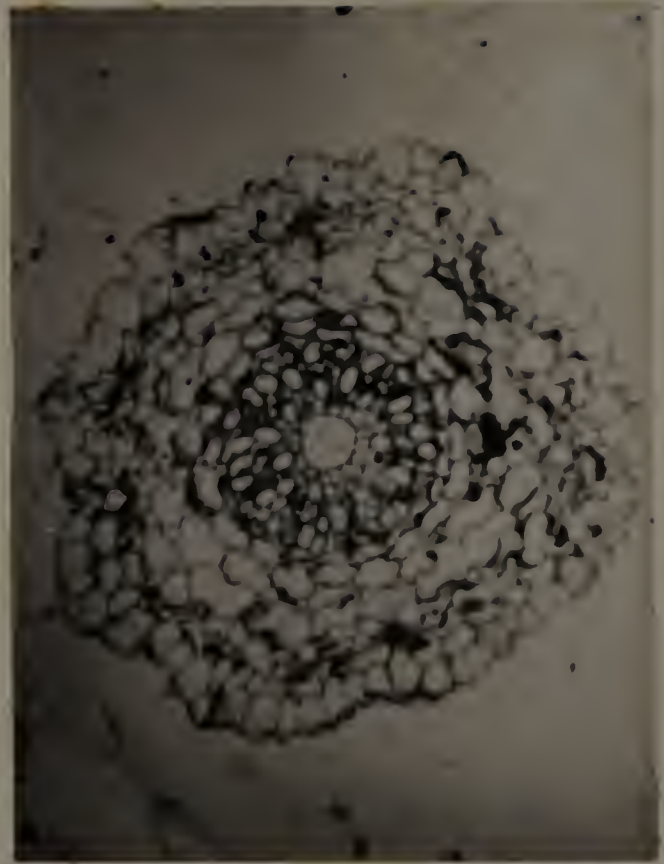


Fig. 4B. Cross section of alachlor treated ( $10^{-4}M$ ) root in region of non-enlargement of cortical cells. Epidermis still intact X100.

coleoptile tissue due to alachlor.

Paraffin embedded material also displayed the same similarity of treated and untreated roots in respect to the number of cells and the degree of lignification in the xylem tissue, but comparison of cortical and epidermal tissues by this method was impossible because of poor preservation.

Roots treated for 4 days with  $10^{-4}M$  alachlor showed a mitotic index of 7 and 2 in untreated and treated root tips, respectively. By day 7 the index for untreated root tips was 5.6, and for treated roots zero. After 7 days of treatment with alachlor, the nuclear envelopes were readily

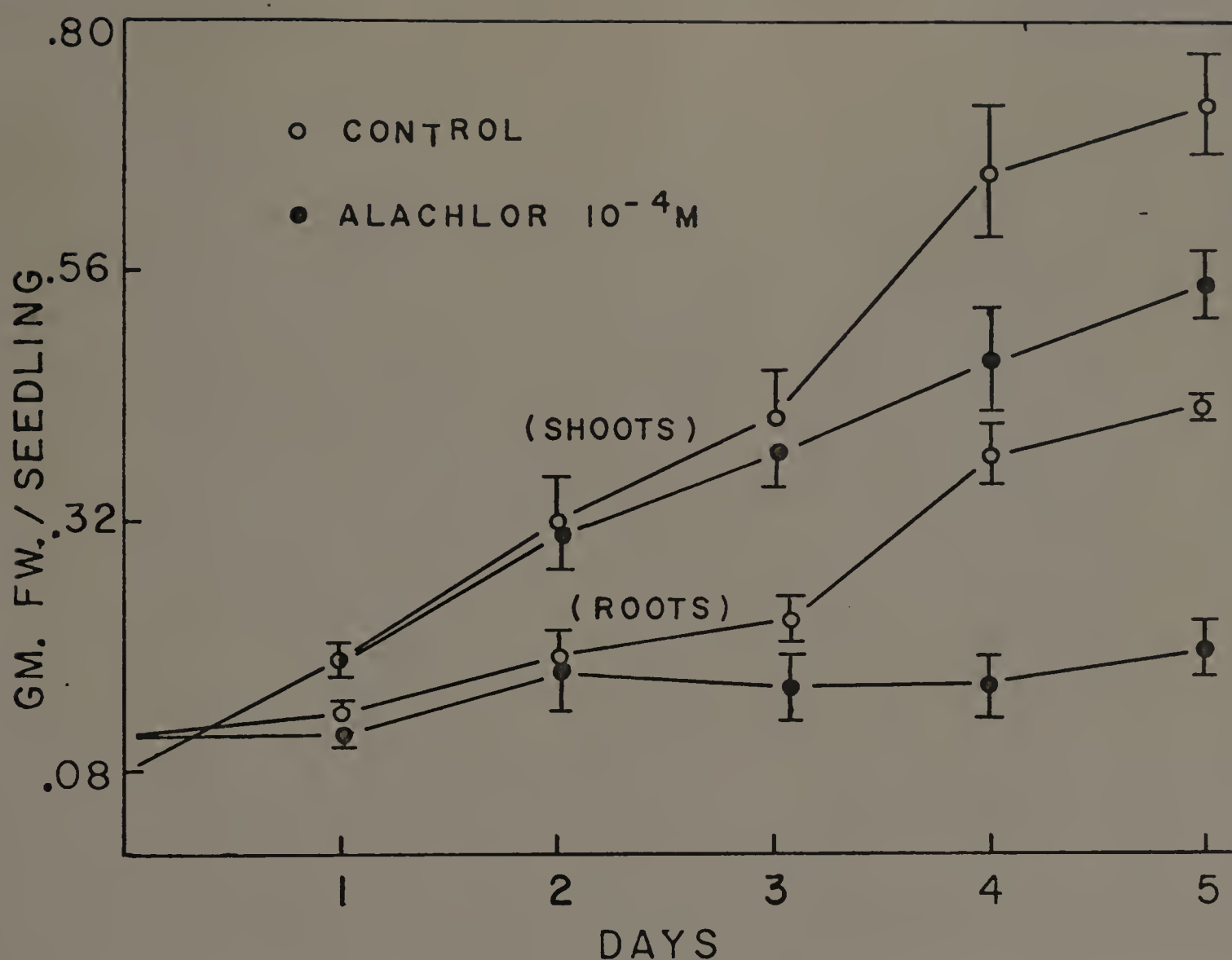


Fig. 5. Time course of increase in fresh weight for oat seedlings treated with and without alachlor ( $10^{-4}$  M).

destroyed in preparing squashes. These observations are consistent with weight measurements of intact seedlings (Figures 5 and 6). The time course of increase in fresh weight shows an alachlor-induced inhibition beginning at 4 days in shoots and at 3 days in roots. The time course of increase in dry weight shows an alachlor-induced inhibition beginning at 4 days in roots but not at all in shoots.

