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I. SUMMARY OF ACCOMPLISHMENTS AND UTILIZATION

A simplified and faster nutrient solution technique for screening Mn toxicity tolerance in wheat and corn was developed. The range of tolerance of wheat and corn genotypes to Mn toxicity was studied. Three levels of Mn tolerance were determined in these species. Varieties with a useful range in Mn tolerance were identified. The association of Mn and Al tolerances in wheats was also investigated. It was found that these mineral imbalances may occur together or separately in different genotypes.

II. OBJECTIVES

1. To develop an improved, simplified method for evaluating Mn tolerance in wheat and corn.
2. To determine the range of genetic variability in wheat and corn for response to excess Mn.
3. To identify varieties that are tolerant to Mn toxicity.
4. To examine the genetic relationships between Al tolerance and Mn tolerance.

III. ACCOMPLISHMENTS

A. WHEAT

1. METHODS AND MATERIALS

a. Nutrient solution method for evaluating the decrease in growth caused by Mn toxicity

About 70 seeds of eight wheat cultivars were planted in a tray floating over water as described previously (Polle et al. 1978a). After 24 hours the trays were transferred to a nutrient solution containing 1.0, 1.0, 0.4 and 0.2 millimoles per liter of KNO_3, $Ca(NO_3)_2$, $MgSO_4$ and KH_2PO_4 respectively. Two days later the seedlings of each variety were transferred to a series of nutrient

solutions containing variable concentrations of Mn and the same concentrations of macro elements as above plus 0.07, 0.01, 0.04, 0.02 and 1 ppm of B, Cu, Zn, Mo and Fe respectively. Boron was added as H_3BO_3 and the other elements as $CuSO_4.5H_2O$, Zn $SO_4.7_2O$ and MoO_3. Half of the iron was supplied as Fe $SO_4.7H_2O$ and the rest as Fe-chelate (Fe Sequestrene, Geigy). The Mn concentrations used (as $MnCl_2.4H_2O$) were 0.13, 8, 16 and 32 ppm. Three seedlings of each variety were secured with cotton through holes 0.6 cm in diameter in a polyethylene vial 2.5 cm internal diameter and 2.5 cm high. Eight varieties, each with three replicate vials, were placed in holes in a styrofoam lid 2.5 cm thick and positioned at the top of a polyethylene container filled with 10 liters of test solutions (Figure 1). The vials supporting the plants were adjusted in such a way that the seeds were just covered with the nutrient solution. The holes in the sytrofoam lid were arranged to have 24 vials with three plants each evenly spaced in the area of 24.5 x 32 cm corresponding to the solution area in the container. Aeration was provided during the whole period of growth of the plants. The containers were placed in a growth chamber with an eight hour dark period at 13°C and a 16 hour light period at 22°C. The light energy of the fluorescent lamps recorded at the level of the styrofoam lids was 0.001862×10^{-6} V/Wm^{-2}. The nutrient solutions were changed the 4th, 8th, 11th, 14th, and 17th days after transferring the plants to the Mn treatments. The plants were grown for 23 days after the initial planting in water. At this time the tops of the plants were cut just above the seed. The total fresh weight of the tops of the three plants

in each vial and that of the corresponding roots after blotting in absorbent paper tissue were recorded. The dry weight was recorded after drying the plants at 60°C.

The Mn effect on plant growth was evaluated for each variety by two estimates: Mn concentration for 30 per cent decrease in yield: the Mn concentration required to decrease the fresh weight yield on the tops by 30 per cent over the yield of the 0.13 ppm Mn treatment was estimated from a graph.

$$\text{Relative yield (R.Y.)} = \frac{\text{fresh weight on the tops in the 32 ppm Mn treatment}}{\text{fresh weight on the tops in the 0.13 ppm Mn treatment}}$$

b. Analytical Methods

(1) Mn analysis: Plants previously dried at 60°C were ground in a ceramic ball mill. The Mn in the plants was analyzed by atomic absorption spectroscopy after dry ashing at 550°C, digestion in 10 N HNO_3 in a steam bath for 15 minutes and appropriate dilution with water. The analytical results were rounded to the first two significant figures in the Tables and in the text.

(2) Colorimetric method for Mn detection:

Morgan Reagent: 10 g Cryst. $NaC_2H_3O_2$ and 9 ml $HC_2H_3O_2$ in water to make 100 ml, pH 4.8

Saturated aqueous solution of KIO_4

Methane base solution: Dissolve one gram of 4,4' - Methylenebis - (N, N-dimethylaniline), $((CH_3)_2NC_6H_4CH_2C_6H_4N(CH_3)_2)$ (Eastman Organic Chemicals) to 100 ml in acetone.

Procedure: Place in each depression of a porcelain plate one piece of a leaf about one cm^2, grind the piece with a blunt plastic rod and add 1 ml of Morgan reagent. After 15 minutes add one drop of saturated KIO_4, stir with a clean rod. Add one drop of Methane base

solution and stir. The blue color will develop in about 30 seconds and will remain without substantial change for about two minutes.

c. Dry weight determination as affected by Mn

Stacks of styrofoam strips 8 x 29.5 cm and 0.7 cm thick were drilled with 39 holes 1 cm diameter. A layer of a hexagonal nylon mesh (opening 0.3 cm) was "sandwiched" between two layers of stryofoam fastened with rubber bands (Figure 2). The styrofoam strips were then floated in water with the screen in contact with the water. Three seeds of each variety were planted seam down (one seed/hole), and covered with Saran Wrap. Air was bubbled in the water. After 24 hours the strips were transferred to a nutrient solution as in the solution method described under III.A.1.a. On the third day the strips were transferred to a nutrient solution containing 32 ppm of Mn, plus macro and minor elements as before. The plants were grown in a growth chamber under the same conditions described in III.A.1.a. The nutrient solution was changed the 7th and the 11th day and the experiment was sampled the 14th day after the initial planting in water. This was done by cutting from each plant the complete first leaf and one-half of the second leaf. The fresh weight of the leaves was determined with an analytical balance immediately after cutting. The leaves were then placed in numbered plastic vials (1.3 cm internal diameter, 5.5 cm high) arranged in a wooden rack and placed in an oven at 60°C overnight (Figure 3). Each sample was then weighed on the same balance and the percentage dry weight calculated.

2. RESULTS AND DISCUSSION

Results of three experiments with the nutrient solution method

described in III.A.1.a. are in Table 1. In this table the wheat genotypes tested are listed in order of decreasing value of their relative yield as displayed in column 5. In the experiment of Table 1, as well as in several repetitions, cultivar Atlas appears to be less tolerant than Monon, in agreement with Foy et al. (1973). However, Table 1 indicates that compared to highly Mn tolerant Monon, there are other wheat cultivars far less tolerant than Atlas.

The concentration of Mn required to decrease the fresh weight yield 30 per cent was estimated graphically as indicated in III.A.1.a. The results in Table 1, column six, show an ordering for Mn tolerance of the cultivars differing very little from the one in column five. Because of the simplicity of the method, the relative fresh weight yield (R.Y.) method was used for further work.

In Table 1, column five, the Al tolerance for the same accessions is indicated. No relationship is apparent between Al and Mn toxicity tolerances in the wheat genotypes examined.

The elapsed time employed with the nutrient solution method described in III.A.1.a. seems to be the minimum time in which measurable Mn toxicity effects on growth can be distinguished. With this method, it was observed that during the first week most of the varieties experienced a stimulation in growth at all Mn levels of above 0.13 ppm. This stimulative effect faded with time. Only in the third week of growth were visual symptoms of Mn toxicity shown by the susceptible genotypes. These toxicity symptoms included the appearance of irregular variations in the intensity of green color in older leaves followed in some cases by yellowing or necrotic spots in the tip of the oldest leaves of the seedling. However,

these symptoms were expressed by some but not all of the genotypes whose growth were markedly affected by the higher Mn concentrations.

The Mn concentrations calculated on fresh weight basis (Table 1, Column 7) showed a small increase for the average Mn content of the medium tolerant genotypes. This result is due in part to the increase in dry matter content in the less Mn tolerant genotypes, which will be discussed later. The analysis calculated on dry weight basis (Table 1, Column 8) showed a trend suggesting that the more tolerant genotypes were at the same time those that accumulated more Mn than the less tolerant. However, some genotypes did not follow this general trend. Since this discrepancy could be related to the age of the plants and/or to the fact that Mn analyses in Table 1, Column 7 refer to the whole of the shoots in each plant, an attempt was made to find if, by choosing the appropriate leaf and time of sampling for Mn analyses, a better correlation between Mn accumulation and Mn tolerance could be established. During the course of these experiments Mn was determined with both the atomic absorption technique in III.A.1.b.(1) and the colorimetric method described in III.A.1.b.(2). The colorimetric method was not quantitative but permitted a comparison between concentrations in different parts of the plant, between different genotypes and different Mn absorption times.

A series of experiments was performed with 10 of the wheat genotypes from Table 1 that showed a distinct range in Mn tolerance. The following genotypes were included in this study in order of decreasing Mn tolerance according to their R.Y. value: Monon, Fielder, IAS54, Atlas 66, Vila Velha,

Druchamp, Gaines, Sonora 64, Brevor and Trintani. In the following experiments the change in shoot Mn concentration with time was detected with atomic absorption analyses. The nutrient solution in these experiments contained 32 ppm of Mn, with the other nutrients and growing conditions as described in III.A.1.a.

It can be seen in Table 2 that in most of the wheat genotypes the Mn concentration increases almost linearly up to the second week and remains relatively stationary afterwards. In 15 days the more tolerant varieties accumulate 1900 to 2800 ppm of Mn. The less Mn tolerant genotypes accumulate 1700 to 1800 ppm with the exception of Sonora 64, which accumulates 2400 ppm Mn. By the 21st day, the separation of Mn tolerance in the wheats improves. At this stage the more tolerant genotypes accumulate 2200 to 3100 ppm of Mn. The less tolerant cultivars accumulate 1400 to 1700 ppm Mn. Sonora 64 is an exception with 2100 ppm Mn. The intermediate tolerant Druchamp accumulates 1700 ppm Mn. The tissue analyses with colorimetric method in III.A.1.b.(2) indicated that in a growing plant the average Mn concentration determined by analysis of the whole plant is not sufficiently sensitive to estimate differential Mn accumulation associated with Mn tolerance. By comparative colorimetric analyses of plant parts it was found that Mn tends to accumulate first in the older leaves starting at the tip of the leaf. Thus, a quantitative study of the Mn content in different parts of the plant was carried out with the varieties of Table 2. This study was performed on plants 19 days old (Table 3). In Table 3 we observe that the best separation of genotypes for Mn tolerance in terms of Mn accumulation can be observed using the second leaf. Here the less tolerant genotypes accumulate approximately half the Mn of the more

tolerant genotypes. These results confirm the preliminary hypothesis that the more Mn tolerant plants tend to accumulate more Mn and to grow with larger internal concentrations of this element than those less tolerant. However, the fact that these differences require almost three weeks of growth is undesirable for practical screening.

