MORPHOLOGICAL EFFECTS OF NAPHTHALIC ANHYDRIDE AND/OR THIOBENCARB ON IR-36 AND RD-19 RICE SEEDLINGS

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Portion of Ph.D. dissertation conducted by the author in U.P. at Los Baños, College, Laguna.

ABSTRACT

The root and shoot elongation as well as the anatomy of IR-36 and RD-19 rice seedlings treated with different concentrations of NA (1,8-naphthalic anhydride), thiobencarb (S-4-chlorobenzyl diethylthiocarbamate) and NA + thiobencarb was observed. Root elongation in the 2 cultivars was adversely affected by thiobencarb but not by NA treatment. Generally, the presence of NA in antidote-herbicide treatments seemed to reduce the inhibitory effect of thiobencarb on the root. Shoot growth was inhibited by thiobencarb and NA + thiobencarb to a greater extent than root growth. Inhibition of root and shoot elongation was more pronounced in IR-36 than in RD-19 seedlings. Root growth inhibition by thiobencarb was not accompanied by any phytotoxic symptom at the histological level. In the shoot, thiobencarb injury was expressed by kinking of linear rows of cells and alteration of physical arrangement of mesophyll cells in developing leaves within the coleoptile. This anatomical effect was noted in all thiobencarb treatments with or without NA in both cultivars except in the NA + 5 ppm thiobencarb treatment on RD-19 seedlings. Such anatomical observation can be correlated with the abnormal growth and emergence of leaves from grass coleoptiles which are the major phytotoxic symptoms induced by thiobencarb and other thiocarbamate herbicides.

Ann. Trop. Res. 4: 245-258.

KEY WORDS: Morphological effects. Naphthalic anhydride. Thiobencarb. Anatomy. Rice seedlings. Root. Shoot. Kinking. Elongation. Coleoptile.

INTRODUCTION

Carbamate and thiocarbamate herbicides generally provide very effective control of most grass weeds. However, they occasionally become phytotoxic to crops which also belong to the grass family.

Application of these herbicides to the soil primarily causes growth inhibition of emerging shoots particularly of grasses. Injured plants are characterized by abnormal growth and emergence of the leaves from the coleoptiles. Leaf emergence is completely inhibited at high rates of application. At low rates, the leaves may emerge but may remain longitudinally rolled or just emerge through the base of the coleoptile.

Thiobencarb (S-4-chlorobenzyl diethylthiocarbamate) is one of the thiocarbamate herbicides used for weed control in rice. It may become non-selective if sprayed to a field planted with a susceptible rice variety.

The selectivity of thiobencarb and related compounds on rice can be greatly improved with the application of naphthalic anhydride or NA (1,8-naphthalic anhydride). NA treatment has shown promise for protecting rice against molinate (S-ethyl hexahydro-1H-azepine-1-carbothioate) and makes possible the selective control of the very closely related red rice *(Oryza punctata)* in rice (Parker and Dean, 1976). Henry (1972) reported that NA protects rice against a number of other herbicides including thiobencarb. More recently, Madrid (1980) observed that NA provided increased protection from thiobencarb damage to IR-40 and Nam Sagui 19 rice cultivars.

Although several reports indicate the effectiveness of NA against thiobencarb injury in rice, no attempt has been made earlier to determine the effect of these 2 chemicals on rice at the morphological and histological level. Hence, this study was conducted to look into the morphological responses of susceptible and tolerant rice varieties to treatments with NA, thiobencarb, and NA + thiobencarb.

MATERIALS AND METHODS

Seeds of IR-36 and RD-19 rice cultivars were germinated in petri dishes containing 10 ml of 0, 1, 2 and 5 ppm of NA suspension or 0, 5, 10 and 25 ppm of thiobencarb emulsion. Some seeds were coated with 0.5% (w/w) of NA and then germinated in petri dishes with 10 ml of 0, 5, 10 and 25 ppm of thiobencarb. All treatments with 4 replicates each were kept inside the laboratory. Root and shoot length was measured 1 week after treatment. The seedlings were also observed for any morphological abnormality.

For histological study, treated and untreated seedlings were washed thoroughly with distilled water. Afterwards, root and shoot apices were cut off and fixed in FAA [formalin (5 ml): glacial acetic acid (5 ml): 70% ethyl alcohol (95 ml)] solution. The tissues were dehydrated through a graded series of tertiary butyl alcohol and embedded in paraffin. Longitudinal sections about 12-15 microns thick were cut and stained following the safranin 0-fast green schedule. The prepared slides were examined and photomicrographed.

RESULTS AND DISCUSSION

Root and Shoot Elongation.

The root length of IR-36 and RD-19 rice seedlings one week after treatment is presented in Table 1. Generally, the pattern of response of the 2 cultivars to the different treatments was almost the same. NA at any level did not cause root inhibition in both cultivars. However, application of thiobencarb at all levels seemed to be more inhibitory to root growth than treatments with NA alone or NA-thiobencarb combinations (Figs. 1a, 1b, 2a, 2b). Root length also progressively decreased as the level of thiobencarb either applied alone or combined with NA was increased. All combinations of NA with thiobencarb caused the same degree of root inhibition but this was less than that noted in any treatment with the herbicide alone. The protective effect of NA on the 2 cultivars was more evident when it was combined with 10 and 25 ppm than 5 ppm thiobencarb. Seedlings treated with 5 ppm thiobencarb with or without NA exhibited comparable suppression of root growth whereas NA decreased in-

Table 1. Root and shoot length of IR-36 and RD-19 rice seedlings 1 week after treatment with NA, thiobencarb, and NA + thiobencarb.

Treatment	IR-36 Length (mm)[1]		RD-19 Length (mm)[1]	
	Root	Shoot	Root	Shoot
Distilled water (without NA and thiobencarb)	34.4 ab	29.2 b	55.9 ab	24.0 ab
1 ppm NA	36.8 a	27.7 b	54.0 ab	25.4 ab
2 ppm NA	28.9 abc	26.9 b	63.9 a	28.8 a
5 ppm NA	26.3 abc	30.1 b	57.9 ab	30.8 a
5 ppm thiobencarb	20.3 cd	5.6 cd	37.9 bcd	7.0 c
10 ppm thiobencarb	11.5 de	3.4 d	24.3 cd	5.7 c
25 ppm thiobencarb	3.7 e	3.1 d	15.6 d	5.5 c
Coated with NA + distilled water	21.8 bcd	37.8 a	30.8 cd	33.9 a
Coated with NA + 5 ppm thiobencarb	19.8 cd	7.6 c	39.2 bc	23.6 ab
Coated with NA + 10 ppm thiobencarb	21.9 bcd	7.3 c	37.3 bcd	15.2 bc
Coated with NA + 25 ppm thiobencarb	18.4 cd	5.6 bcd	37.4 bcd	6.3 c

[1] Average of 4 replications. In a column, means followed by a common letter are not significantly different at the 1% level by DMRT.