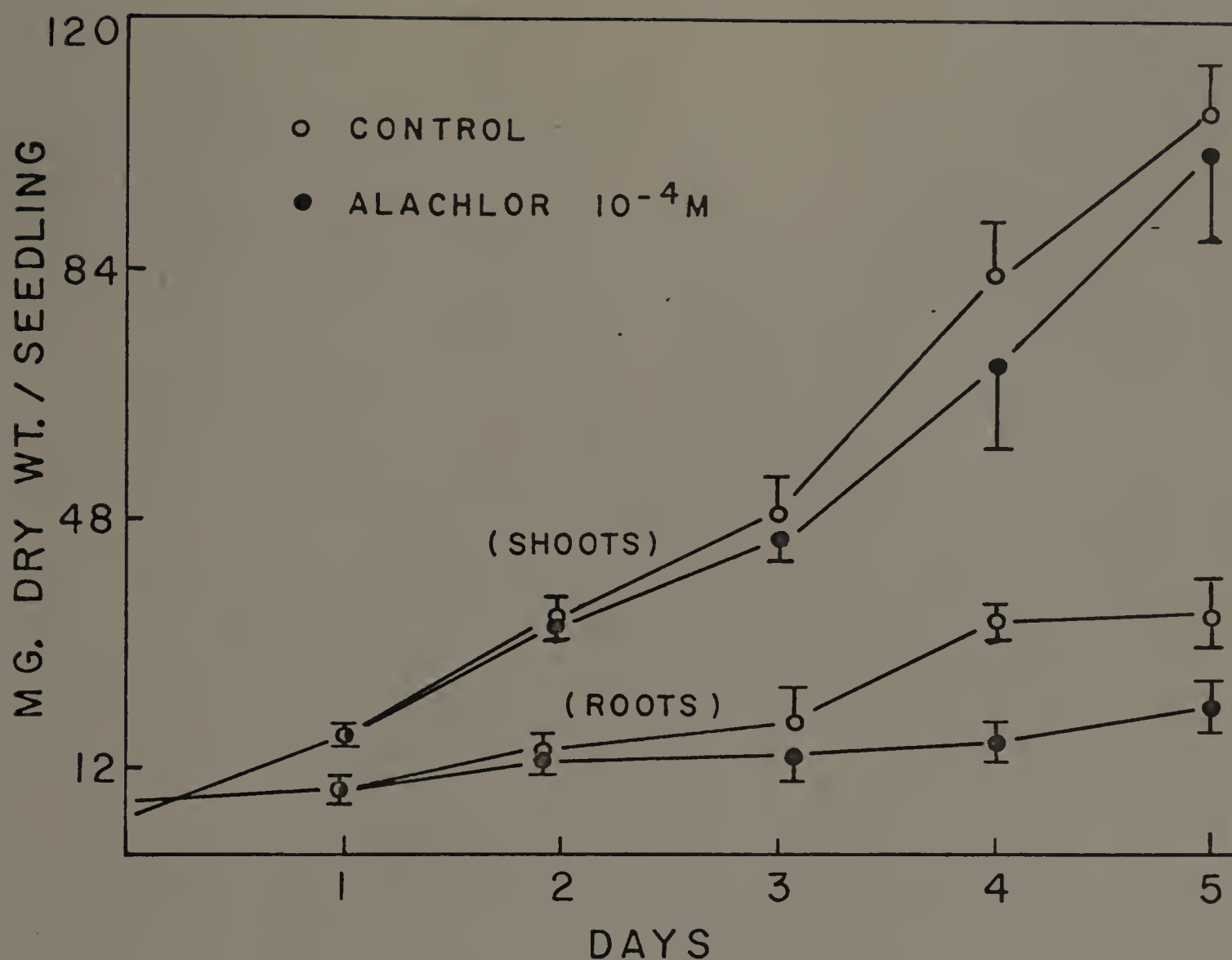


Fig. 6. Time course of increase in dry weight for oat seedlings treated with and without alachlor ( $10^{-4}$  M).

The results of these experiments suggest the site of action of alachlor is located in the root system of Avena. Chandler (5) showed that in soybean and wheat alachlor accumulated in the roots and was not translocated to the shoots. The lack of inhibition by alachlor of dry weight increase in Avena shoots indicates that the alachlor-induced inhibition of seedling height reported by Chang is not due directly to alachlor but to an inhibition of water uptake.

The occurrence of inhibited water uptake in Avena seedlings by a subirrigation treatment of alachlor has been reported by Marsh et al (11). Bates (3) noted that exposing oat root sections to alachlor ( $10^{-4}$  M) resulted in strong inhibition of uptake of a broad range of solutes. This inhibition of solute uptake by the roots would result in a decrease in accompanying water uptake as well as limiting solute transport to the growing points, which would undoubtedly lead to growth inhibition.

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SECTION III

INHIBITION OF SOLUTE UPTAKE BY ALACHLOR  
IN AVENA ROOT SECTIONS

### Abstract

2-Chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide (alachlor) at a concentration of 0.1 mM inhibited uptake of leucine,  $\alpha$ -amino isobutyric acid, 3-O-methyl glucose and potassium but not calcium. Inhibition of leucine uptake was noted within 30 minutes. Examination of leucine incorporation into protein indicated that alachlor does not inhibit this process. In addition, no effect of alachlor on respiration or on membrane leakage could be detected.

### Introduction

Growth inhibition of Avena seedlings by alachlor (a chloroacetamide herbicide) has been well established by Chang (4) and Bardzik (1). The inhibition of root growth by alachlor noted by Eshel (7) has been shown to be the site of action of alachlor by Bates (2). These findings are in agreement with the findings of Chandler et al (3) who showed that alachlor once taken up by the roots of soybean or wheat remains primarily in the root system and is not translocated to the shoot in any great amount. Rao (12) working with alachlor reported that alachlor may inhibit protein synthesis. This would seem likely since other chloroacetamides such as propachlor (6) and CDAA (8) have been shown to interfere with protein synthesis. However

our preliminary experiments indicated that protein synthesis was not inhibited although leucine uptake was. It will be shown here that alachlor has no effect on leucine incorporation into protein within the time period when growth inhibition is complete by alachlor but that it does inhibit the uptake of a broad range of solutes without affecting respiration or causing membranes to become leaky.

### Materials and Methods

Plant Material: Oat seedlings (Avena sativa var. orbit) were grown on moist paper toweling or on vermiculite in plastic boxes. Seedlings were grown at room temperature in the dark. Three day old and four day old seedlings were used as a source of root and coleoptile sections respectively.

Uptake studies: Sections 1 cm in length (from the tip back) of three day old primary roots were weighed and placed in 25 ml beakers (15 sections/beaker) containing 1.5 ml of 10 mM potassium phosphate buffer (pH 6.5) with and without alachlor. Beakers were then placed on a shaker for various preincubation periods after which time uptake was initiated by addition of 0.1 uci of labeled solute ( $^{14}\text{C}$ -1-leucine 62 uci/mM,  $^3\text{H}$ -3-O-methyl glucose 60 uci/mM, and  $^{14}\text{C}$ -alpha-amino isobutyric acid 50 uci/mM). After a 15 minute uptake period, the solution was aspirated and the sections washed in 5 ml of cold distilled water for 15 minutes. The wash

solution was aspirated and the sections placed in 5 ml of scintillation fluid (Beckman Ready-Solv Solution VI) and the radioactivity determined on a Beckman scintillation counter.

Studies of  $^{45}\text{Ca}$  (2.3mci/mg  $\text{Ca}^{++}$ ) uptake were conducted by the same procedure with the exception of a 15 minute wash in 1.0 mM calcium chloride instead of distilled water.

Leucine incorporation assay: Sections 1 cm in length (from the tip back) of three day old primary roots were weighed and placed in 25 ml beakers (15 sections/beaker) containing 1.5 ml of 10mM potassium phosphate buffer (pH 6.5) with and without 0.1 mM alachlor and 0.1  $\mu\text{ci}$  of  $^{14}\text{C}$ -1-leucine (62  $\mu\text{ci}/\text{mM}$ ). Beakers were placed on a shaker (80 oscillations/min) throughout the incorporation period. At various times the incubation medium was aspirated and sections washed in cold distilled water for 15 minutes. Sections were then boiled in ethanol. The ethanol was evaporated off and the sections were then homogenized in 1.5 ml of 1N sodium hydroxide. The extent of incorporation of  $^{14}\text{C}$ -leucine into protein was assayed according to Mans and Novelli (11). Three-tenths ml aliquots of the homogenate were spotted on 3 cm filter paper discs and air dried. The discs were placed in an ice cold 10% TCA solution for one hour, then transferred to 5% TCA ( $85^{\circ}\text{C}$ ) for 15 minutes, after which they were put through a series of ethanol-ether dehydration washes. Finally the filter paper discs

were air dried and placed in 5 ml of scintillation fluid (Beckman Ready-Solv Solution VI) and their radioactivity determined. A 0.2 ml aliquot of the homogenate was placed directly into 5 ml of scintillation fluid and counted to determine total leucine uptake. The radioactivity remaining in the filter paper discs was assumed to represent leucine incorporation into protein. An additional 0.3 ml aliquot was used for protein determination by the Lowry et al method (10).

K<sup>+</sup> determination: Sections 1 cm in length (from the tip back) of three day old primary roots were weighed and placed in 25 ml beakers (15 sections/beaker) containing 1.5 ml of distilled water, various concentrations of potassium phosphate with and without alachlor (0.1 mM). Beakers were placed on a shaker (80 oscillations/min) for 3 hours for K<sup>+</sup> uptake after which the incubation solution was aspirated and the sections washed in cold distilled water for 15 minutes. The wash solution was aspirated and the sections digested using nitric acid. Tissue digests were assayed for potassium content using a flame atomic absorption spectrophotometer.