In Table 1, Column 10, it can be seen that there is a marked tendency for the less Mn tolerant varieties to exhibit higher dry matter content. This behavior was investigated with ten cultivars as shown in Table 4. For all the varieties the dry matter content increases with age and the increment is larger for the less Mn tolerant varieties.

Next, the dry matter content of 19 day old plants was determined for each of the three leaves, the remainder of the plant and the whole plant (Table 5). There was considerable variation in the dry matter content, depending on the type of leaf sampled. However, the same trend of higher dry matter content in the least Mn tolerant varieties is present. The first leaf was the most suitable for relating dry content and Mn tolerance.

Then the plants were grown as before, but in nutrient solutions containing 0.13, 32 and 64 ppm Mn, the first leaf was sampled at three different times and the second leaf was sampled for the 14 day treatment (Table 6a and 6b). It is evident that the earliest age at which the dry matter content allows separation of the genotypes for Mn tolerance is 14 days. The Mn concentration most effective for differentiating Mn toxicity tolerances among the genotypes is 32 ppm.

To test the previous methods on a larger group of genotypes, 45 wheat accessions from a CIMMYT (Mexico) crossing block (plus Brevor, Fielder and Atlas 66 for reference) were tested with the solution

culture method of III.A.1.a. One replicate (of 3 plants) of each genotype was treated at 0.13 and 32 ppm of Mn for 23 days. The fresh weight of the tops was obtained and the relative yield (R.Y.) calculated (Table 7). The various genotypes are considered Mn tolerant, less Mn tolerant, and Mn susceptible when R.Y. is higher than 0.7, between 0.55 and 0.70 or less than 0.55 respectively.

The same wheat genotypes were included in another experiment with these accessions grown according to the procedure in III.A.1.c. In Table 8 the genotypes are classified according to their average dry matter content. Mn tolerant, Mn less tolerant, and Mn susceptible accessions have dry weights less than 11.25 per cent, 11.25 to 14 per cent or higher than 14 per cent respectively. Comparing Tables 8 to 7 shows that classification on the basis of dry matter content is essentially the same as on the R.Y. value (only Torim 73, Quetzal 75 and Cinto "S" changed to a neighboring group). Figure 4a, b, and c show the growth effect of 32 ppm of Mn 23 days after planting in varieties CM11683-A-1Y-1M-1Y-7M-OY, Ticus and Kafue, to be tolerant, intermediate and sensitive to Mn toxicity according to the methods described here. Use of the dry matter method, with a single test solution, is much more convenient for screening Mn tolerance in wheat. This screening method is sensitive enough to classify tolerance with a single good quality seedling. Thirty-six genotypes were then tested to determine if the dry matter method for Mn tolerance could be successfully employed on the same plants already used for the hematoxylin method for classifying Al tolerance. The 36 wheat genotypes were planted (one replicate/variety) in the styrofoam strips and floated

in water as described in III.A.1.c. The seedlings were then treated with hematoxylin as described by Polle et al. (1978a). After the staining, the plants were placed in water for one hour and then in the nutrient solution containing the macro elements used in the Mn experiments. The next day the plants were transferred to the nutrient solution containing 32 ppm Mn. This nutrient solution was changed on the 8th and 12th days after the initial planting in water. The dry matter content was determined when the plants were 15 days old. No appreciable difference was found in the grouping of genotypes as compared with previous results (only four genotypes changed to a neighboring class) for Mn tolerance.

B. CORN

1. METHODS AND MATERIALS

a. Nutrient solution method for evaluating the decrease in growth caused by Mn toxicity

About 60 seeds of each maize variety were placed in a tagged 12 x 8 cm nylon mesh bag (Polle et al. 1978a, Fig. 5). Bags carrying 20 different varieties were suspended from the border of a cylindrical container. The container was filled with 5 liters of deionized water and the seeds were kept immersed. Air was bubbled through the water. After 24 hours at room temperature (about 22°C), the water was discarded and replaced by 5 liters of Terraclor ($C_6Cl_{15}NO_2$, Olin Mathieson Chemical) suspension (0.2 grams per liter). Air was again bubbled. After 30 minutes the seeds were spread on the surface of a moistened filter paper in a shallow pan and covered with a plastic film as described by Polle et al. (1978b, Fig. 3). The seeds were then placed in

an incubator at 20°C and kept in the dark for 2 days. Next, for each variety about 20 seeds of about the same root length were transferred to perforated styrofoam strips floating on 10 liters of a nutrient solution containing 1 mM of each KNO_3 and $Ca(NO_3)_2$, 0.4 mM of $MgSO_4$ and 0.2 mM KH_2PO_4. The tray was covered with a clear plastic film. Air was bubbled. The tray was kept at room temperature (about 22°C). Two days later the plants were transferred to a series of nutrient solutions containing variable concentrations of Mn and the same concentrations of macro elements as above, plus 0.07, 0.01, 0.04, 0.02 and 1 ppm of B, Cu, Zn, Mo and Fe respectively. Boron was added as H_3BO_3 and the other elements as $CuSO_4.5H_2O$, $ZnSO_4.7H_2O$ and MoO_3. Half of the iron was supplied as $FeSO_4.7H_2O$ and the rest as Fe-chelate (Fe Sequestrene, Geigy). The Mn concentrations used (as $MnCl_2.4H_2O$) were 0.13, 8, 16 and 32 ppm. The plants were placed in these solutions as described by Polle et al. (1978b) for Al tolerance screening. After transplanting, the seedlings were sprayed with a suspension of Benomyl (Benlate, Dupont) 1.25 g/liter to control fungus growth on the seeds. The plants received alternate periods of 12 hours of light and dark per day. Incandescent light 8.5 μ Einsteins sec^{-1} m^{-2} (400-700nm waveband) was used. The containers were kept in a water bath at 25°C. The solutions were replaced by fresh solutions four days after transplanting and thereafter every 3 days. Twenty-one days after transplanting the plants were cut above and below the seed and the fresh weight of roots and tops recorded.

2. RESULTS AND DISCUSSION

A 21 day growth period seems to be the minimum necessary for evaluating differential growth responses to Mn in corn. In a number of shorter term experiments with comparable light and temperature conditions, differences in response to Mn toxicity were difficult to recognize.

Symptoms of plant response to Mn started to appear two days after the plants were placed in the nutrient solutions with Mn concentrations of 8 ppm or more. Symptoms were the appearance of chlorotic strips along the leaves, in some cases accompanied by tip burning. Five days after planting the first and second leaf generally showed more intense symptoms then the third leaf (Fig. 5). In some varieties the chlorosis strips affected the whole leaf, and in others only a part of it. Although, there was a relationship between the intensity of these symptoms and the decrease in growth experienced by the varieties 21 days after planting, this relationship was not general and could not be accurately used for detecting Mn tolerance in the varieties examined.

Seventeen corn lines used in Washington State were examined with respect to their Mn tolerance with the above described method. The results are shown in Table 9. In this Table the relative yield of tops (R.Y.) with respect to the control (0.13 ppm Mn) is defined as before by:

$$\text{R.Y.} = \frac{\text{fresh weight on the tops in the 32 ppm Mn treatment}}{\text{fresh weight on the tops in the 0.13 ppm Mn treatment}}$$

The following levels of Mn tolerance were determined from the relative yield data. A tolerant group, where growth in the treatment with 32 ppm of Mn exceeds the control: Earli King, Bulkpack, PX 15, Early Vee, PX 26 and Morning Sun. A medium tolerant group where R.Y. was no less than 60% the growth in the control: Popcorn R, NK 199, Northern Vee, PX 485, Span Cross, PX 74, FM Cross and Golden Cross Bantam. A Mn susceptible group, with R.Y. less than 60% of the control: PX 610, Polar V and Popcorn W. Figure 6 shows hybrid maize Bulkpack, PX 485 and Polar Vee classified as Mn tolerant, medium tolerant and susceptible according to their relative yield.

Efforts were made to develop a faster and more efficient method for screening Mn tolerance than the one based on the reduction in growth response. However, Mn analyses of the plant tops did not show varietal differences associated with growth response after 21 days of growth in high Mn solutions. The dry matter content (plant dry weight) as a response to Mn tolerance was also examined. A general tendency for the dry weight of the plant tops to increase as the Manganese concentration in the nutrient solution increased, was observed. However, varietal differences in dry weight content were not measurable.

C. RECOMMENDATIONS TO COOPERATORS

The dry-matter content method as a way to predict Mn tolerance in wheat can be applied in breeding programs. A single Mn treatment is necessary and requires only a 2 week period of growth with little additional work after planting. The growth-reduction method of evaluating response to Mn toxicity in corn is not an efficient

way to evaluate tolerance responses. It is too time consuming and requires substantial space for screening large collections. In addition to these disadvantages, it is not suitable for breeding purposes because it requires at least 2 Mn treatments per variety and thus is useful only for screening collections at a rather slow pace.

Also, both methods need to be checked with field results. Little information is available on growth responses in soils with Mn toxicity. We are in the process of developing a standard artificial Mn toxic soil in order to check our solution methods. With this soil we have been able to induce visual symptoms of Mn toxicity in barley seedlings. Barley is very sensitive to Mn toxicity (Vlamis and Williams, 1967), readily developing necrotic spots under conditions of Mn toxicity. The artificial test soil is made of peat, quartz sand and a standard bentonite clay. As a source of Mn, we use Mn carbonate. This artificial soil can be made Mn toxic to barley at pHs above 5, where no Al toxicity is expected. It is anticipated that within the span of several months, sufficient progress will be made with the artificial soil to make further recommendations on detailed procedures.