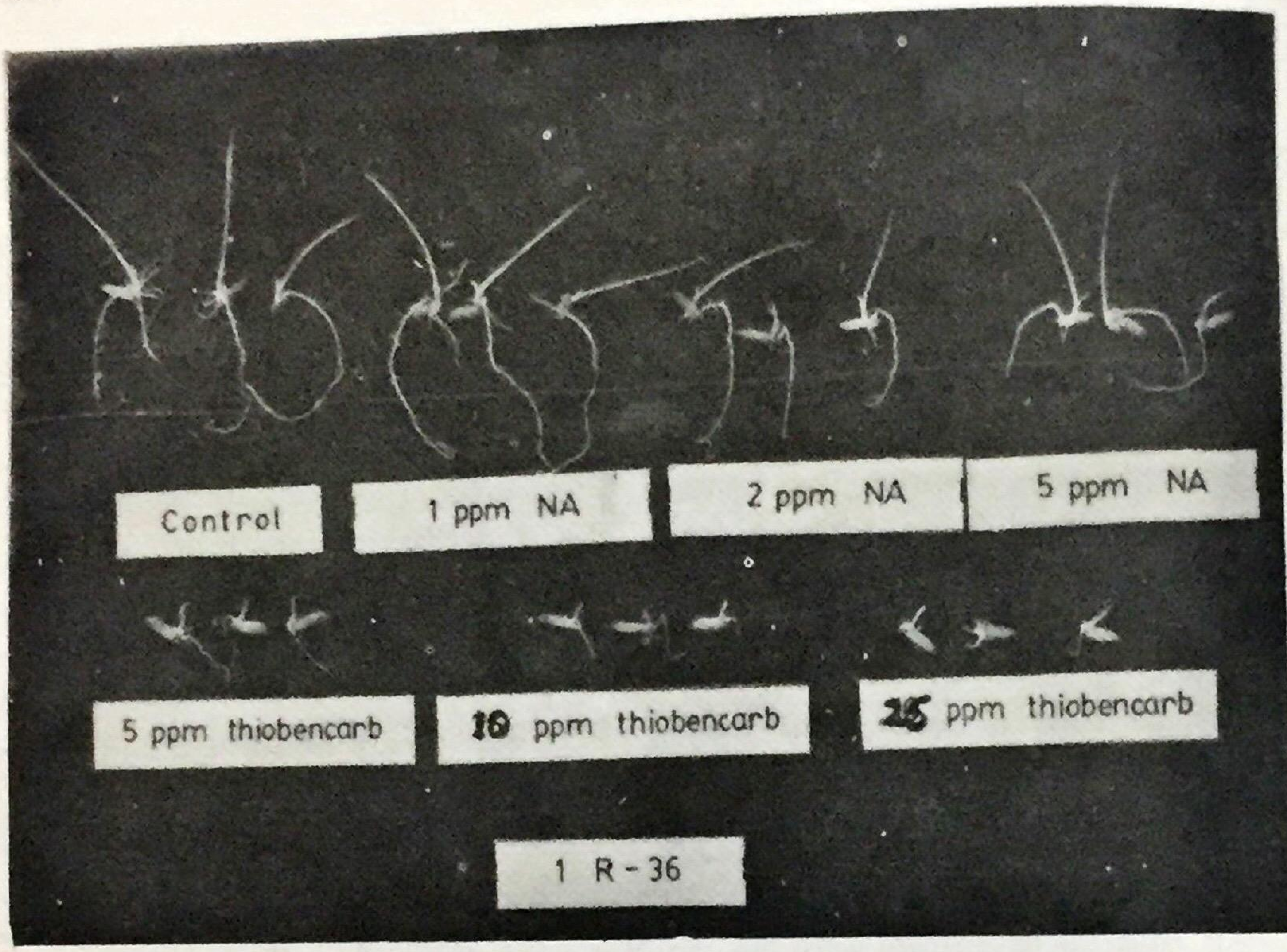


Fig. 1a. IR-36 rice seedlings treated with different concentrations of NA and thiobencarb.

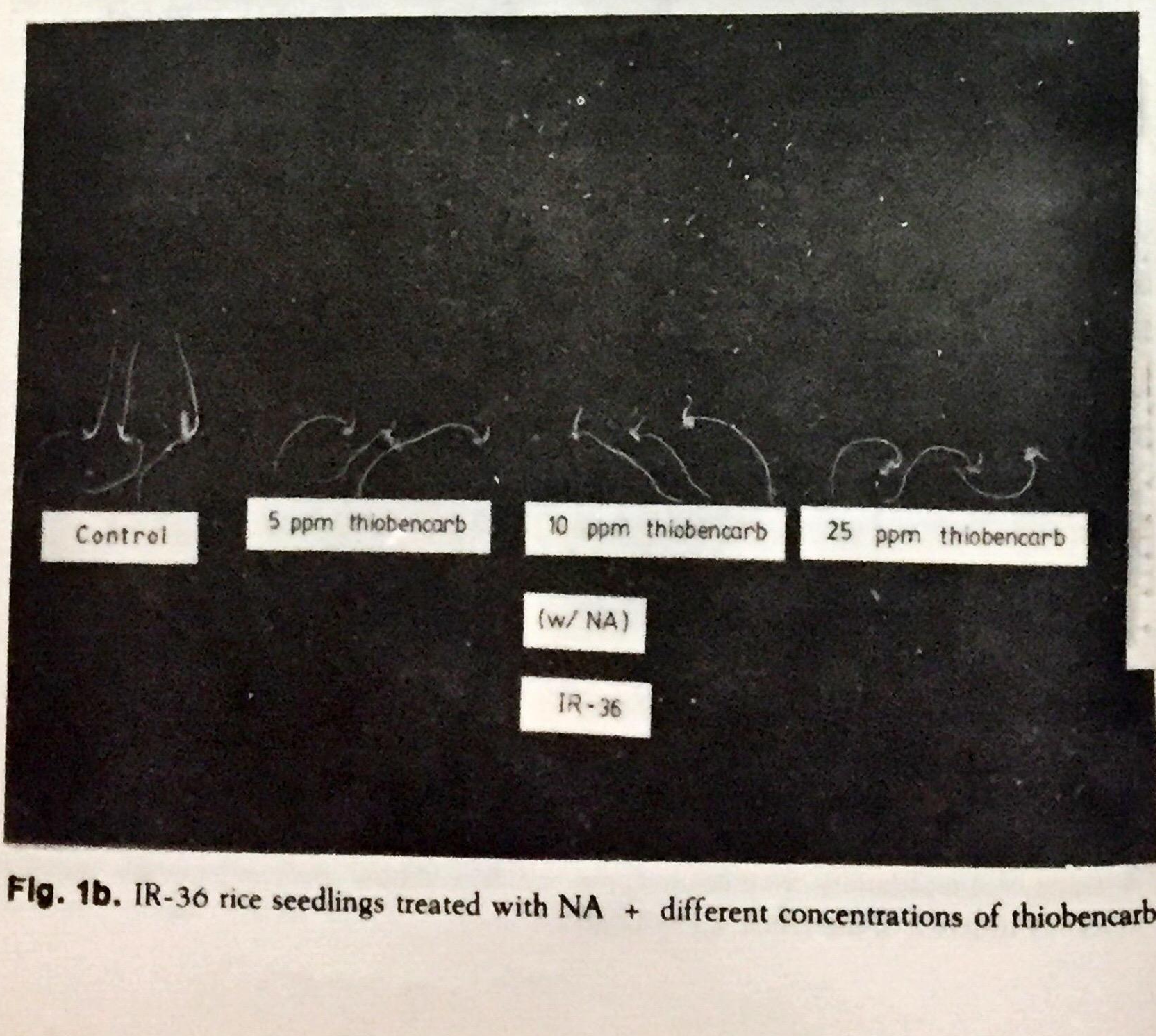


Fig. 1b. IR-36 rice seedlings treated with NA + different concentrations of thiobencarb.