Respiration measurements: Sections 1 cm in length (cut 3 mm from the tip back) of isolated 4-day old coleoptiles were placed in warburg flasks containing 3 ml of 50 mM trizma/potassium phosphate buffer (pH 7.2) with and without 0.1 mM alachlor. Oxygen uptake was measured on a Gilson manometer.

Leakage studies: Root systems of 3-day old seedlings were excised and groups of equal weights were placed in 25 ml beakers containing 15 ml of distilled water and 0.15M mannitol with and without 0.1 mM alachlor. Conductivity measurements were made using a Serfass conductivity bridge.

All experiments contained in this section were conducted two or more times.

### Results and Discussion

Results presented in Figure 1 show the inhibitory effect of 0.1 mM alachlor on the uptake of  $^{14}\text{C}$ -1-leucine by oat root sections. The onset of inhibition occurs between 15 and 30 minutes after exposure to alachlor. The percent inhibition increases greatly with the time of incubation up to approximately 70% inhibition at 3 hours. The increasing rate of leucine uptake in the control tissue is similar to that noted by Leonard and Hanson (9) and is the result of prolonged immersion of root sections in water.

To see if alachlor behaves like other chloroacetamides with regard to inhibition of protein synthesis, the incorporation of  $^{14}\text{C}$ -leucine was examined. Figure 2 depicts the effect of 0.1 mM alachlor on  $^{14}\text{C}$ -leucine incorporation in the oat root sections. As can be seen by the hypothetical curve, there is no inhibitory effect due to alachlor on  $^{14}\text{C}$ -leucine incorporation up to 1 hour after exposure to alachlor. However, between 1 and 3 hours after exposure to

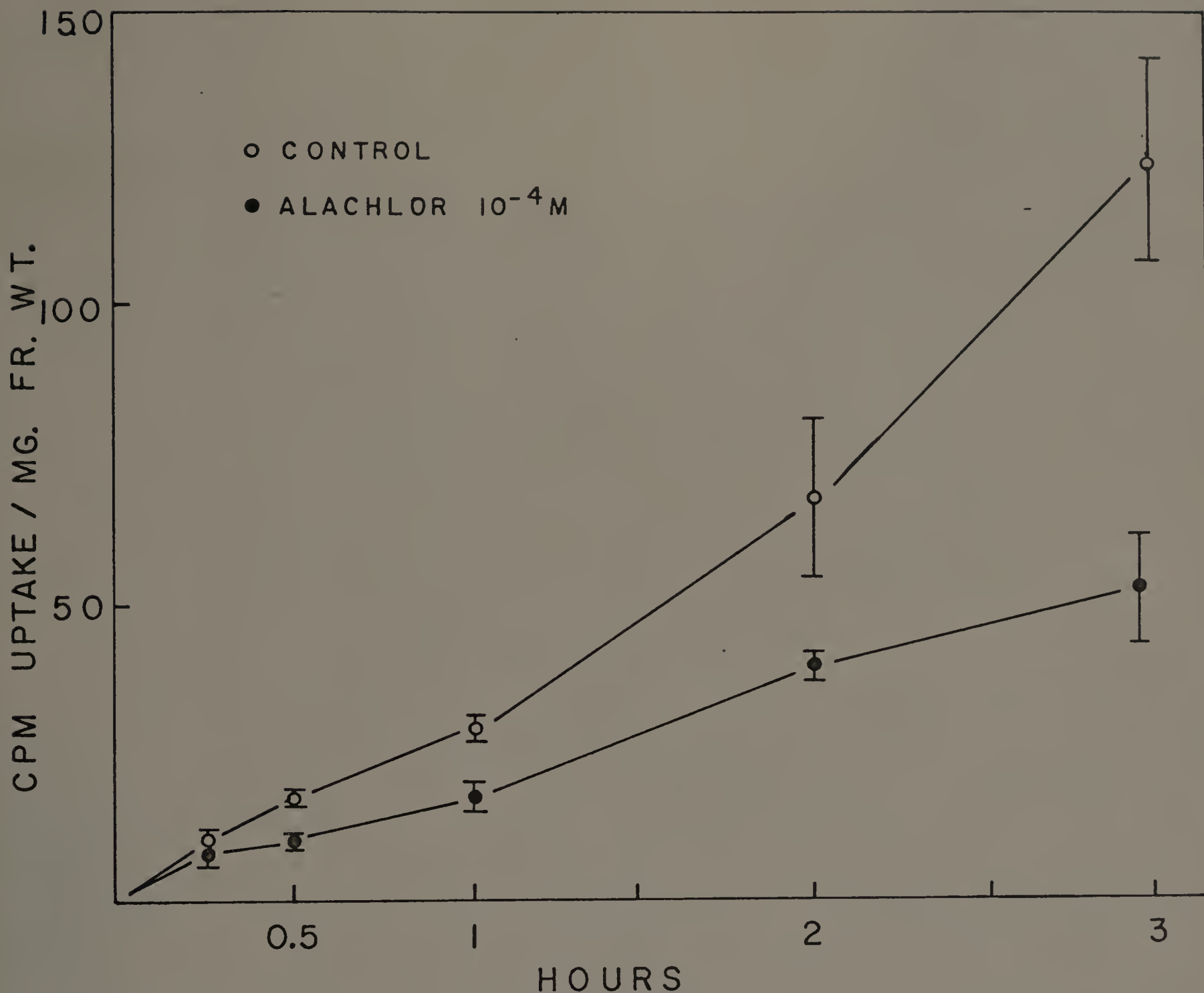


Fig. 1. Time course of leucine uptake by oat root sections with and without 0.1 mM alachlor.

alachlor there is an apparent stimulation of leucine incorporation.

In order to determine if the alachlor-induced inhibition of leucine uptake was reversible, an experiment was conducted in which root sections were exposed to 0.1 mM alachlor for one hour and then transferred to a medium free