IV. DISSEMINATION AND UTILIZATION OF RESEARCH RESULTS

A. PUBLICATIONS AND MEETINGS

Part of the findings in this report were communicated to the 70th Annual Meeting of the American Society of Agronomy in Chicago, Illinois, December 3-8, 1978.

B. USES BY OTHERS

Part of this work has been communicated to Dr. Glenn Anderson, CIMMYT, Mexico. A set of 500 wheat lines from CIMMYT is being

screened for Mn tolerance.

C. INFORMAL TRAINING AND GUIDANCE

Dr. H. I. Hashmi, from the Radiation Genetics Institute in Pakistan, participated in the earlier part of this work. Mohammed Boukhnafer, a graduate student from Morocco, has received training in the use of the methods described in this report.

D. INVOLVEMENT BY MINORITY PERSONNEL AND WOMEN

Ms. Elaine Wright, Vickie Nichols and Swapna Ray were part-time assistants on this project.

E. PROJECTIONS OF THE RESEARCH WORK

Although the dry weight technique developed for screening in wheat is a substantial improvement over the longer term methods based on growth reduction, we think that a more direct and simpler method would be desirable. For reasons not well understood at present, Manganese toxicity in wheat is observable under the environmental conditions reported here only after at least two weeks. Visual symptoms of toxicity in this species are not general to all sensitive varieties. We are continuing research along two lines: One is to vary the environmental conditions (including temperature and composition of the nutrient solution) to accelerate the appearance of symptoms, i.e. either visual symptoms or those related to leaf growth. The second approach involves investigating the effects of total salt concentration together with the ratio of P/Ca in the nutrient solution.

Data in the literature for species other than wheat or corn (Horst and Marschner, 1978) suggest that Mn tolerance may be associated with the ability of the plant to maintain low concentration

of unbound Mn in the tissues. We are investigating different fractions of Mn in varieties that exhibit differential Mn tolerance.

In one of the earlier studies on the toxic effects of Mn, Barton and Trelease (1927) reported that toxicity symptoms in the form of dark narrow rings in wheat roots developed in seedlings grown for 48 hours in solutions containing very dilute Mn and Ca concentrations. We have been able to reproduce this symptom under similar nutrient conditions. However no differential response to this symptom was found between varieties that had previously shown different Mn tolerance according to our procedures. We will investigate further if an appropriate nutrient solution is able to induce a differential response in this symptom with varieties of known response to Mn.

In corn we have observed that the chlorotic banding along the leaves can be induced by foliar application of Mn solutions and that leaves of less tolerant varieties tend to wilt in a few hours after being placed in an Mn solution. We are investigating these effects toward a possibly more rapid method for this species. We expect in the near future to complete these lines of research and to be in a position to write a Bulletin with improved methods of screening for Mn tolerance.

V. ACKNOWLEDGEMENTS

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Berkeley are gratefully appreciated. Seed material provided by Dr. Glenn Anderson, CIMMYT, Mexico is also acknowledged.

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Table 1. Weight and Mn content of shoots of 23 day old wheat plants.

Cultivar	Mn 0.13 ppm Ave. Fresh Wt. in g 3 plants		Mn 32 ppm Ave. Fresh Wt. in g 3 plants		Relative Yield Mn 32 / Mn 0.13 (fresh wts.)	ppm of Mn in solution to reduce 30% growth	Mn in shoots grown in nutrient solution with 32 ppm of Mn			% Dry Matter in 32 ppm Mn
	roots	shoots	roots	shoots			Conc. fresh wt. basis ppm	Conc. dry wt. basis ppm	Uptake by 3 plants µg	
Monon	1.47	2.60	1.63	2.70	1.04**	>>32	250	2600	670	9.7
Fielder	2.43	3.97	1.90	3.30	0.83***	>32	250	2800	840	9.0
IAS 54	1.40	3.23	1.70	2.67	0.82***	>32	280	2800	750	10.1
Atlas 66	1.60	3.30	1.60	2.57	0.78***	>32	190	1600	480	11.5
Vila Velha	2.27	4.57	2.17	3.40	0.74***	>32	360	3800	1250	9.6
Kharkov	1.80	2.60	1.40	1.80	0.69*	>32	290	2300	520	12.7
Thatcher	2.33	2.93	1.53	2.03	0.69*	26	270	2300	550	11.4
Erechim	2.43	5.93	2.00	4.00	0.67***	26	310	3500	1230	8.9
BH 1146	2.37	4.10	2.00	2.77	0.68***	24	270	2600	760	10.7
INIA 66	2.33	3.03	1.90	1.97	0.65**	28	360	2900	720	12.5
Druchamp	2.43	3.73	1.47	2.37	0.63**	28	250	1900	580	12.7
CI 15003	1.67	3.07	1.10	1.93	0.63	25	380	3000	730	12.5
PI353388	2.37	4.93	1.87	3.03	0.61***	26	240	2500	730	9.7
Wakooma	2.30	3.10	1.30	1.77	0.57*	18	360	2300	650	15.8
Gaines	1.30	2.87	0.80	1.50	0.52*	14	184	1400	260	12.3
C. Spring	3.13	6.07	1.83	3.10	0.51**	13	260	2500	820	10.5
Thorne	1.60	4.53	1.10	2.33	0.51***	17	260	2100	610	12.7
Urquie	1.73	3.13	1.03	1.53	0.49*	23	250	1900	380	13.3
Akadaruma	2.33	3.57	0.90	1.63	0.46*	14	280	1500	460	18.2
Sonora 64	1.40	3.53	0.73	1.60	0.45***	10	260	2100	420	12.7
Brevor	1.90	3.80	0.70	1.63	0.43*	14	220	1500	550	14.9
Trintani	3.17	6.70	1.37	2.63	0.39***	9	220	1400	580	15.5
PI344180	2.47	5.20	1.10	1.93	0.37**	14	240	1600	470	15.7
PI157567	2.30	5.37	0.87	1.87	0.35	12	340	2400	640	14.4

* Low Al tolerance ** Moderate Al tolerance *** High Al tolerance

Table 2. Changes of Mn concentrations with time in the shoots of wheat plants grown in a nutrient solution containing 32 ppm of Mn.

	Mn ppm (ave. of 2 plants)		
Cultivar	3 days	15 days	21 days
Monon	1200	2700	2300
Fielder	1000	2100	2600
IAS 54	1600	2600	2300
Atlas 66	1500	1900	2200
Vila Velha	1300	2800	3100
Druchamp	1100	1800	1700
Gaines	800	1800	1700
Sonora 64	1100	2400	2100
Brevor	900	1800	1300
Trintani	1500	1700	1700

Table 3. Leaf Mn analysis of 19 day old wheat plants grown in a nutrient solution containing 32 ppm of Mn.

	Mn ppm (dry weight basis)			
Cultivar	1st leaf	2nd leaf	3rd leaf	Remainder
Monon	4600	4400	2000	2200
Fielder	4100	3500	2000	2200
IAS54	3500	2600	1900	2200
Atlas 66	3900	2200	1500	2800
Vila Velha	4400	3800	1900	1500
Druchamp	4100	800	1700	3000
Gaines	2100	1300	1400	1900
Sonora 64	2800	1300	2600	4100
Brevor	1200	1400	1700	3800
Trintani	2200	900	1400	2100

Table 4. Change in dry matter content of whole plant shoots in time, when grown in 32 ppm Mn solution (average of 2 reps. with 3 plants each).

Cultivar	% dry matter		
	8 days	15 days	21 days
Monon	9.8	10.0	11.7
Fielder	10.7	10.6	11.7
IAS 54	10.7	11.1	12.8
Atlas 66	10.9	12.4	14.2
Vila Velha	10.7	10.4	11.7
Druchamp	11.8	13.8	16.0
Gaines	11.8	13.3	16.1
Sonora 64	11.7	13.9	16.9
Brevor	14.0	16.9	19.7
Trintani	11.1	13.3	16.7

Table 5. Dry matter content of 19 day old plants, grown in 32 ppm Mn solution (average of 3 leaves).

	% dry matter				
Cultivar	1st leaf	2nd leaf	3rd leaf	Remainder	Total Plant
Monon	10.8	13.3	12.1	9.2	11.2
Fielder	11.9	13.9	12.3	9.2	11.9
IAS 54	12.7	15.1	12.7	8.6	12.2
Atlas 66	12.6	16.3	13.8	9.9	13.2
Vila Velha	11.8	14.2	12.5	8.9	11.5
Druchamp	14.7	20.4	16.3	9.9	15.5
Gaines	14.5	20.1	17.0	10.3	15.9
Sonora 64	19.0	22.0	14.2	10.1	17.5
Brevor	22.8	24.1	16.3	10.3	19.2
Trintani	14.5	22.6	4.5	9.0	13.3

Table 6a. Dry matter content of the first leaf. (3 leaves)

Cultivar	% dry matter								
	9 days Mn ppm			14 days Mn ppm			18 days Mn ppm		
	0.13	32	64	0.13	32	64	0.13	32	64
Monon	9.6	10.1	10.1	8.4	9.3	11.3	8.5	9.1	13.6
Fielder	9.9	9.6	10.3	10.4	10.0	11.1	9.4	9.9	13.9
IAS 54	9.9	10.2	10.6	9.1	11.8	11.8	9.1	12.9	12.3
Atlas 66	9.5	10.6	10.2	9.1	11.9	18.2	8.9	13.9	19.6
Vila Velha	9.9	9.6	10.4	9.7	10.0	13.0	9.0	13.7	13.3
Druchamp	10.7	11.2	11.8	10.4	12.6	16.6	10.5	14.8	18.2
Gaines	11.0	13.0	13.6	10.9	13.8	16.0	9.9	14.3	16.0
Sonora 64	10.1	12.1	11.4	10.0	14.5	15.5	9.1	15.1	12.4
Brevor	10.8	14.7	14.1	11.1	20.0	20.8	10.4	22.0	25.5
Trintani	9.3	10.8	11.6	10.8	13.1	12.9	9.0	12.9	15.7

Table 6b. Dry matter percentage of second leaf of 14 day old plants. (3 leaves)

Cultivar	Mn ppm 0.13	32	64
Monon	9.3	9.6	13.5
Fielder	10.0	10.0	14.8
IAS 54	9.8	11.5	15.5
Atlas 66	10.2	13.2	-
Vila Velha	10.8	11.0	13.0
Druchamp	10.6	12.8	16.5
Gaines	10.7	14.6	14.9
Sonora 64	11.0	14.3	16.0
Brevor	11.1	15.4	17.2
Trintani	9.8	12.2	15.0

Table 7. Classification of Mn tolerance according to relative growth.