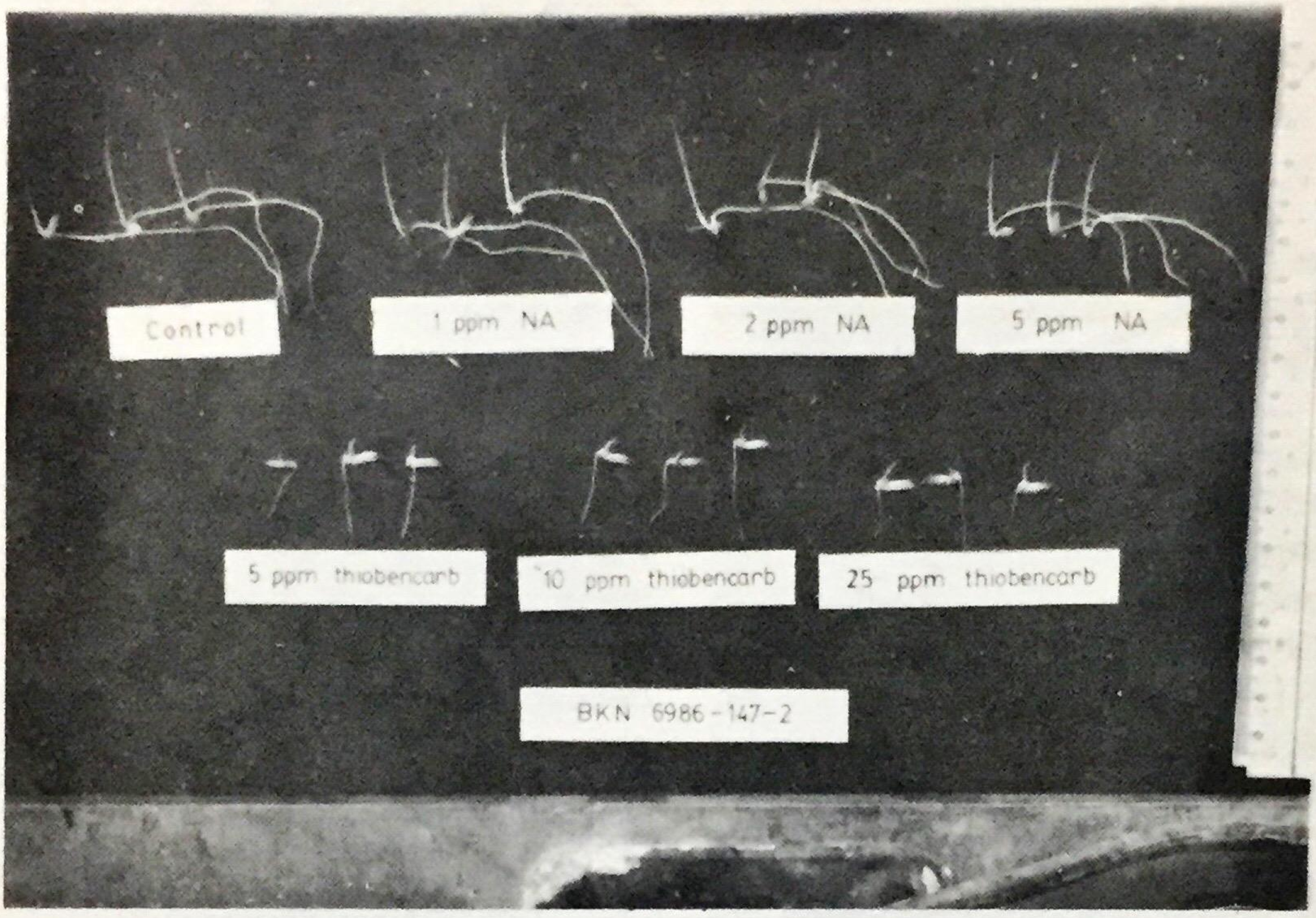


Fig. 2a. RD-19 (or BKN 6986-147-2) rice seedlings treated with different concentrations of NA and thiobencarb.

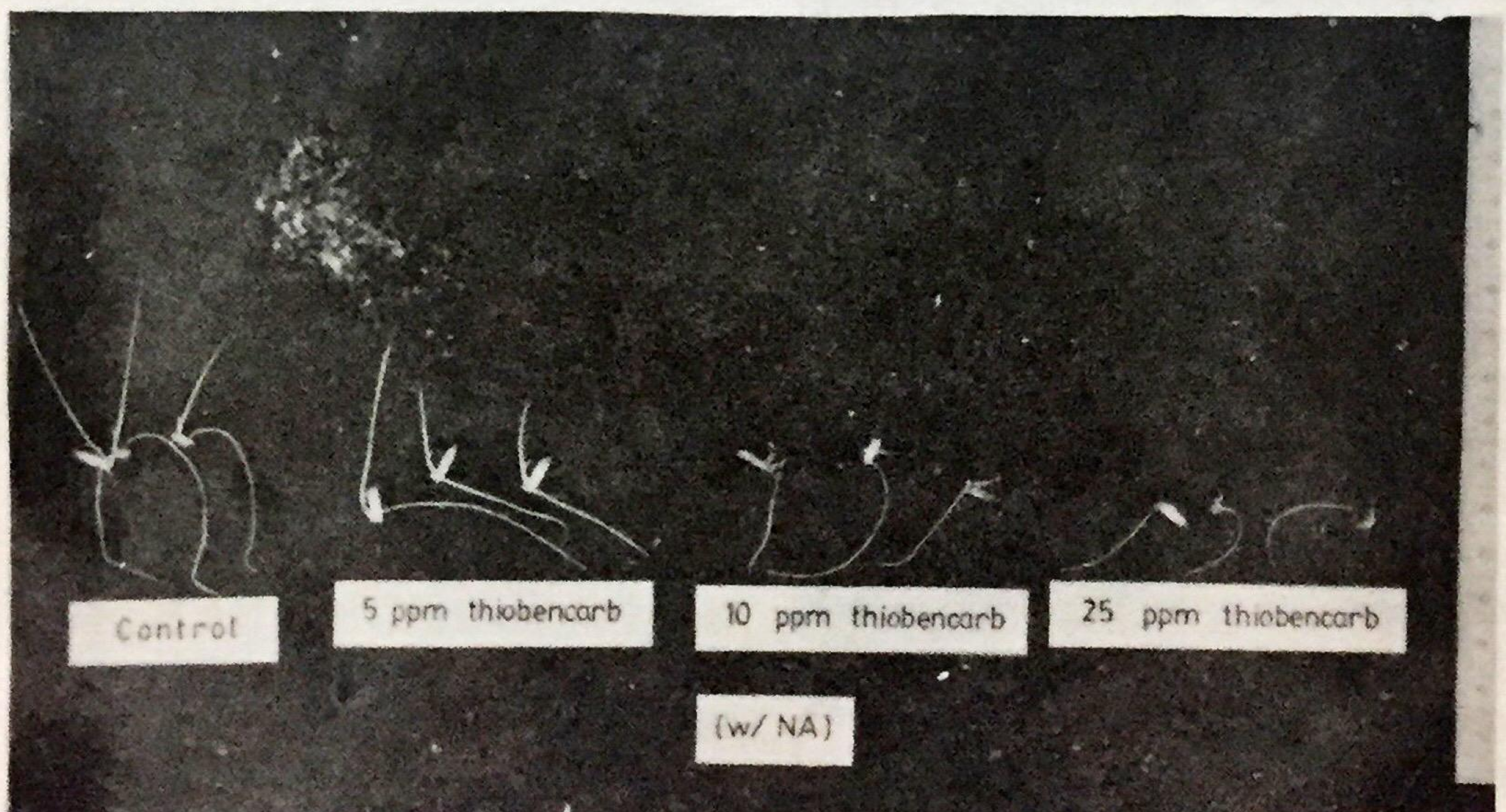


Fig. 2b. RD-19 (or BKN 6986-147-2) rice seedlings treated with NA + different concentrations of thiobencarb.