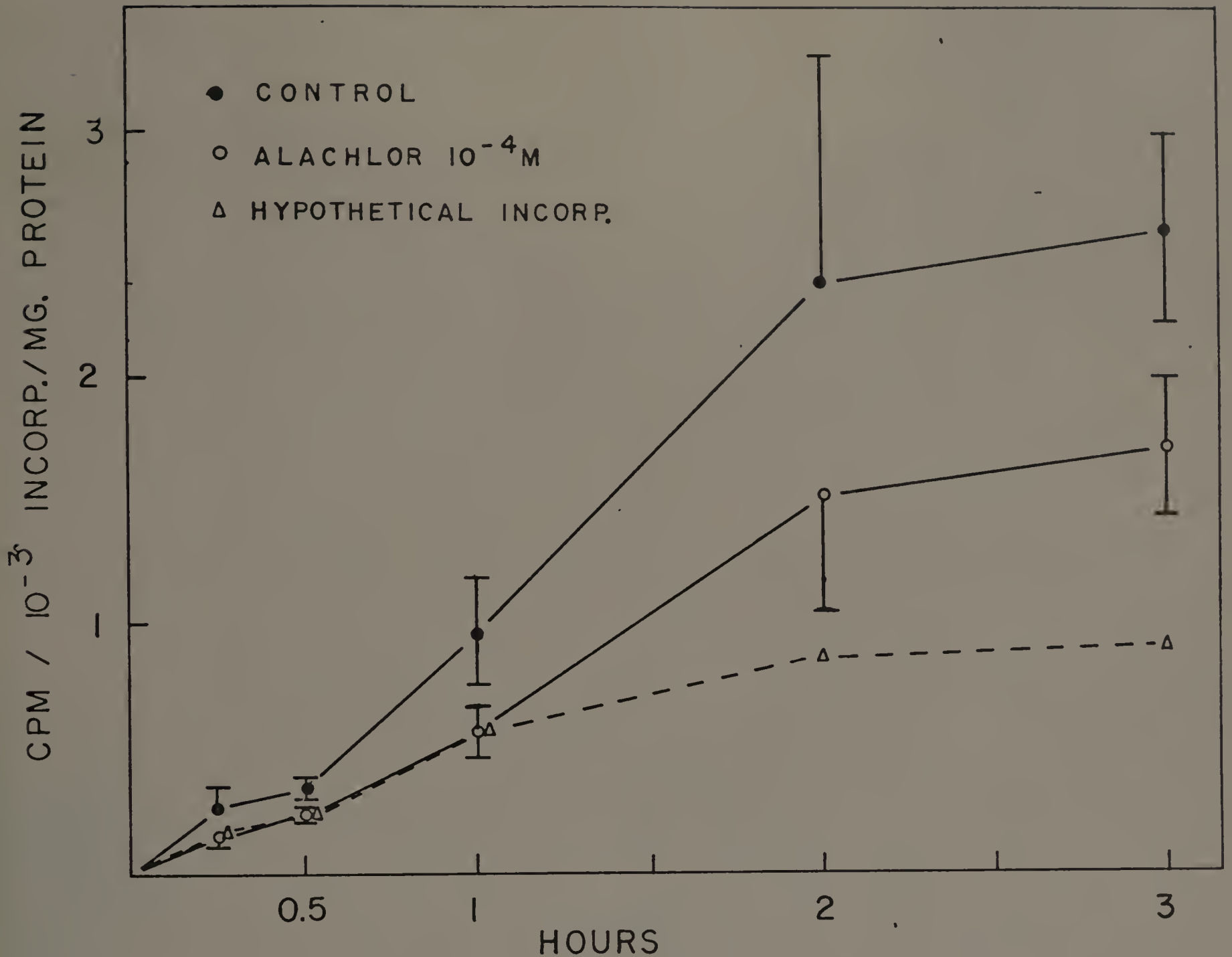


Fig. 2. Time course of  $^{14}\text{C}$ -1-leucine incorporation in oat root sections in the presence of 0.1 mM alachlor (○) and without alachlor (●). The dotted line (Δ) represents the hypothetical amount of leucine that should be incorporated based on the amount of free leucine (cpm uptake/mg protein) in treated tissue in relation to untreated tissue.

of alachlor for a further two hour incubation. Sections were then exposed to  $^{14}\text{C}$ -1-leucine for a 15 minute labeling period. The results of this experiment (Table 1) show

Table 1Leucine Uptake  
by Avena Root Sections\*cpm/mg. fr. wt./hr.

<u>TREATMENTS</u>	<u>1 hr.</u>	<u>3 hr.</u>
Control	14.7 $\pm$ 1.6	30.2 $\pm$ 0.4
Ala $10^{-4}$ M	8.3 $\pm$ 0.5	_____
**Ala $10^{-4}$ M	_____	45.0 $\pm$ 2.6

\* Values are an average of 3 repetitions of 15 sections each.

\*\* Exposure for 1 hr, then transferred to control conditions for remainder of 3 hr treatment.

that not only is the alachlor-induced inhibition reversible but that the washing out of alachlor has a stimulatory effect on leucine uptake.

Table 2 shows the effects of various concentrations of alachlor on the uptake of various solutes. Leucine uptake is strongly inhibited by  $10^{-4}$ M and  $5 \times 10^{-5}$ M alachlor. In a separate experiment  $10^{-6}$ M alachlor increased leucine uptake two-fold (data not shown). Alpha-amino isobutyric acid ( $\alpha$ -AIB, a non-incorporated amino acid) was looked at in order to study the effects of alachlor on amino acid uptake independent of incorporation of the amino acid into protein. As seen here  $10^{-4}$ M alachlor strongly inhibited uptake of  $\alpha$ -AIB but  $10^{-6}$ M alachlor has no effect. A non-metabolized sugar 3-O-methyl glucose (3-O-CH<sub>3</sub>-Glu) was used to examine effects of alachlor on sugar uptake. Results were similar to those of  $\alpha$ -AIB, with  $10^{-4}$ M alachlor

Table 2

Solute Uptake  
by Avena Root Sections

<u>Treatment</u> (3 hr)	<u>*cpm/mg. fr. wt./hr.</u>			
	<u>Leucine</u>	<u>-AIB</u>	<u>3-O-CH<sub>3</sub>-Glu</u>	<u>Ca<sup>++</sup></u>
Control	133.6 $\pm$ 6.5	39.7 $\pm$ 4.0	10.1 $\pm$ 1.5	62.3 $\pm$ 7.9
Ala 10 <sup>-4</sup> M	48.3 $\pm$ 5.2	16.3 $\pm$ 1.0	4.9 $\pm$ 0.4	60.0 $\pm$ 4.3
Ala 5X10 <sup>-5</sup> M	79.8 $\pm$ 5.6	_____	_____	_____
Ala 10 <sup>-5</sup> M	225.0 $\pm$ 25	_____	5.1 $\pm$ 0.2	_____
Ala 10 <sup>-6</sup> M	_____	32.4 $\pm$ 1.0	11.1 $\pm$ 3.8	61.7 $\pm$ 3.4

\* Values are an average of 3 repetitions of 15 sections each.

strongly inhibitory and 10<sup>-6</sup>M alachlor having no effect.

Since it has been reported that alachlor inhibits chloride ion uptake in oat coleoptiles (1), the absorption of calcium and potassium were examined for possible effects by alachlor. Alachlor had no effect on calcium absorption (Table 2) at any of the concentrations tried. Results of experiments with calcium absorption under aerobic and anaerobic conditions yielded no difference (data not shown), indicating that calcium absorption in Avena roots is not an active uptake process. Additional experiments monitoring calcium wash out (data not shown) indicate that a large amount of the calcium taken up in the 15 minute labeling period may in fact be adsorbed and not absorbed. The absorption of potassium (Table 3) was inhibited by 10<sup>-4</sup>M alachlor but only when the potassium phosphate concentration

Table 3Potassium Uptake by  
Avena Root Sections\* $\mu\text{m K}^+$ /gr. fr. wt.Incubation Medium

<u>TREATMENT</u>	<u>DIST. H<sub>2</sub>O</u>	<u>0.2 mM KPO<sub>4</sub></u>	<u>2.0 mM KPO<sub>4</sub></u>	
			<u>I.</u>	<u>II.</u>
Original	_____	_____	_____	44.5 $\pm$ 1.5
Control (3 hr)	36.9 $\pm$ 4.6	42.6 $\pm$ 4.1	55.8 $\pm$ 1.8	51.2 $\pm$ 3.3
Ala $10^{-4}$ M (3 hr)	35.3 $\pm$ 3.1	42.0 $\pm$ 2.6	46.0 $\pm$ 7.1	43.6 $\pm$ 2.8

\* Values are an average of 3 repetitions of 15 sections each.

in the medium was 2.0 mM. Treated and control sections both lost potassium when placed in distilled water probably due to leakage.

Examination for effects by alachlor on respiration (Figure 3) were conducted using oat coleoptile tissue. Results here show that  $10^{-4}$ M alachlor has no effect on respiration up to 4 hours after exposure and only a slight effect at 5 hours. These results are similar to those of Bardzik (1) with alachlor and those of Duke (6) with propachlor. Additional experiments using isolated corn mitochondria showed no effect on respiration due to alachlor.

An investigation of possible alachlor-induced membrane leakage as an explanation for the inhibition of solute uptake was conducted. Root systems of 3-day old seedlings