Mn Tolerant R.Y. > 0.70	R.Y.
Desconocido-Frocor	1.16
Fielder	1.04
CM 11683-A-1Y-1M-1Y-7M-0Y	1.00
Colotana	.97
Huacamayo "S"	.97
Alondra "S"	.94
Torim 73	.94
Nova Prata	.93
Tob 66	.93
7 Cerros	.89
Tezanos-Pinto Precoz	.87
Yding "S" x Kal-Bb/Hork "S"-Mo73	.87
Kal-Bb x Alondra "S"	.86
Vulture "S"	.86
Junco "S"	.85
Valdivia "S"	.84
Azteca 67	.83
Colonias	.83
Super X	.83
Blue Jay-Torim 63	.80
IAS 20 x Wte[3]-Nar59	.80
(Rfu[2] x 908-Fn/M2824-KFS) x Cfu x Tob-8156(R)	.80
Nipigon "S"	.75
Pichihuila "S"	.75
CM 11683-A-1Y-1M-3Y-0M	.74
Hork "S"	.74

Mn Less Tolerant 0.55 < R.Y. < 0.70	R.Y.
Emu "S"	.68
Pavon "S" (Bb-Cuo "S" x Jar/Orizaba "S")	.67
Ticus	.67
Atlas 66	.66
7C-Alondra "S"	.66
Pavon 76	.65
Cinto "S"	.64
PF-70354	.64
Barpet-Tal x Rap	.62
Buck Buck "S"	.61
PF-70354	.61
Novafen	.60
Bb-Kal x Alondra "S"/Y50E-Kal[3] x Emu "S"	.58
PF-70354	.58

Mn Susceptible R.Y. < 0.55	R.Y.
Etoile de Choisy	.52
Zacatecas 74-Bon	.43
Chiroca "S"	.40
Glenlea	.40
Brevor	.36
Quetzal 75	.36
Kafue	.35

Table 8. Mn tolerance according to dry matter percentage in 14 day old plants. (average of 3 plants)

Mn Tolerant dry matter < 11.25%	dry matter %	Mn Less Tolerant 11.25 < dry matter < 14%	dry matter %	Mn Susceptible dry matter > 14%	
Nova Prata	9.0	PF-70354	11.3	Chiroca "S"	14.1
Kal-Bb x Alondra "S"	9.4	Novafen	11.4	Cinto "S"	14.7
(Rfu[2] x 908-Fn/M2824-KFS) x Cfu x Tob-8156(R)	9.9	Pavon 76	11.4	Glenlea	14.8
Alondra "S"	10.1	PF-70354	11.5	Etoile de Choisy	15.8
Colotana	10.1	PF-70354	11.7	Zacatecas 74-Bon	16.0
Tezanos-Pinto Precoz	10.1	Torim 73	11.8	Kafue	16.4
Yding "S" x Kal-Bb/Hork "S"-Mo73	10.1	Pavon "S" (Bb-Cuo "S" x Jar/Orizaba "S")	12.0	Brevor	19.1
Hork "S"	10.2	T4-C306	12.1		
Junco "S"	10.2	Atlas 66	12.5		
Valdivia "S"	10.2	Barpet-Tal x Rap	12.5		
Blue Jay-Torim 63	10.3	Bb-Kal x Alondra "S"/Y50E-Kal[3] x Emu "S"	12.5		
Fielder	10.3	Ticus	12.9		
Colonias	10.4	Quetzal 75	13.5		
CM 11683-A-1Y-1M-3Y-0M	10.5	Buck Buck "S"	13.8		
CM 11683-A-1Y-1M-1Y-7M-0Y	10.5	Emu "S"	13.9		
Nipigon "S"	10.5	7C-Alondra "S"	13.9		
Huacamayo "S"	10.6				
7 Cerros	10.6				
Vulture "S"	10.6				
Pichihuila "S"	10.7				
Super X	10.9				
Azteca 67	11.0				
Desconocido-Frocor	11.2				
IAS 20 x Wte[3]-Nar59	11.2				
Tob 66	11.2				

Table 9. Yield of corn plant shoots 21 days after transplanting to the nutrient solution.

Variety	Fresh wt. of 3 plants (g) Mn ppm in nutrient solution				Relative yield Mn ppm in nutrient solution			
	0.13	8.0	16.0	32.0	0.13	8.0	16.0	32.0
Earli King	1.5	2.3	4.2	3.1	1	1.53	2.80	2.10
Bulkpack	3.1	4.4	3.3	4.4	1	1.39	1.04	1.40
PX 15	5.9	6.7	10.2	7.2	1	1.13	1.73	1.22
Earli Vee	2.1	1.4	2.1	2.6	1	0.70	1.00	1.20
PX 26	5.6	7.4	8.0	6.3	1	1.32	1.43	1.12
Morning Sun	2.2	2.5	3.7	2.3	1	1.13	1.68	1.04
Popcorn R	2.2	2.0	2.5	2.1	1	0.91	1.14	0.95
NK 199	2.8	1.7	2.3	2.4	1	0.61	0.82	0.86
Northern Vee	2.8	1.8	2.5	2.2	1	0.64	0.89	0.78
PX 485	5.9	7.9	7.5	4.3	1	1.34	1.27	0.73
Span Cross	3.2	3.1	1.2	2.1	1	0.98	0.37	0.66
PX 74	8.4	4.4	6.7	5.2	1	0.52	0.80	0.62
FM Cross	4.3	3.4	4.9	2.6	1	0.80	1.15	0.60
Golden Cross Bantam	5.1	4.0	2.7	3.3	1	0.78	0.53	0.60
PX 610	11.3	9.1	5.2	6.8	1	0.77	0.44	0.58
Polar Vee	3.4	1.70	1.5	1.2	1	0.50	0.44	0.35
Popcorn W	3.9	3.3	1.3	1.3	1	0.85	0.33	0.33

LEGEND FOR FIGURES

Fig. 1. Tray with styrofoam lid used in the nutrient solution method for evaluating the decrease in growth caused by Mn toxicity.

Fig. 2. Styrofoam strips for growing wheat seedlings for dry weight determination after nutrient solution culture with 32 ppm of Mn.

Fig. 3. Wooden rack with polypropylene plastic vials used for drying leaves for dry weight determination.

Fig. 4. (a) Wheat selection CM 11683-A-1Y-1M-1Y-7M-OY Mn toxicity tolerant; (b) Wheat cultivar Ticus, moderately Mn toxicity tolerant; (c) Wheat cultivar Kafue, Mn toxicity susceptible; wheat genotypes grown 23 days in nutrient solutions containing 0.13 (left) and 32 ppm of Mn.

Fig. 5. Hybrid maize seedlings: (a) Bulkpack and Polar Vee, (b) Earli King and Span Cross, (c) PX 26 and PX 610 after growing 5 days in a nutrient solution containing 0.13 (left) and 32 ppm (right) of Mn.

Fig. 6. (a) Hybrid maize Bulkpack, (b) PX 485 and (c) Polar Vee grown 21 days in nutrient solutions containing 0.13, 8, 16 and 32 ppm of Mn (left to right).