hibition due to thiobencarb in the 2 other treatment combinations. These results imply that thiobencarb and not NA adversely affected root elongation of IR-36 and RD-19 rice seedlings. Moreover, seed treatment with NA could lessen the inhibitory effect of thiobencarb on the root.

The data likewise show that the roots of RD-19 seedlings were less sensitive to NA and thiobencarb than the roots of IR-36. Reduced root growth was observed only in RD-19 plants treated with 10 and 25 ppm thiobencarb whereas in IR-36 seedlings, all concentrations of the herbicide and NA + 25 ppm thiobencarb produced the same effect. This difference in response of the 2 cultivars could probably be attributed to the inherent tolerance of RD-19 plants.

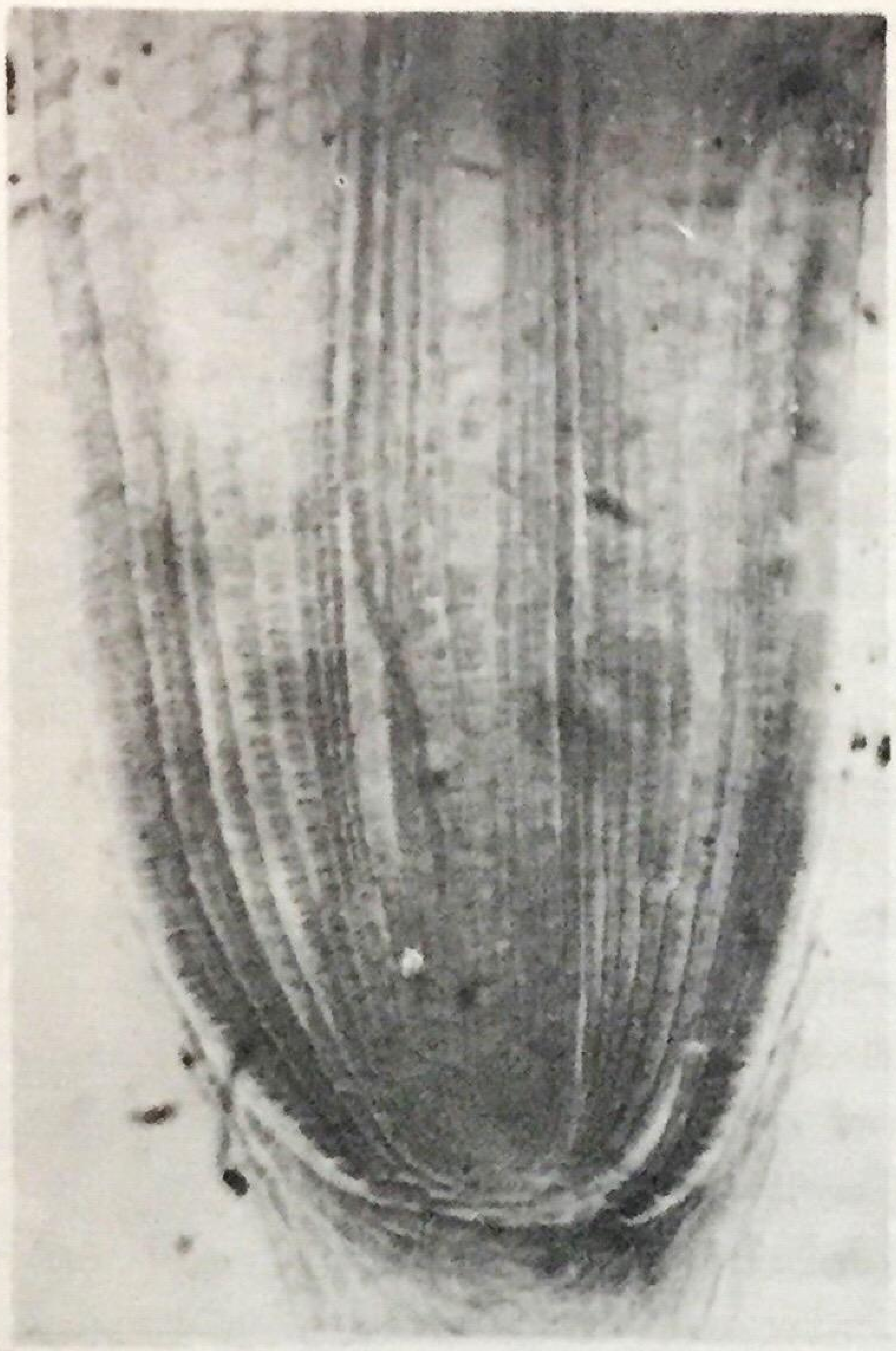
The shoot elongation of IR-36 seedlings was not adversely affected by direct treatments with NA suspension (Table 1). The seedlings treated with different concentrations of NA produced shoots of almost similar length. Plants with NA + distilled water treatment exhibited the longest shoot. This might indicate that NA has some stimulatory effects on shoot growth in the absence of thiobencarb. Shoot growth was greatly suppressed by all thiobencarb treatments (Figs. 1a, 1b). The degree of suppression progressively increased as the concentration of the herbicide became higher. This was observed both in thiobencarb treatments alone and in NA + thiobencarb combinations. Application of NA appeared to counteract the phytotoxic effects of thiobencarb. Unlike its effect on root growth, NA and thiobencarb combinations caused pronounced inhibition of shoot elongation. These data show that the shoots of IR-36 cultivar were more sensitive to the treatments than the roots.

Like IR-36 cultivars, the shoots of RD-19 seedlings seemed to be more sensitive to the treatments than the roots. A week after treatment, thiobencarb at all concentrations as well as NA + 25 ppm thiobencarb caused marked inhibition of shoot elongation (Figs. 2a, b). The other 2 NA-thiobencarb combinations did not considerably reduce shoot length. These observations further prove that RD-19 cultivar is more tolerant to thiobencarb than IR-36. Although both cultivars exhibited suppressed shoot growth in all thiobencarb treatments, the degree of inhibition in IR-36 was greater than in RD-19. All antidote + herbicide treatments used adversely affected the shoots of IR-36 seedlings whereas only NA + 25 ppm thiobencarb treatment caused shorter shoots in RD-19 cultivar.

Macroscopic examination of IR-36 and RD-19 seedlings grown in NA, thiobencarb, and NA + thiobencarb did not show any morphological abnormality due to the treatments. No swelling or kinking of the different plant parts was noted. The cultivars responded to the treatments in terms of inhibited root and shoot growth only.