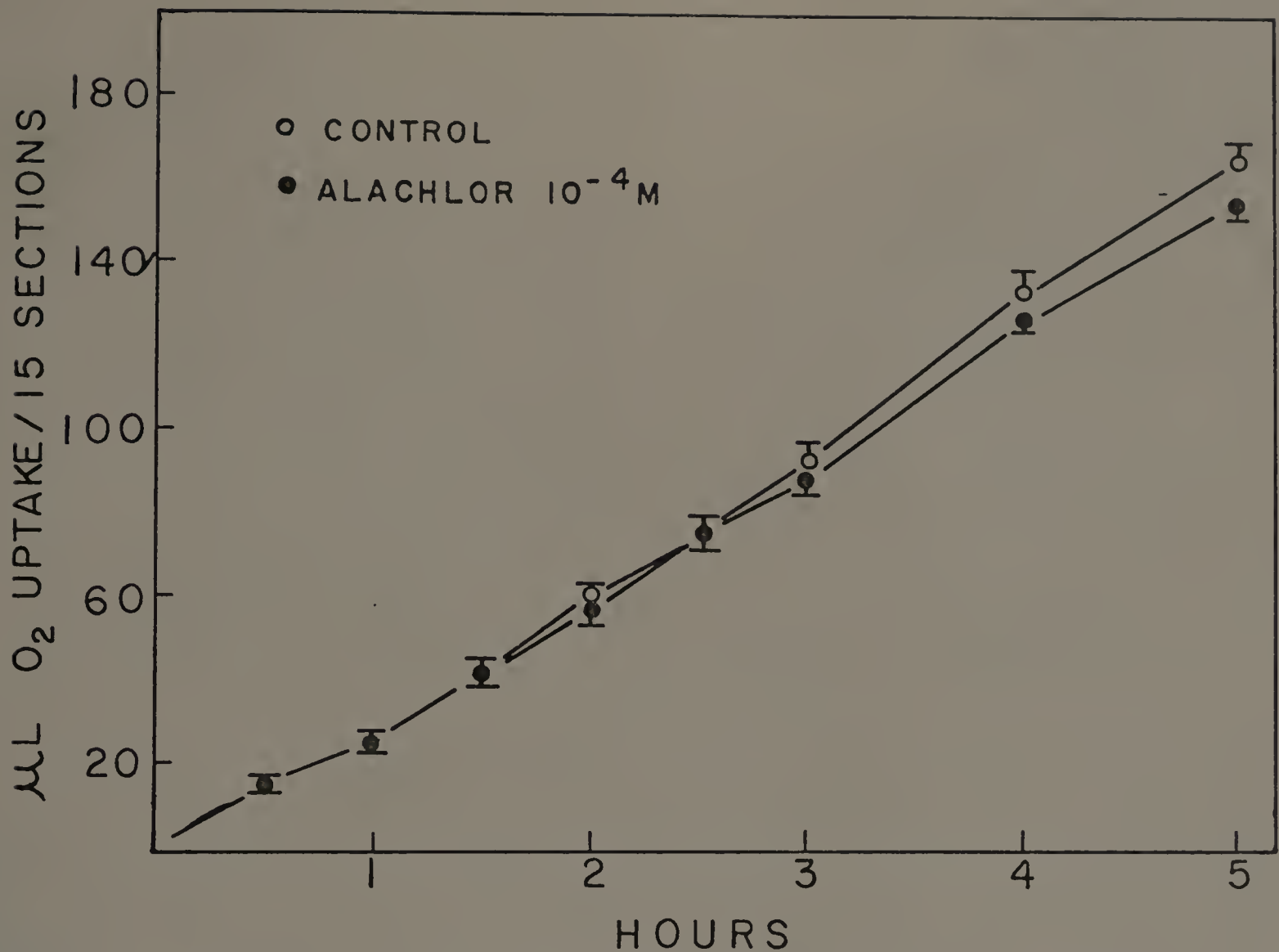


Fig. 3. Time course of O<sub>2</sub> uptake in *Avena* coleoptile sections with and without 0.1 mM alachlor.

were placed in .15M mannitol, a concentration which is far below that needed for plasmolysis (5), to prevent the leakage which results when the tissue is placed in distilled water. Ethanol and dinoseb, a known inhibitor of lipid synthesis which causes membrane leakage (13) were used as checks for membrane leakage. The results presented in Figure 4 show that even after 5 hours exposure to 0.1 mM alachlor there is no increased leakage of ions from the root sections. Additional experiments looking for an effect

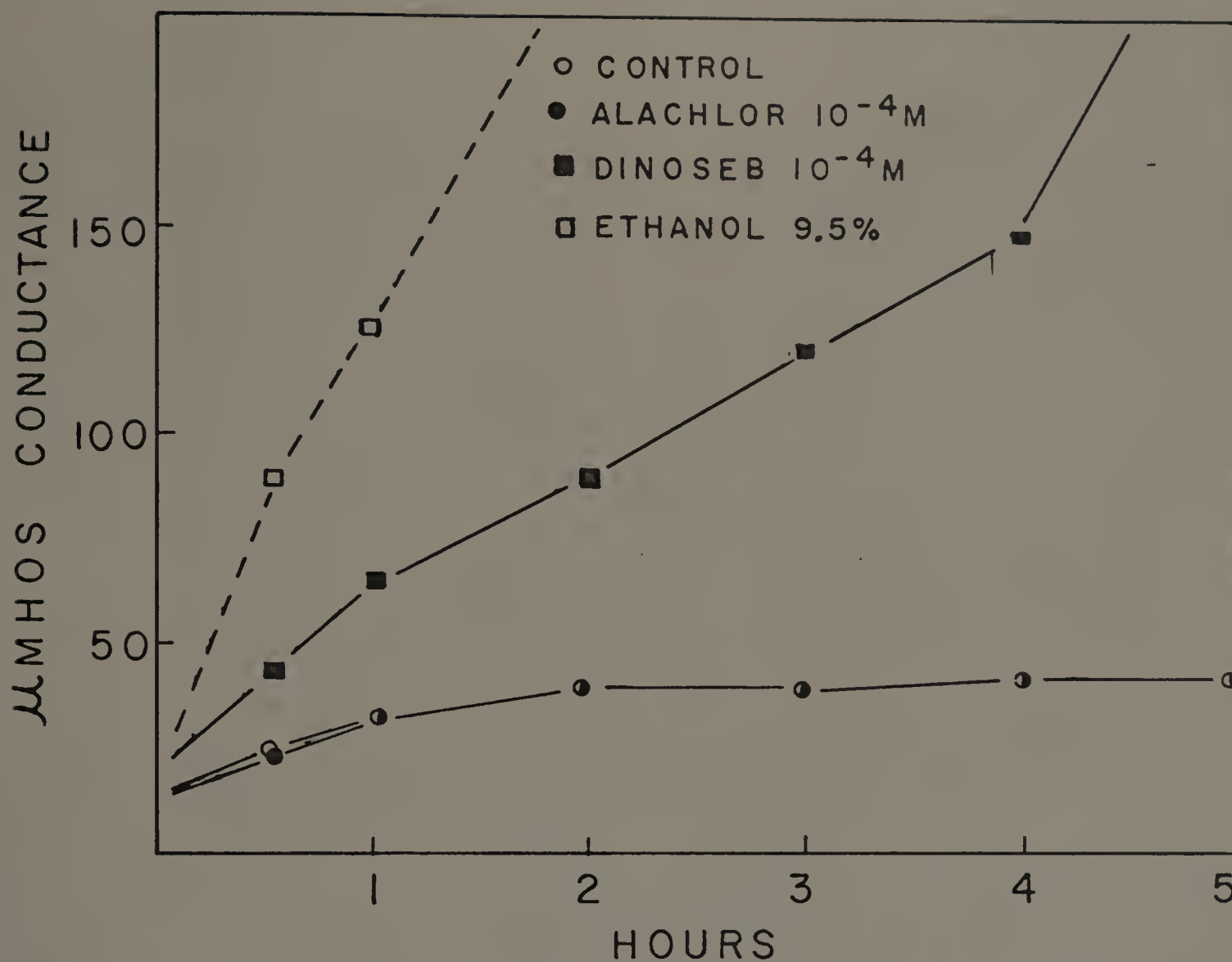


Fig. 4. Time course of electrolyte leakage from oat roots with and without 0.1 mM alachlor.

of alachlor on beta-cyanin leakage from red beets and the cyanin from sorghum seedlings yielded negative results (data not shown). Dinoseb was used as a check here also and resulted in pigment leakage.

#### Summary

Contrary to findings with other chloroacetamide

herbicides (8, 12), the mode of alachlor does not appear to be that of inhibiting protein synthesis. Exposure to alachlor seems to stimulate rather than inhibit protein synthesis. The stimulation of leucine uptake in root sections treated with alachlor which is subsequently washed out resembles the stimulation of uptake by low concentrations of alachlor and is probably due to low concentrations of alachlor caused by the wash out procedure. The fact that leucine uptake was stimulated by low concentrations of alachlor while the other solutes were not indicates that the stimulated leucine uptake is probably due to an apparent stimulation of leucine incorporation. The apparent stimulation of leucine incorporation may be real or may be due to an alachlor interference with leucine pools in the tissue resulting in the labeled leucine which was taken up by the treated tissue comprising a greater percentage of leucine available for available for incorporation than in control tissue. If the apparent stimulation of leucine incorporation is not real then there is no apparent explanation for the stimulated uptake of leucine.

Inhibition by alachlor of uptake of many solutes has been shown with the exception of calcium absorption (due to calcium absorption probably being passive or adsorbed) and since no effect by alachlor on respiration or on membrane leakage could be found, it would appear that the mode of action of alachlor deals directly with solute transport systems.