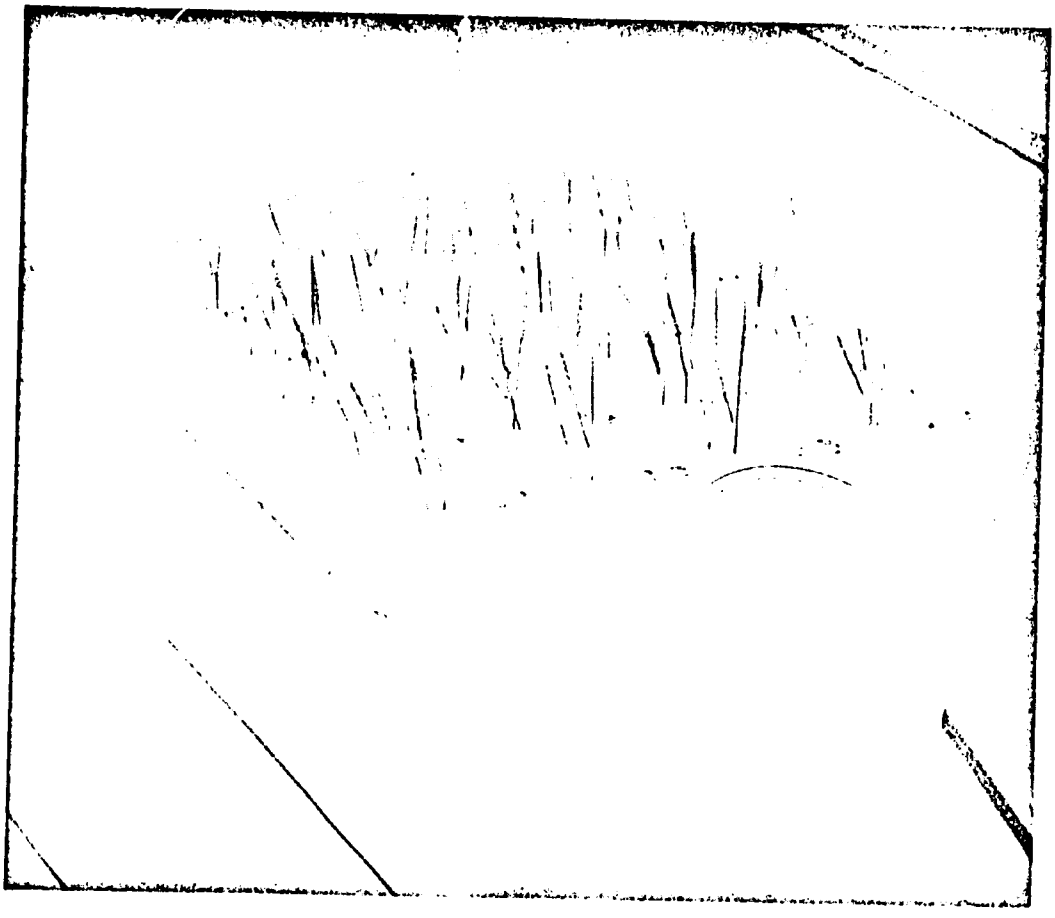


Fig. 1

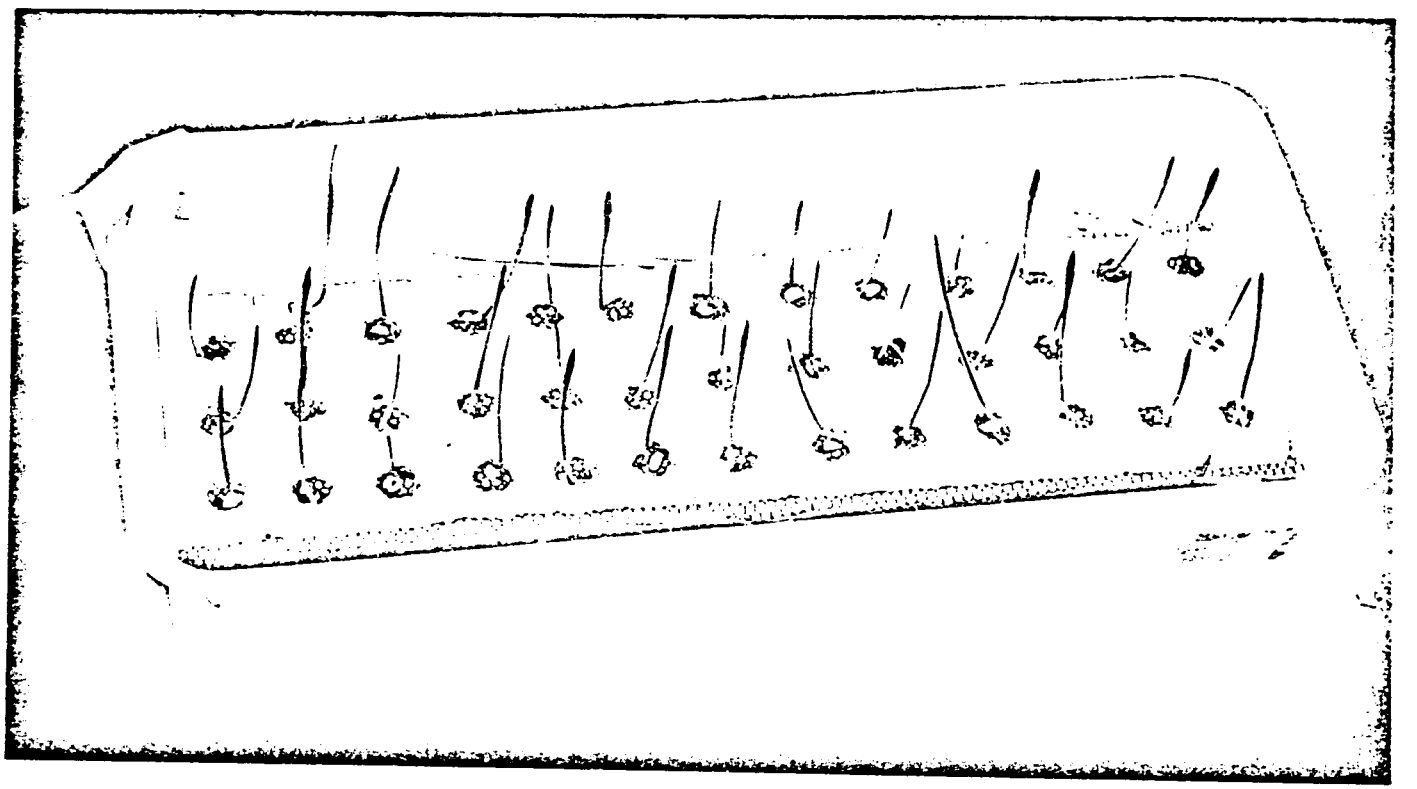


Fig. 2

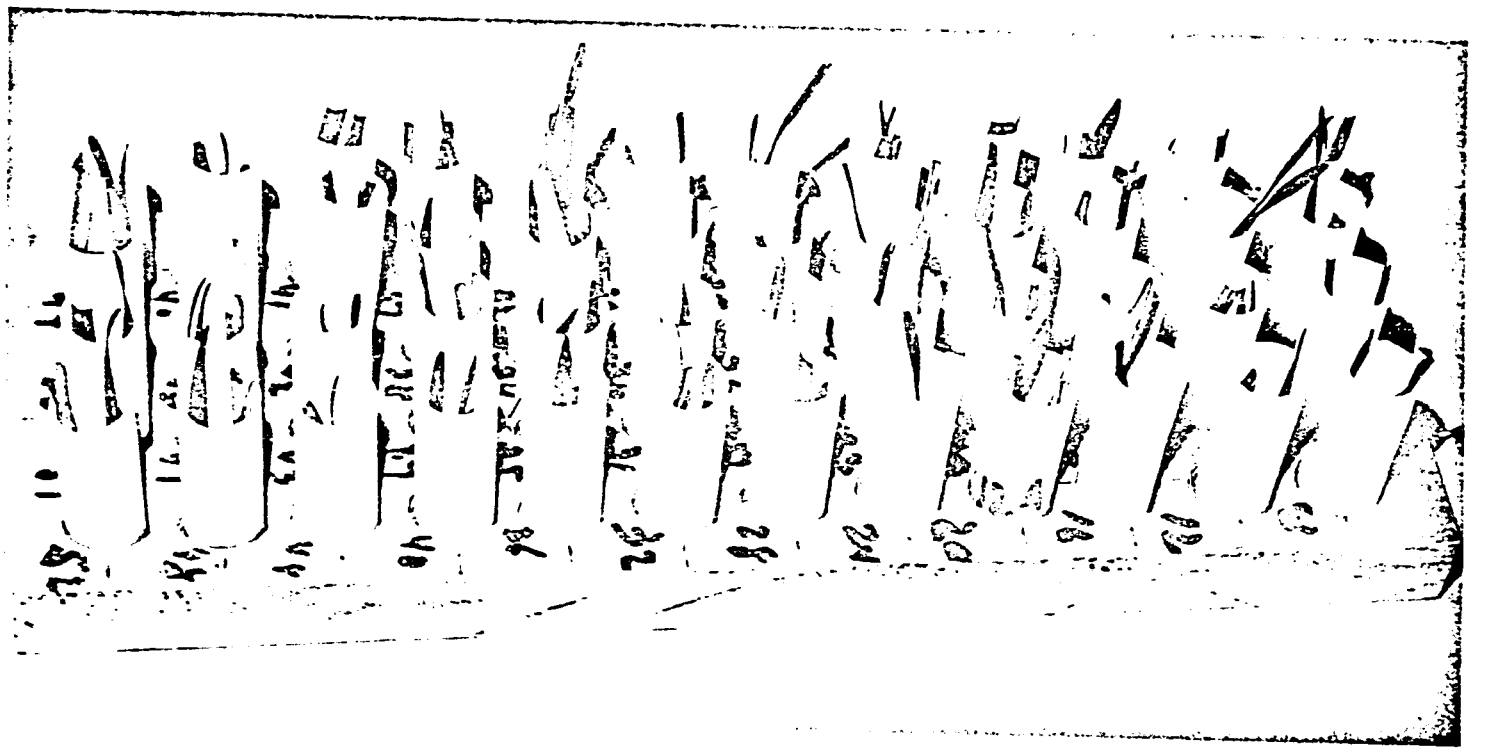


Fig. 3

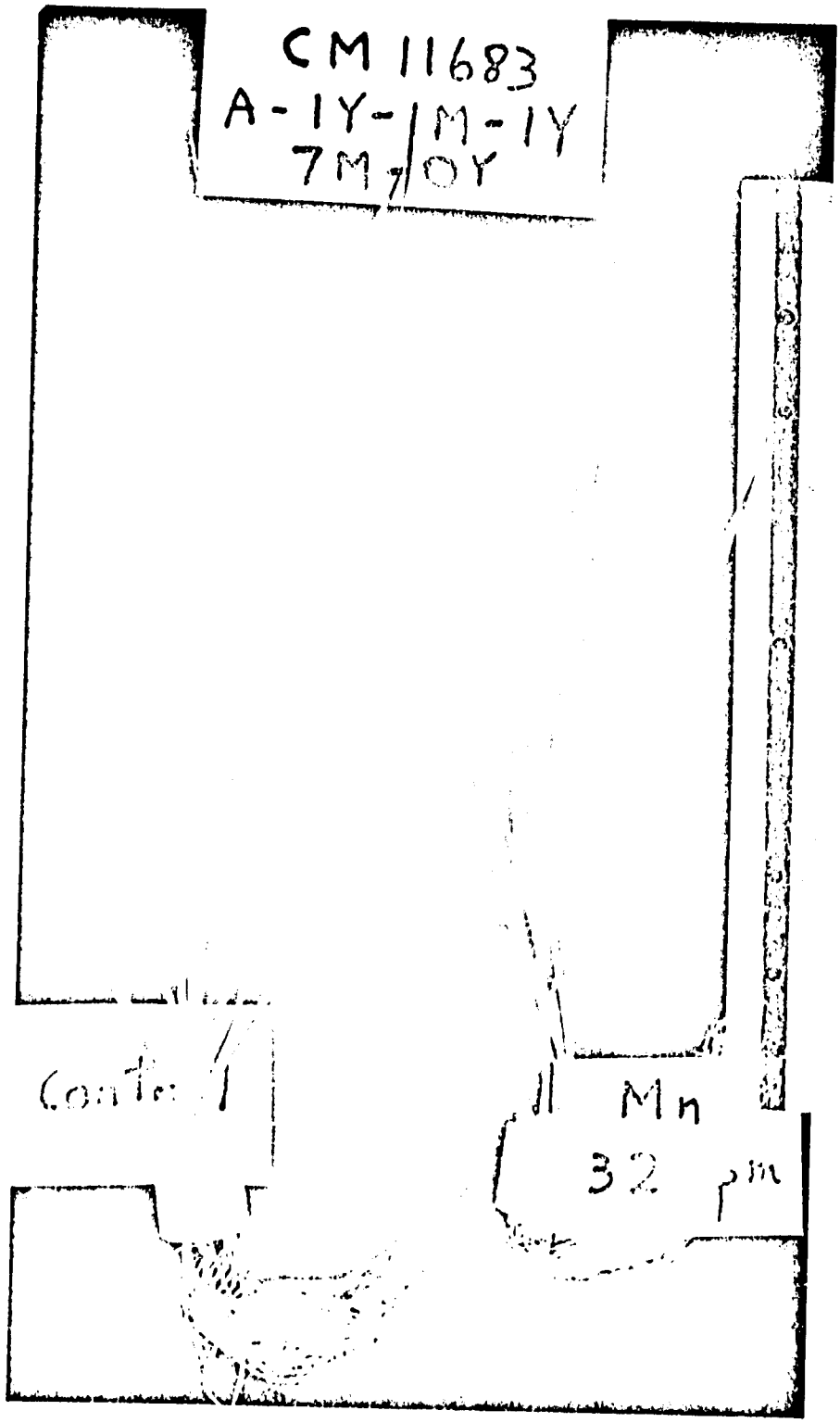


Fig. 4 (a)