Anatomical Effects.

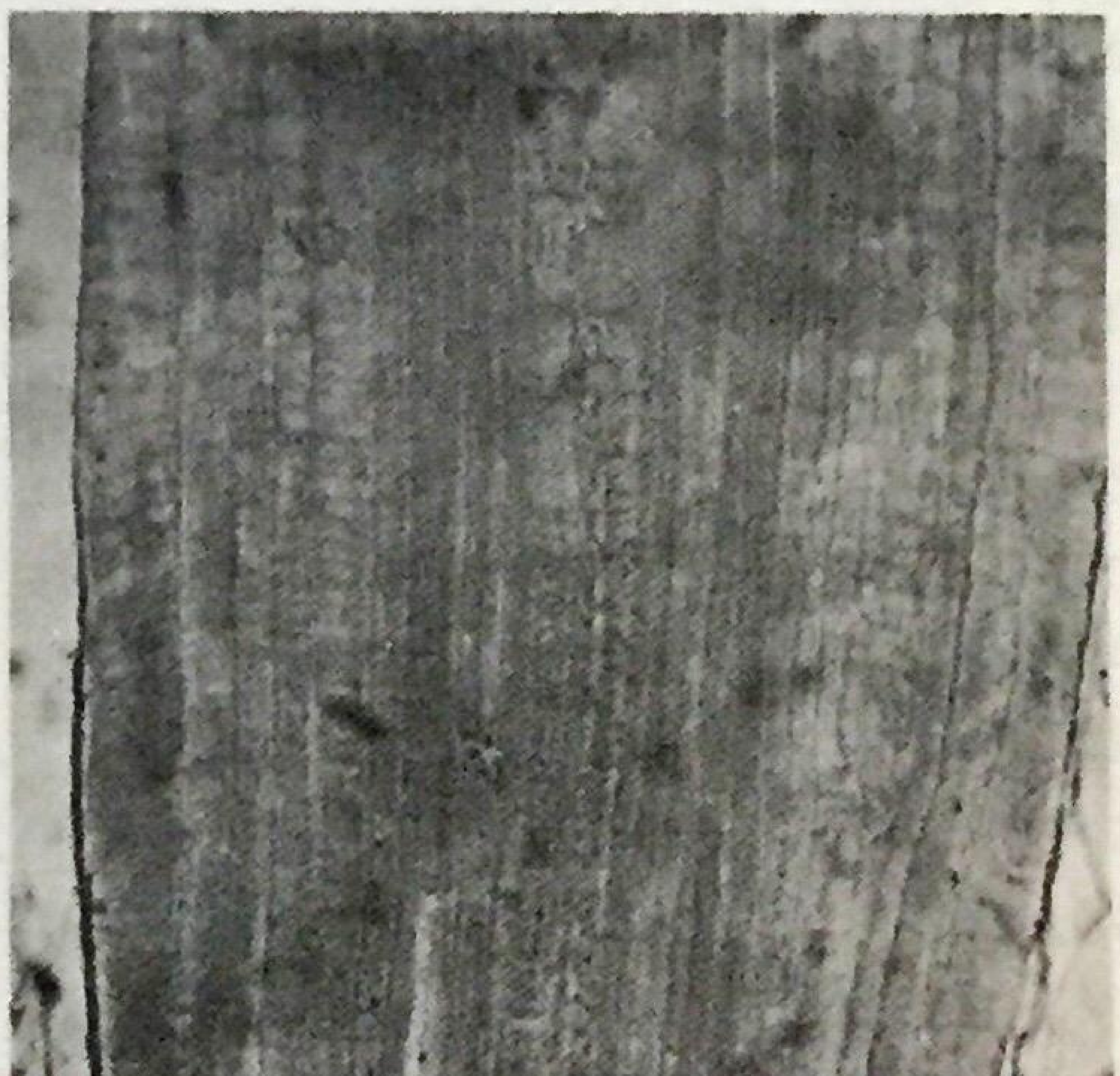
Anatomically, the different treat-



a

b

c



d

Fig. 3. Longitudinal sections of IR-36 rice roots treated with a) distilled water, b) 2 ppm NA, c) 5 ppm thiobencarb, d) NA + 5 ppm thiobencarb (200x).

ments produced no apparent effect on the roots of IR-36 seedlings. The roots exposed to various levels of NA, thiobencarb, and NA + thiobencarb as well as those grown in distilled water were very similar in appearance (Fig. 3). No cell proliferation was observed in all treatments. Root inhibition seems to be primarily due to suppression of cell elongation or expansion.

The differences among the treatments were not evident in the shoot apices of IR-36 seedlings (Fig. 4). The seedlings treated with different levels of NA, NA + distilled water and 5 ppm thiobencarb with or without NA showed almost identical shoot apex appearance and organization to the untreated control. Application of 10 or 25 ppm thiobencarb with or without NA did not seem to affect the shoot apices but disrupted the mesophyll tissues near the coleoptilar node of the seedlings. Figure 4b shows mild tissue disruption in this area after treatment with 10 ppm thiobencarb. The disruption was, however, not as apparent and severe as that on the upper portion of the coleoptile including the developing leaves. The anatomic effects of thiobencarb treatment on this part of the coleoptile are very pronounced. Thiobencarb at any concentration in the presence or absence of the antidote caused kinking and buckling of linear rows of cells (Fig . 5c, d). The physical arrangement of the mesophyll cells was also affected. In the untreated plants, large intercellular spaces were evident whereas these spaces were absent in the treated plants. Seedlings grown in distilled water, in various concentrations of NA or in NA + distilled water did not likewise exhibit such abnormalities (Fig . 5a, b).

The roots of RD-19 seedlings responded to the treatments in a similar manner as IR-36. The longitudinal sections of the roots exposed to the various treatments did not show any difference in appearance (Fig. 6).

The effects of the treatments on the coleoptile of RD-19 seedlings were nearly the same as those on IR-36. No abnormalities were induced in the shoot apex (Fig. 7). Seedlings grown in 10 and 25 ppm thiobencarb with or without NA showed ruptured mesophyll tissues near the coleoptilar node (Fig. 7d). In IR-36 cultivar, the linear rows of cells comprising the developing leaves became twisted and curled whenever thiobencarb was applied regardless of the concentration and the presence or absence of NA. In RD-19 seedlings, treatment with NA + 5 ppm thiobencarb did not cause such herbicide injury (Figs. 7c, 8c). The kinking effect on the developing leaves was induced by 10 ppm thiobencarb with or without NA (Fig. 8b, d).