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SECTION IV

EFFECTS OF ALACHLOR ON CORN SEEDLINGS

### Abstract

The herbicide 2-chloro-2',6'-diethyl-N-(methoxymethyl)acet-anilide (alachlor) has been shown to severely inhibit the root and shoot growth of zero to 3-day old corn (Zea mays L.) growing in sand when applied by sub-irrigation. The roots of 5-day old seedlings were inhibited by the same treatment, but shoots of the older seedlings showed no response. The coleoptile of corn is apparently insensitive to alachlor in that the herbicide neither inhibited elongation of coleoptile segments nor <sup>14</sup>C-leucine uptake by this tissue. Leucine uptake by excised roots, however, was inhibited 40 to 67%.

### Introduction

Alachlor is an effective herbicide for use in corn (Zea mays L.) where it controls the growth of most weed species with no apparent deleterious effect on the growth of the crop. Directions for the use of alachlor call for its preemergence incorporation into the top two inches of soil prior to seeding the corn, which normally is planted at a depth of 1 1/2 to 2 inches. The fact that corn is successfully grown under diverse cultural conditions employing an alachlor weed control program implies that this species is somehow resistant to the herbicide.

Experiments in our laboratory indicated that many crops are sensitive to alachlor at  $10^{-4}$ M, which is about 40% of the concentration recommended for field application (assuming an application rate of 2 lbs. alachlor per acre). It was of interest, therefore, to establish the effects of alachlor, if any, on the growth of corn seedlings.

### Materials and Methods

Corn seedling studies: Corn seeds were sown in 6.5 cm diameter plastic pots filled with quartz sand. Seedlings were grown at room temperature with a 12 hour day of 7000 Lux. Alachlor ( $10^{-4}$ M) in one-half strength Hoagland's nutrient medium was added by subirrigation to the seedlings at various times after imbibition, and exposure to alachlor was continued until termination of the experiment. Daily height measurements as well as observations of root systems were taken.

Coleoptile growth studies: Corn seeds were grown on moist paper toweling in the dark at 25°C. Four day old seedlings were harvested and 6.5 cm coleoptile sections excised. Sections were placed in 10 mM potassium phosphate buffer (pH 6.5) containing indolacetic acid ( $10^{-5}$ M) with and without alachlor ( $10^{-4}$ M). Growth was monitored with a dissecting microscope using a lens micrometer.

Leucine uptake: Dark grown seedlings (as above) were

harvested. Excised coleoptile sections (5 mm in length) and excised primary root sections (5 mm in length) were placed in beakers containing 1.5 ml of 10 mM potassium phosphate buffer (pH 6.5) with and without  $10^{-4}$  M alachlor. One-tenth  $\mu$ Ci of  $^{14}$ C-1-leucine (62  $\mu$ Ci/mM) was added to each beaker for a period of 15 minutes, after which the sections were aspirated and washed for 15 minutes in cold distilled water. Sections were then aspirated and placed in 5 ml of scintillation fluid (Beckman Ready-Solv solution VI) and counted on a Beckman scintillation counter. Peeled coleoptiles were prepared by physically peeling away the cuticle and outer epidermal cell wall layer (3).

### Results

Alachlor inhibited shoot growth of corn seedlings when applied at the time of imbibition, or 3 days after imbibition, the former treatment causing almost total inhibition and the latter approximately 40% inhibition (Fig. 1 and Figs. 2A and B). Treating the seedlings with alachlor 5 days after imbibition caused no detectable effect on the growth of the shoots. Examination of the whole seedlings revealed that the alachlor treatment at the time of imbibition or 3 days after imbibition caused a severe inhibition of root as well as shoot growth (Fig. 2A and B). In contrast to the shoots, the roots of the seedlings which had been treated 5 days after imbibition

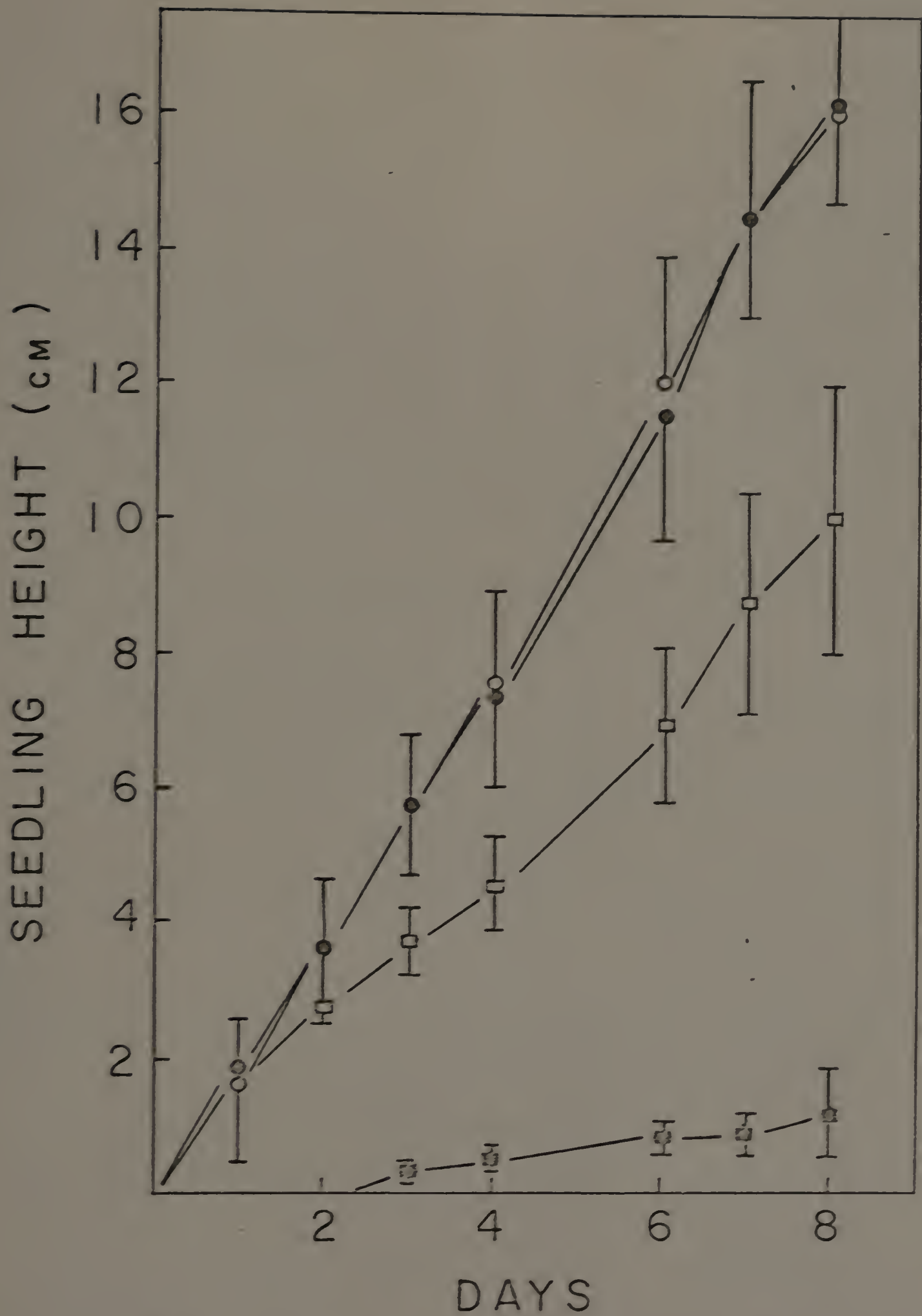


Fig. 1. Time course of corn seedling growth after emergence. Control (●), alachlor ( $10^{-4}$  M) added at imbibition (■), alachlor ( $10^{-4}$  M) added 3 days after imbibition (□) and alachlor ( $10^{-4}$  M) added 5 days after imbibition (○).