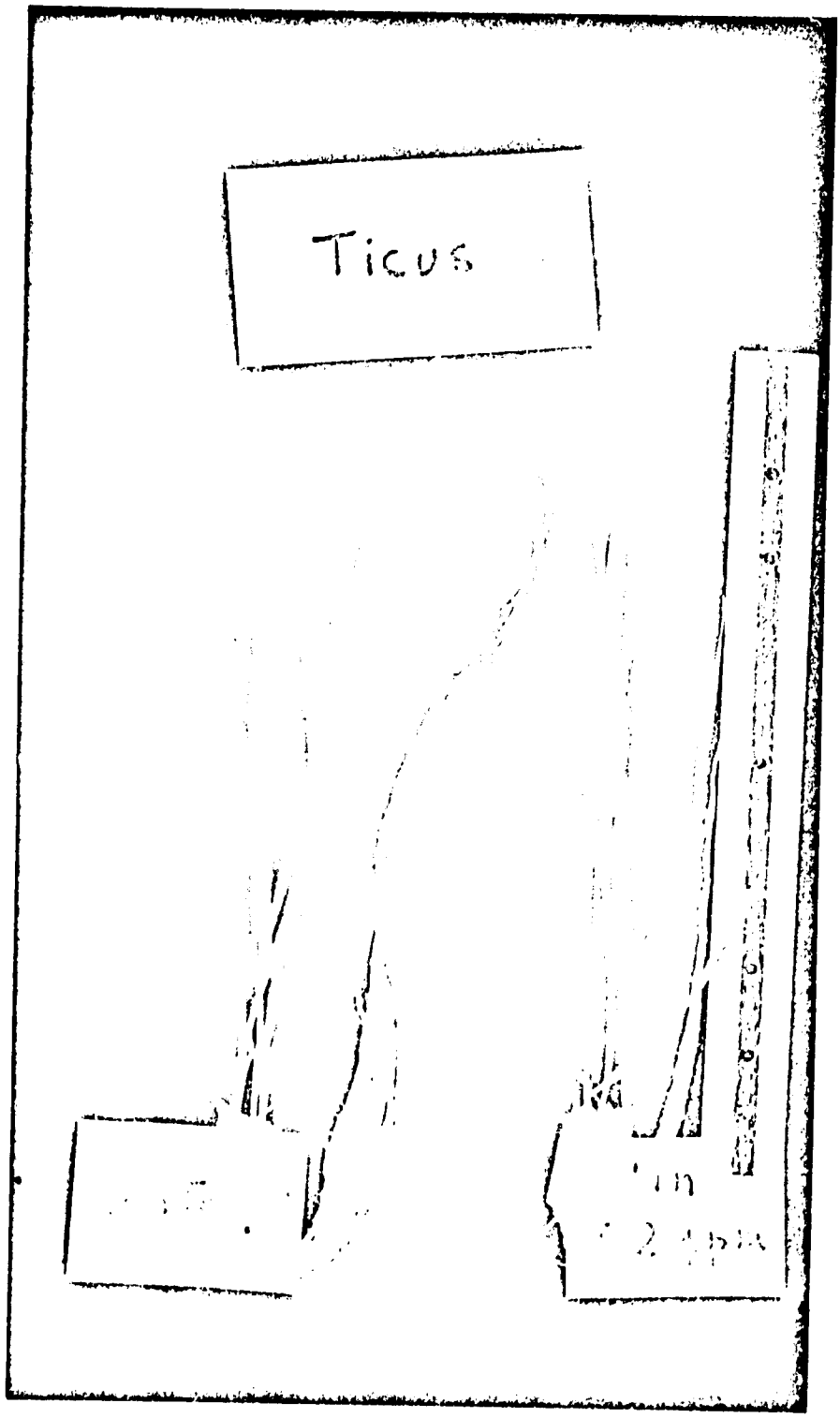


Fig. 4 (b)

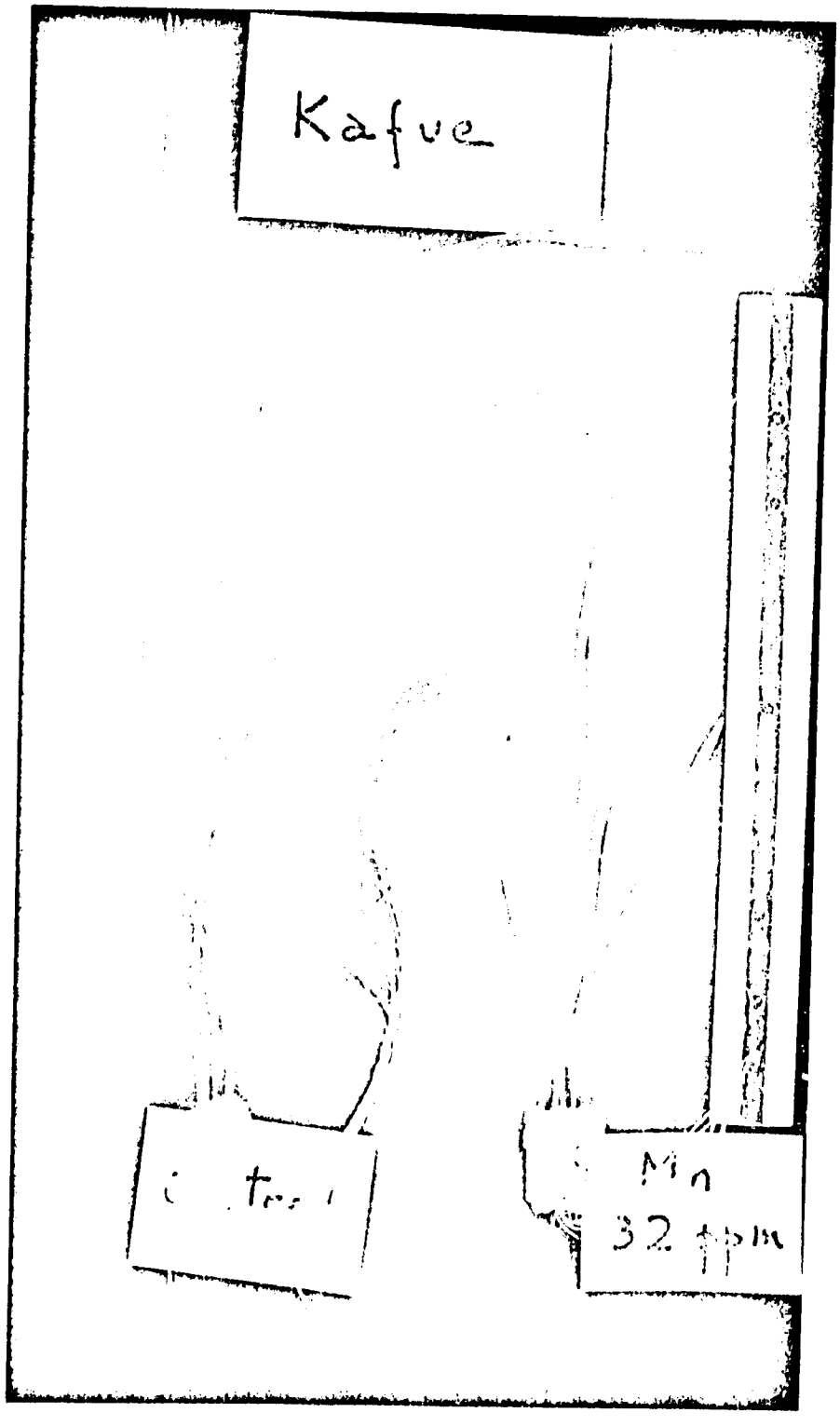


Fig. 4 (c)