These results imply that the inhibited root growth observed in both cultivars exposed to thiobencarb particularly at high levels, with or without the antidote, is primarily due to inhibition of cell division and/or cell elongation. No growth abnormality was induced. In the shoot, injury due to thiobencarb

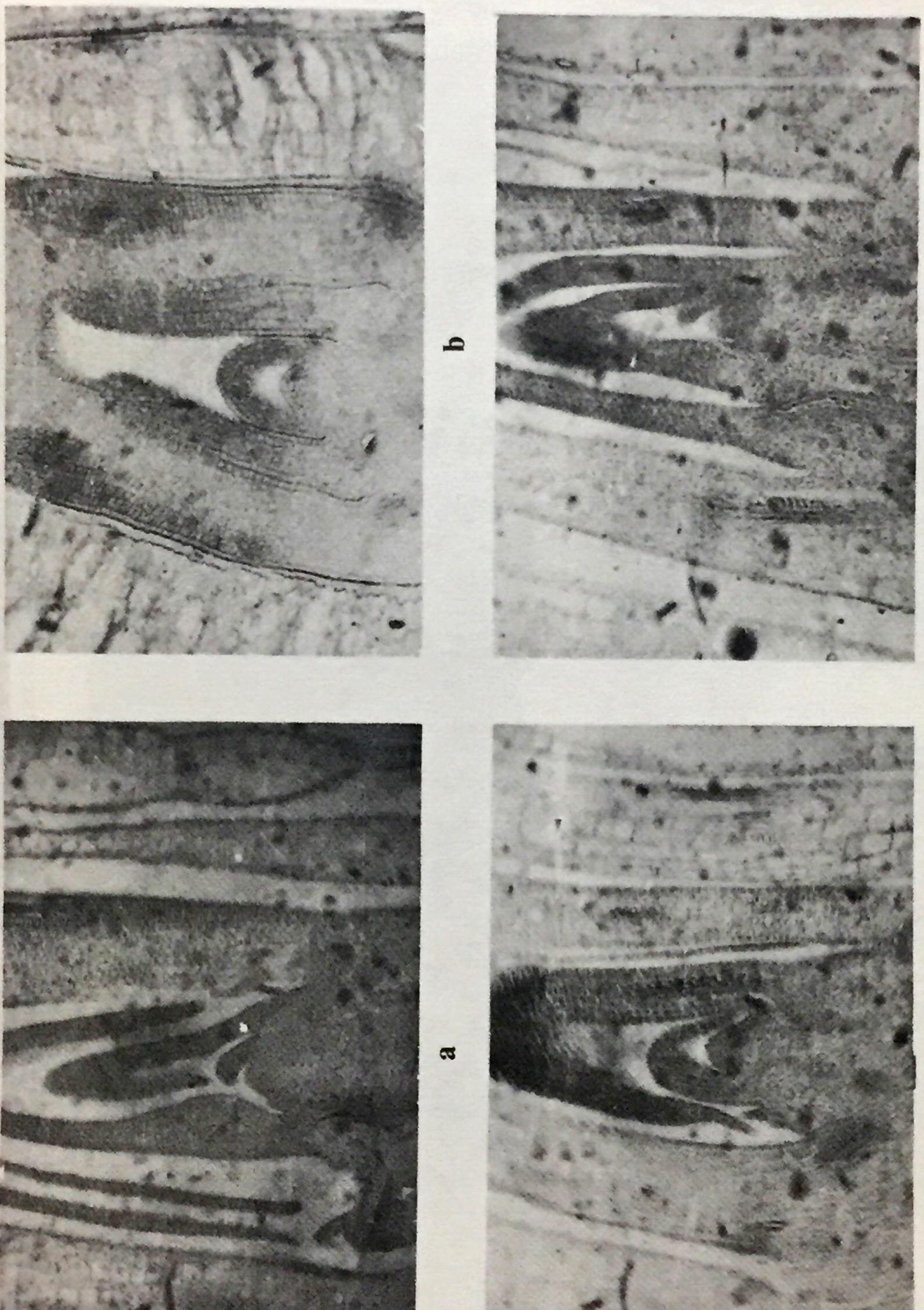


Fig. 4. Longitudinal sections of IR-36 rice coleoptiles treated with a) distilled water, b) 10 ppm thiobencarb, c) NA + distilled water, d) NA + 5 ppm thiobencarb showing shoot apices (100x).

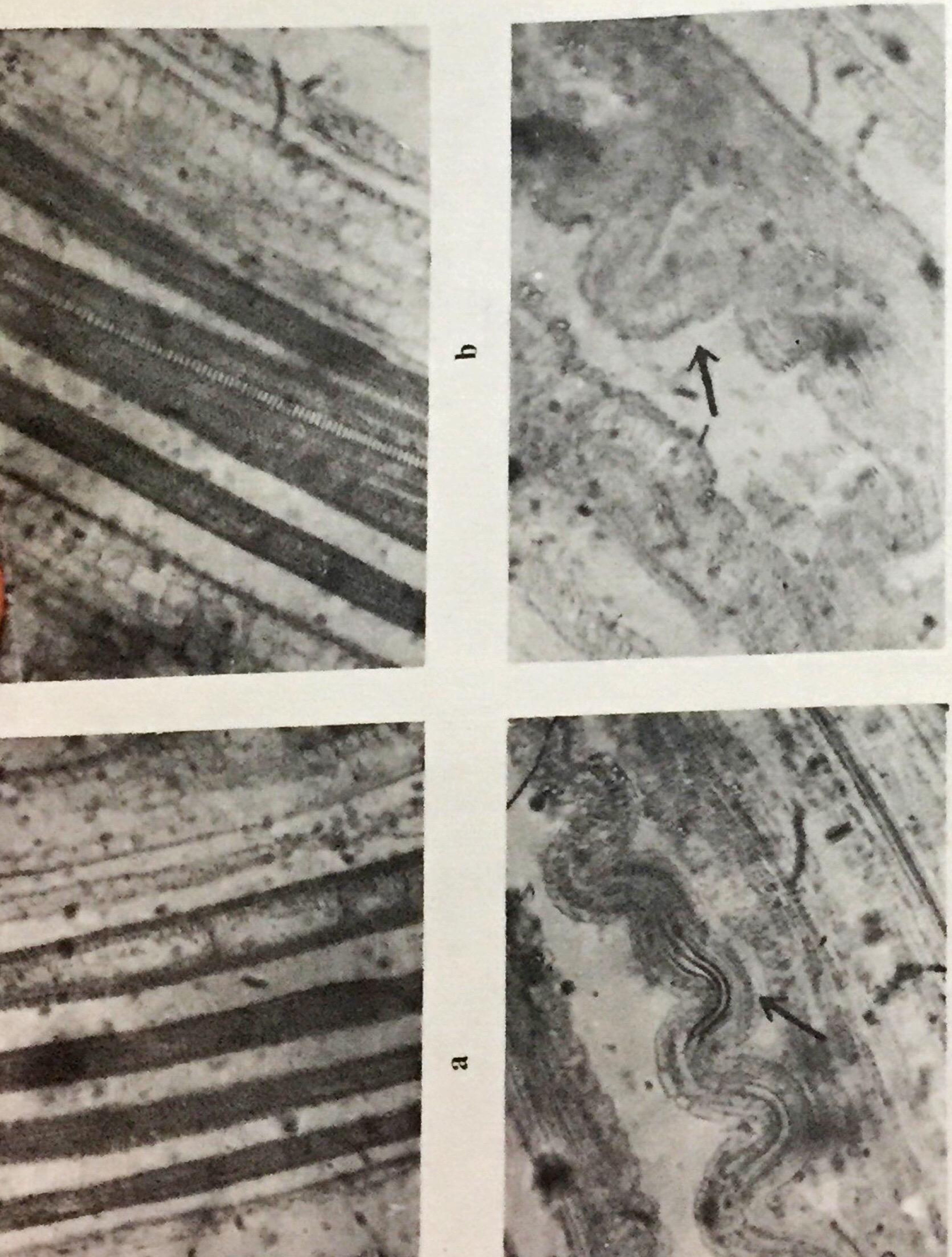


Fig. 5. Longitudinal sections through upper portion of IR-36 rice coleoptiles treated with a) distilled water, b) 5 ppm NA, c) 10 ppm thiobencarb, d) NA + 25 ppm thiobencarb showing developing leaves. Note kinking and buckling of linear rows of