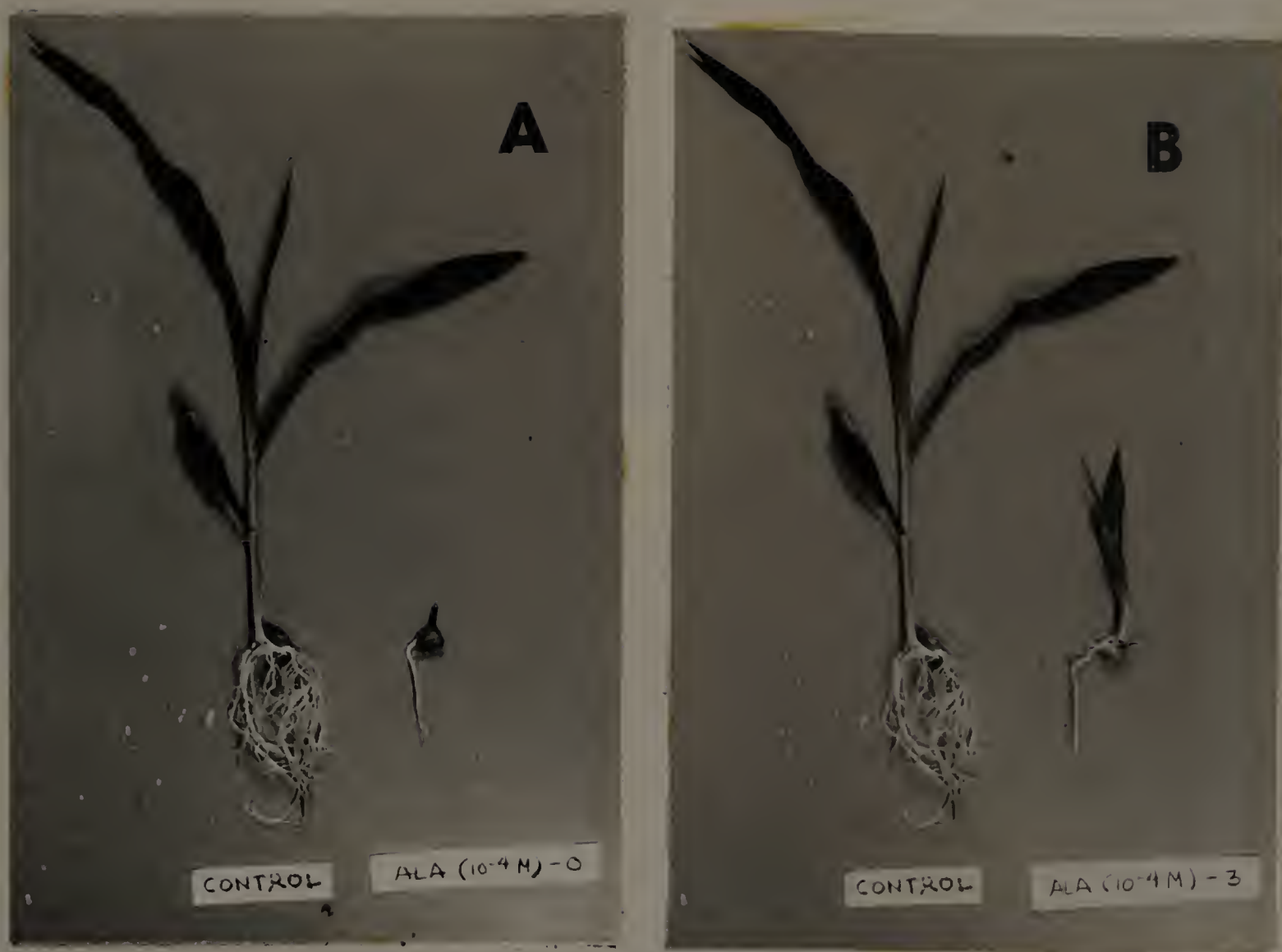


Fig. 2. Corn seedlings treated with alachlor ( $10^{-4}M$ ) at time of imbibition (A) and 3 days after imbibition (B).

were also significantly inhibited (Fig. 3A), although not as severely as those treated earlier (Fig. 3B).

The effect of alachlor on the growth of corn coleoptiles was also examined since extensive work with oats has shown this organ to be especially sensitive to the herbicide (1, 2). Interestingly, elongation of the corn coleoptile appears to be completely insensitive to  $10^{-4}M$  alachlor (Fig. 4), a concentration which causes almost complete inhibition of oat coleoptile growth after a lag period of about 2 hours (1, 2).

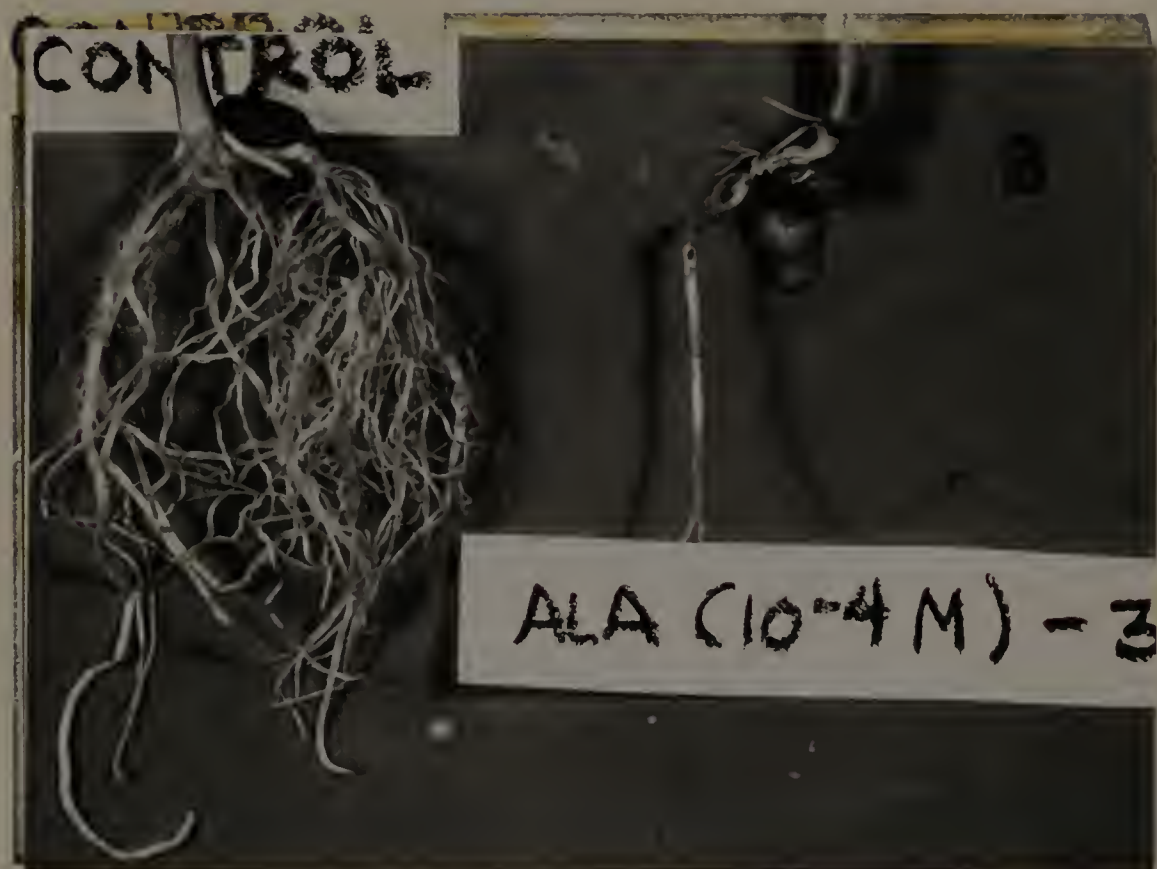
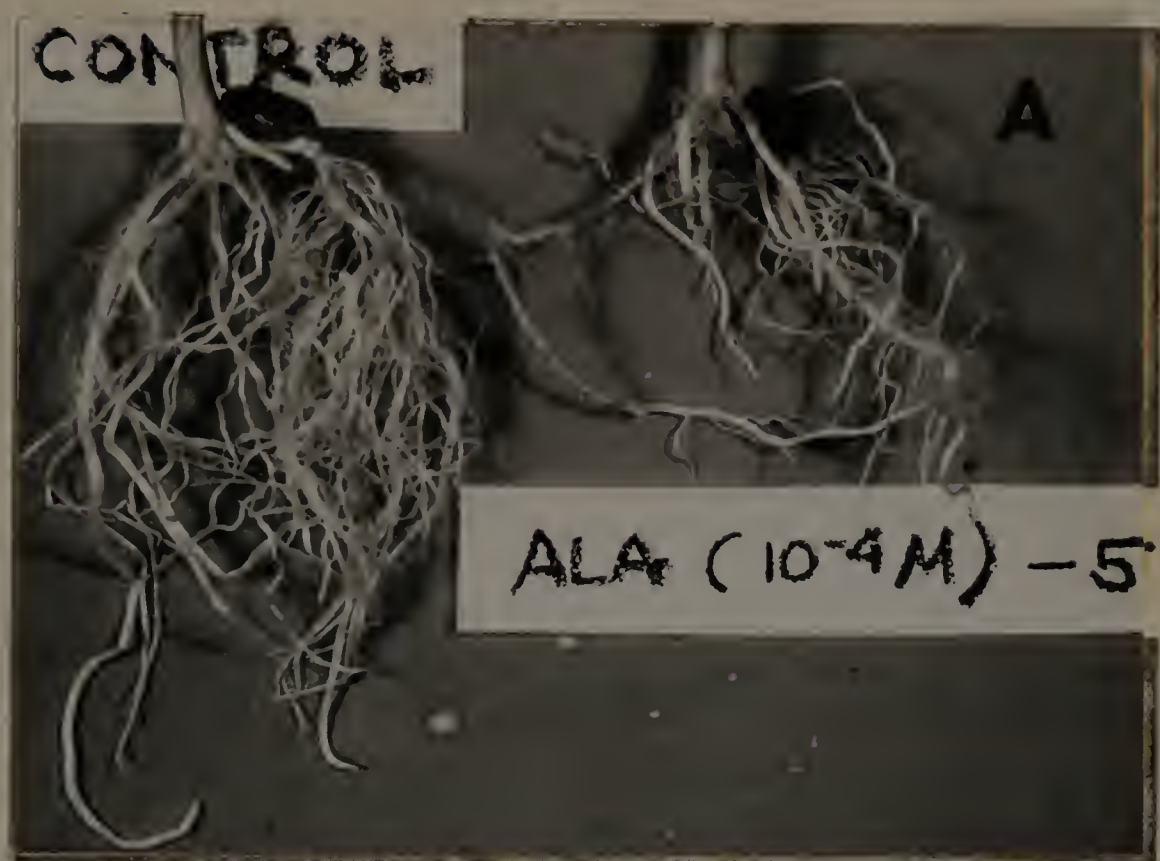