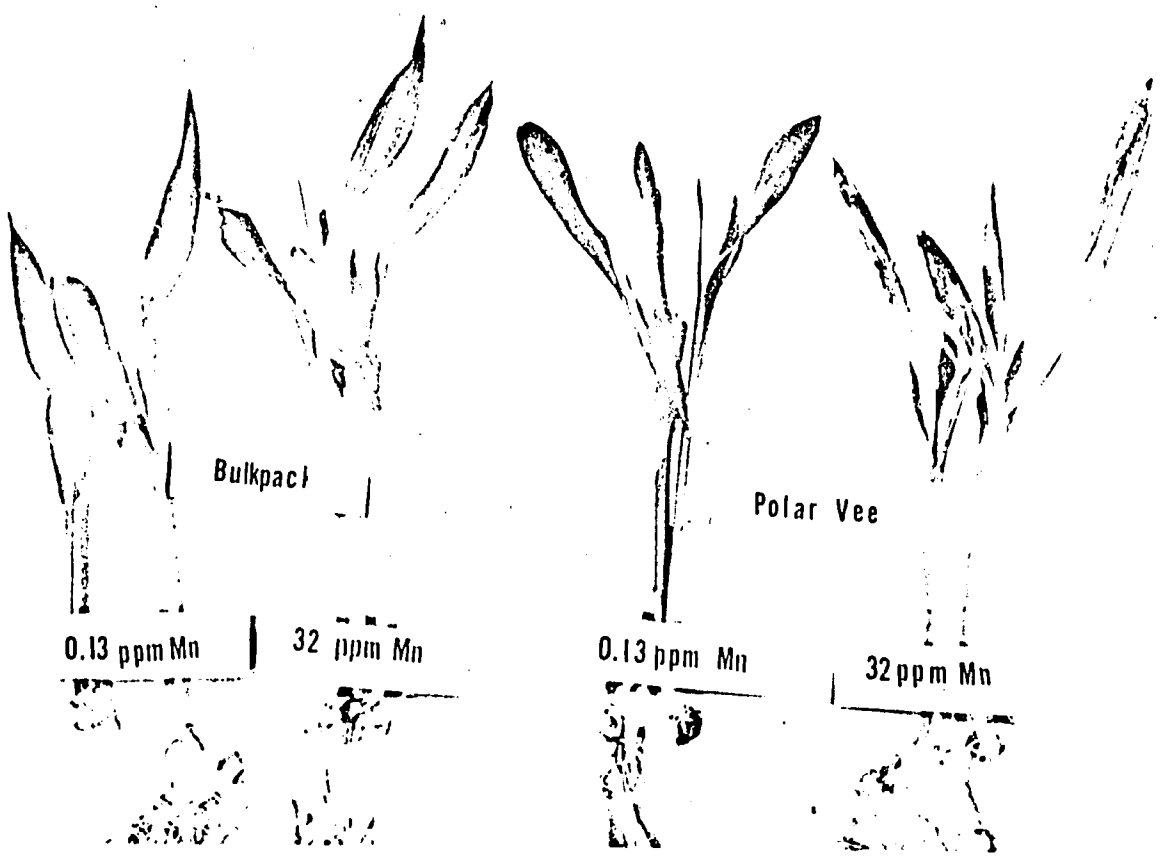


Fig. 5 (a)

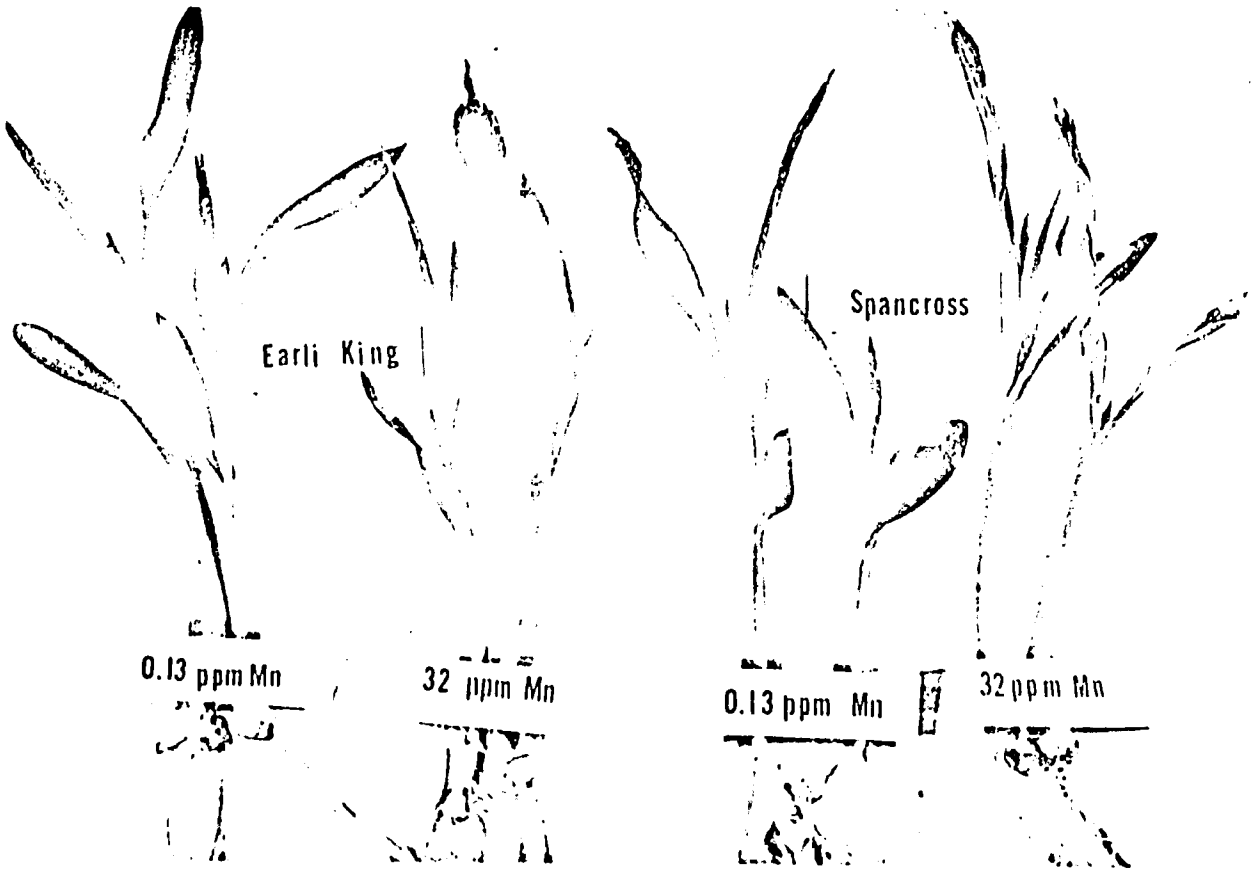
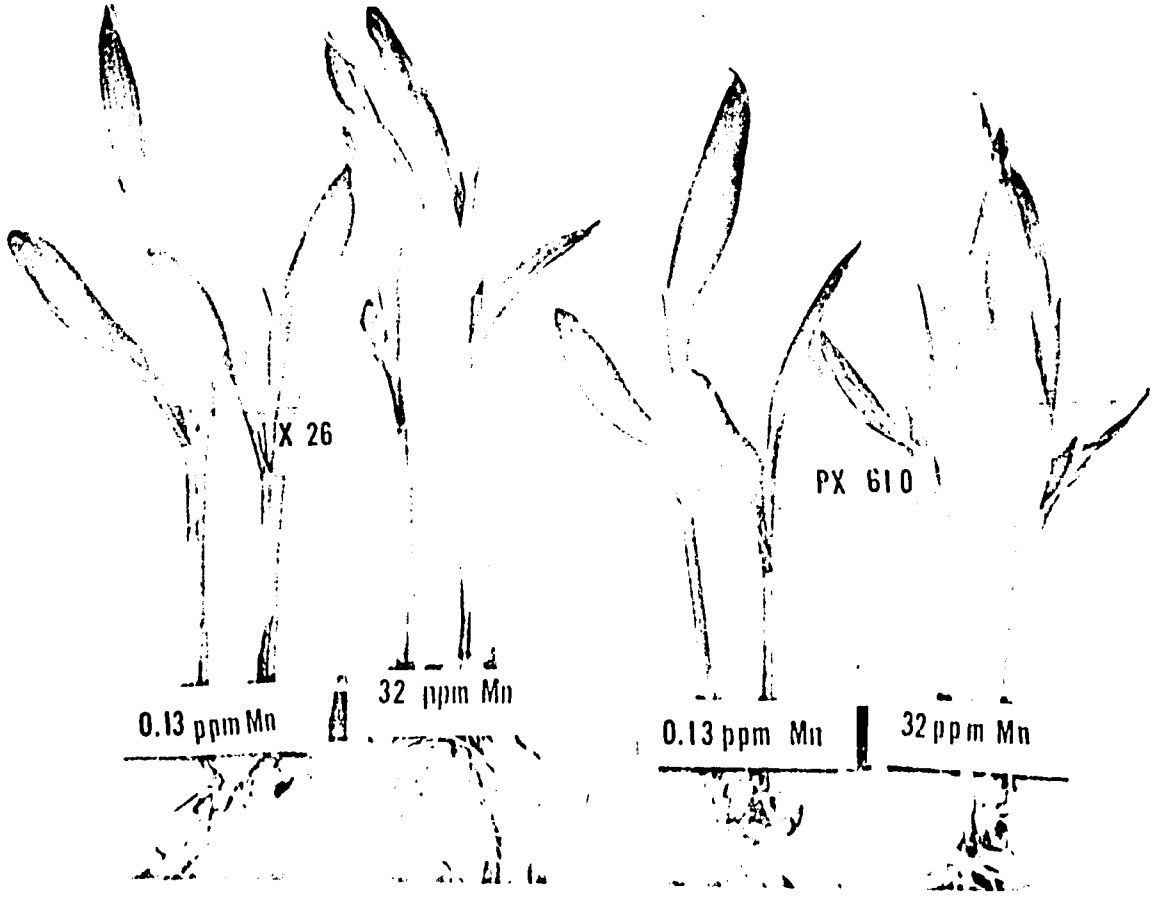


Fig. 5 (b)