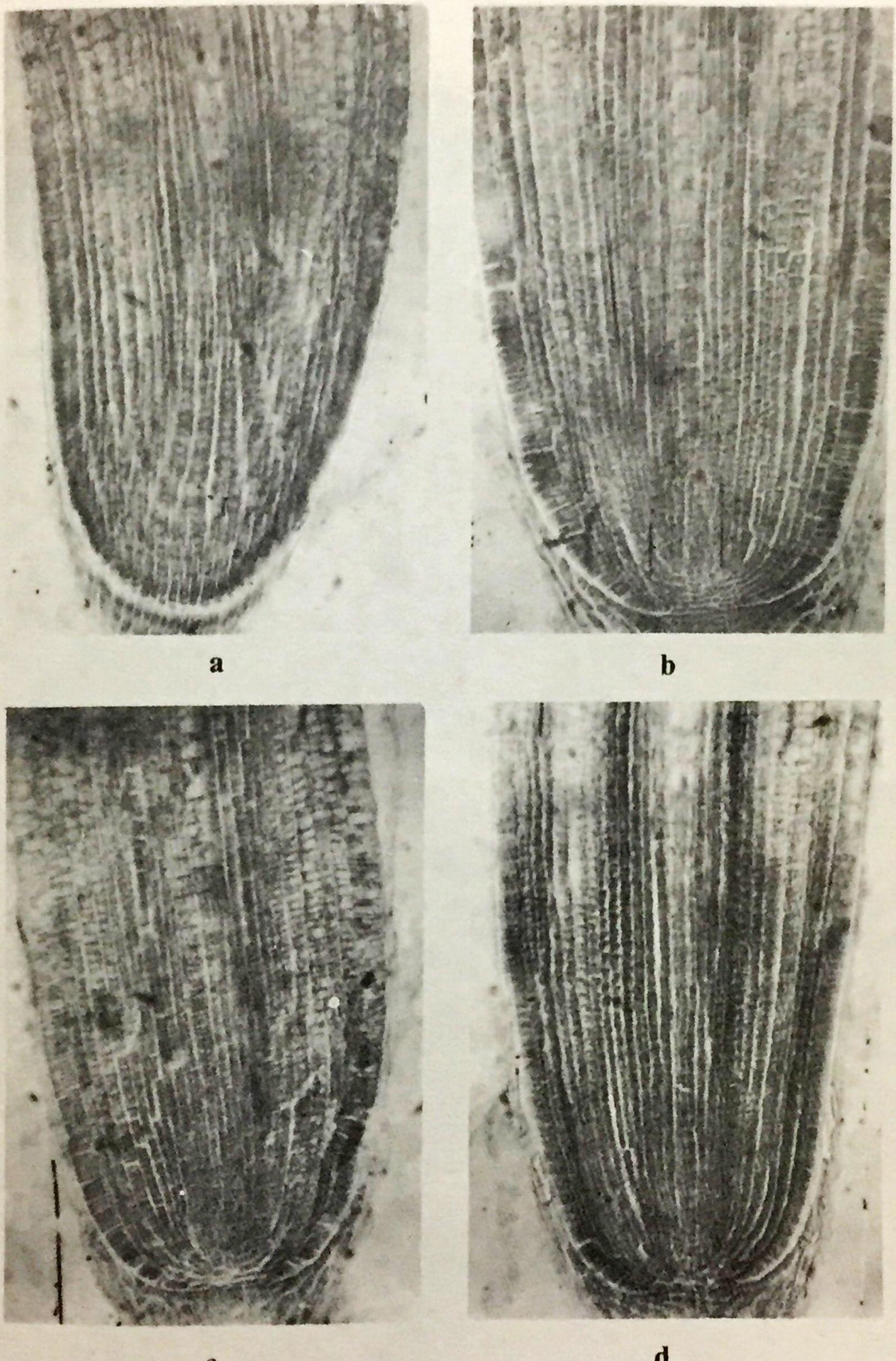


Fig. 6. Longitudinal sections of RD-19 rice roots treated with a) distilled water, b) 25 ppm thiobencarb, c) NA + distilled water, d) NA + 10 ppm thiobencarb (200x).