Fig. 3. Corn roots systems treated with alachlor ( $10^{-4}M$ ) 5 days after imbibition (A) and 3 days after imbibition (B).

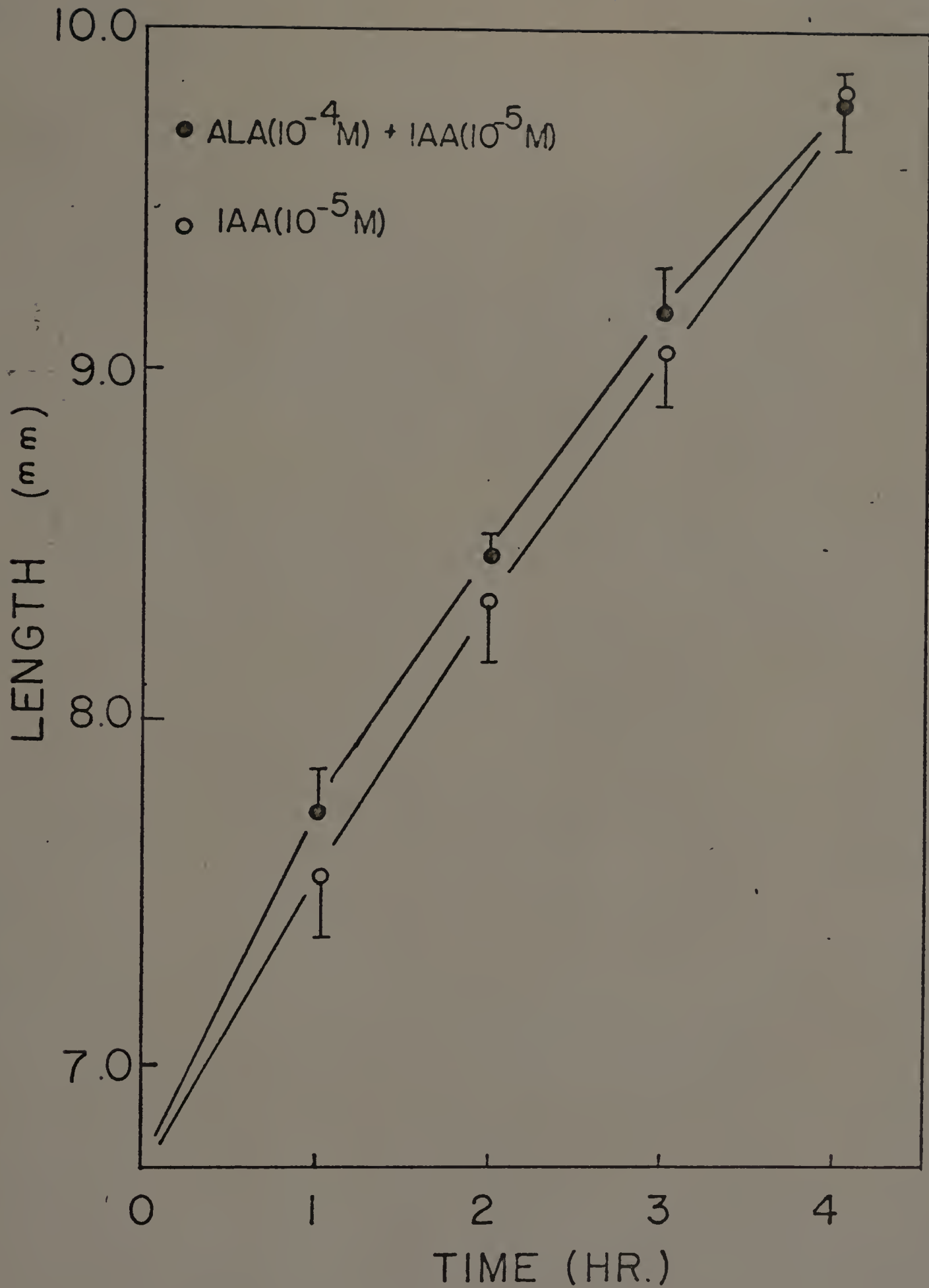


Fig. 4. Time course of auxin-induced corn coleoptile elongation in the presence and absence of  $10^{-4}$  M alachlor.

<sup>14</sup>C-Leucine Uptake by Corn Tissue

<u>Treatment</u> (3 hr.)	<u>Leucine Uptake</u> (cpm/mg. fr. wt./hr.)			
	<u>Root Sections</u>		<u>Coleoptile Sections</u>	
	I	II	<u>Unpeeled</u>	<u>Peeled</u>
Control	16.8 $\pm$ 5.6	20.4 $\pm$ 2.5	11.2 $\pm$ 0.6	19.8 $\pm$ 2.8
Alachlor ( $10^{-4}$ M)	7.3 $\pm$ 0.5	12.4 $\pm$ 4.5	9.6 $\pm$ 0.8	18.4 $\pm$ 1.6
Inhibition (%)	67	40	14	7

Alachlor at  $10^{-4}$ M causes 40 to 60% inhibition of metabolite transport in both oat root and coleoptile tissues (2). Examination of the effect of this concentration of alachlor on <sup>14</sup>C-1-leucine uptake by root and coleoptile sections revealed that uptake by corn roots was as sensitive as in other species (Table). Leucine uptake by the coleoptiles, however, was only slightly inhibited. Since the coleoptile cuticle is a barrier to the transport of some metabolites in other tissues (3), the cuticle was removed and the effect of alachlor on <sup>14</sup>C-1-leucine uptake again monitored. The data presented in the Table indicate that although the cuticle may act as a barrier to leucine uptake, it can not be responsible for the corn coleoptile's insensitivity to alachlor.

### Discussion

It has been demonstrated that under certain conditions a concentration of alachlor within the range recommended for field application can inhibit the growth of corn seedlings. That the herbicide does not normally have such drastic effects as noted here may be attributed to several factors. First, the soil probably binds some of the alachlor, thus reducing its effective concentration to the point that it is not so inhibitory to corn but still inhibits weed seedlings. Second, the corn seeds may be planted just at the lower boundary of where the herbicide is incorporated into the soil, thus the seed may escape direct contact with a significant amount of the herbicide. Thus, when the seed germinates, the sensitive roots can be envisioned as growing down out of the zone of the alachlor while the shoot, protected by the resistant coleoptile, grows up through the alachlor layer.

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