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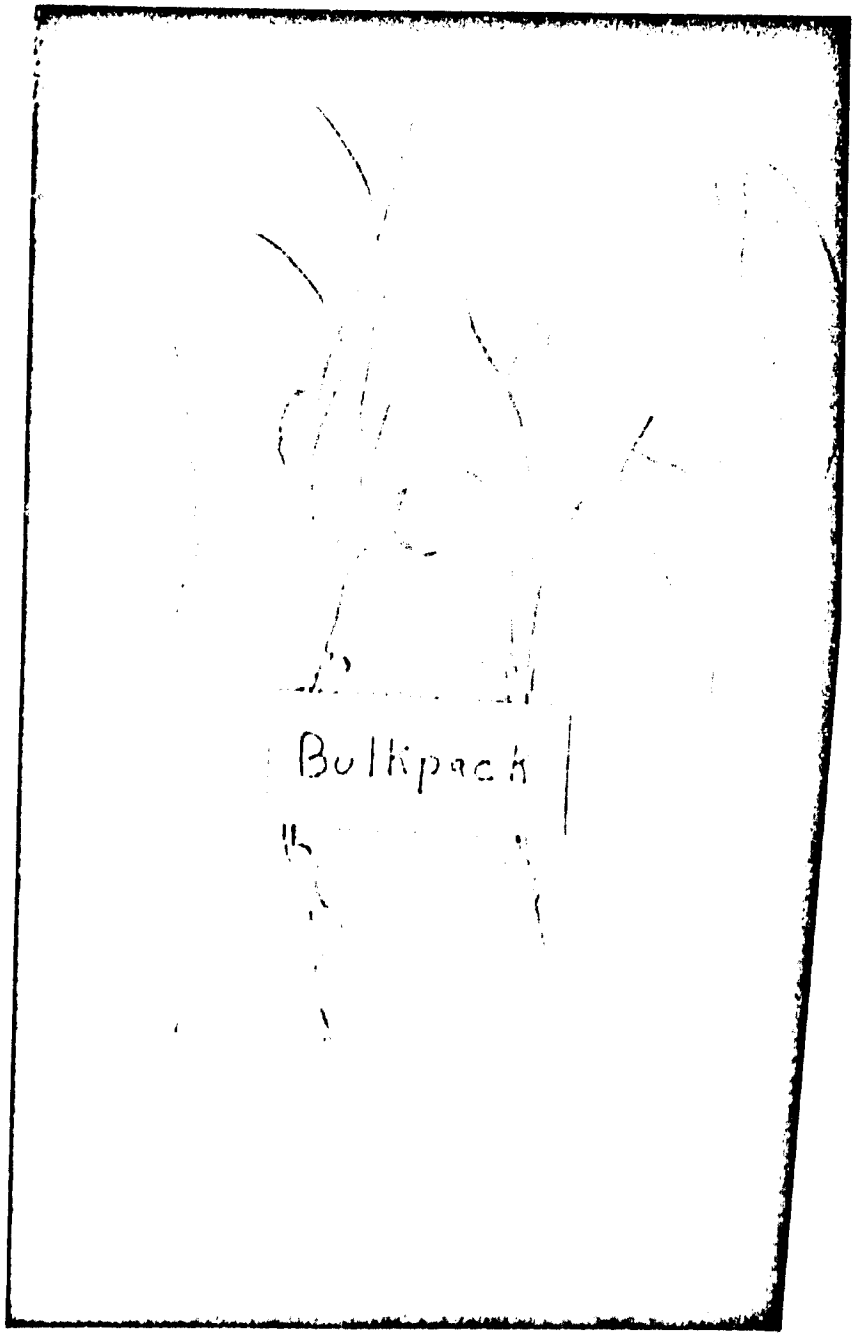

Fig. 6 (a)

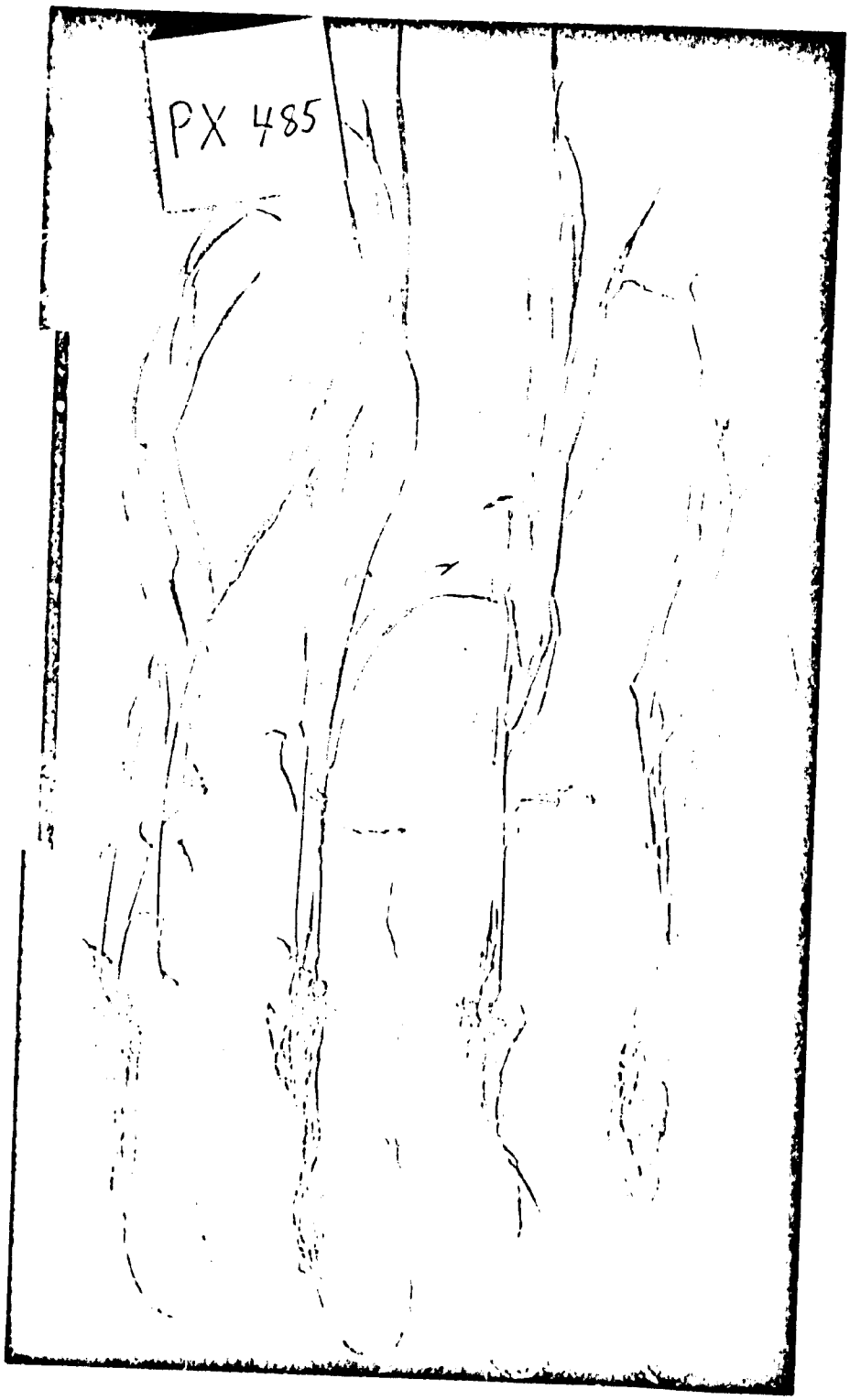

Fig. 6 (b)

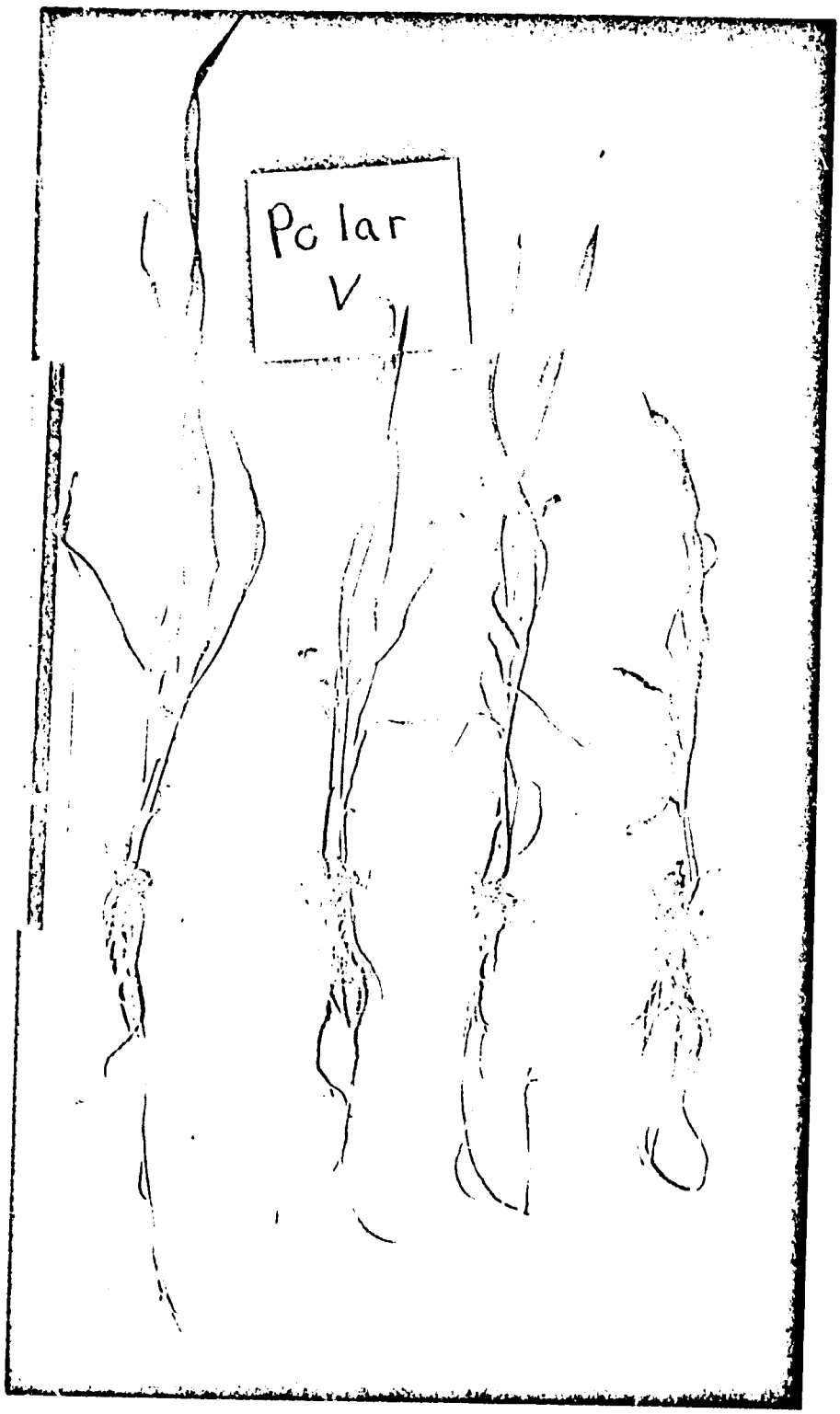

Fig. 6 (c)