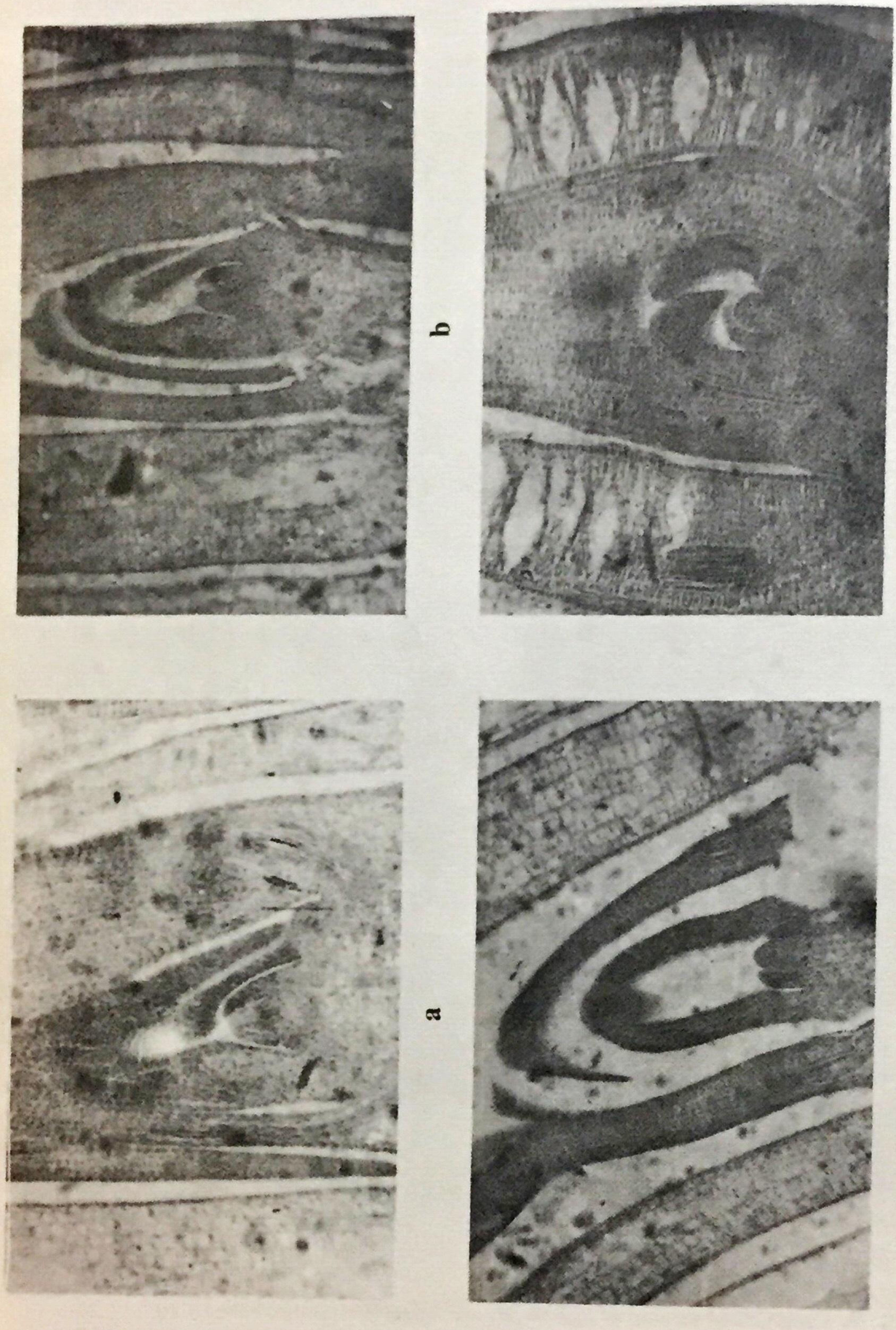


Fig. 7. Longitudinal sections of RD-19 rice coleoptiles treated with a) distilled water, b) 1 ppm NA, c) NA + 5 ppm thiobencarb, d) NA + 25 ppm thiobencarb showing shoot apices (100x).

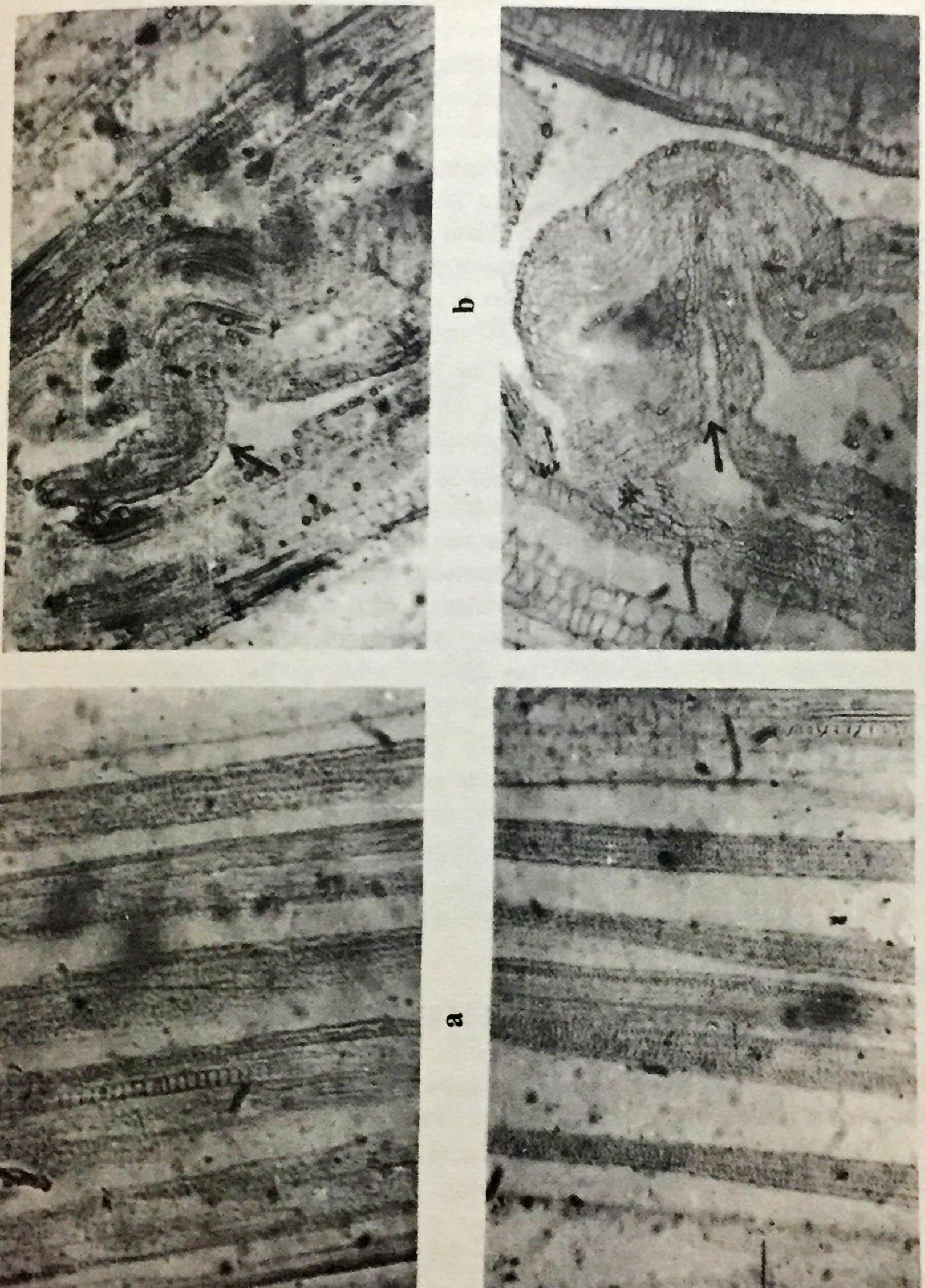


Fig. 8. Longitudinal sections through upper portion of RD-19 rice coleoptiles treated with a) distilled water, b) 10 ppm thiobencarb, c) NA + 5 ppm thiobencarb, d) NA + 10 ppm thiobencarb. Note kinking and buckling of linear rows of cells (arrows) in b and d (100x).

was expressed in alteration of the organization and appearance of the different cells and tissues within the coleoptile. NA + 5 ppm thiobencarb treatment which did not cause marked inhibition of shoot growth in RD-19 seedlings (Fig. 2b) did not also produce any irregularity in internal structure (Fig. 8c).

The effects of thiobencarb on the growth and anatomy of IR-36 and RD-19 rice seedlings were very similar to the results obtained by Dawson (1963) about the effects of EPTC on the development of barnyard grass seedlings. He observed changes in the mesophyll of the developing leaves within the coleoptile. Intercellular spaces were reduced and linear rows of cells were buckled and kinked. However, the kinking of the first internode into a zigzag pattern as well as swelling near the coleoptilar node caused by EPTC was not observed in thiobencarb injured rice seedlings. The studies of Dawson indicated that the injury was concentrated in the mesophyll of the leaves within the coleoptile.

The above observations can be correlated with the mode of action of thiocarbamate herbicides which include thiobencarb. These herbicides when applied to the soil primarily inhibit the growth of emerging shoots of plants, especially grasses. They inhibit the shoot growth of germinating seedlings to a greater degree than the roots. The major phytotoxic symptom induced by these compounds is the abnormal growth and emergence of the leaves from the coleoptiles of grasses. At high rates, the leaves may not emerge from the coleoptile; at lower rates they may emerge but remain longitudinally rolled or just emerge through the base of the coleoptile. The failure of the leaves to emerge from the coleoptile of grasses could be traced back to herbicidal effect at the histological level. In the present study, thiobencarb caused the kinking and buckling of the developing leaves which would most probably result in abnormal development and/or emergence of the leaves.

Herbicidal activity of thiobencarb is concentrated in the shoot, thus injuring leaf tissues. The roots are less of a target for this chemical.

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