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Effects of fusaric acid on *Zea mays* L. seedlings

Efectos del ácido fusárico en plántulas de *Zea mays* L.

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Abstract. The effects of fusaric acid, a mycotoxin produced by *Fusarium* sp, were investigated in relation to its action on corn seedlings. The mycotoxin inhibited the development of corn seedlings at 0.2 mM, causing root length reduction. Anatomically, alterations were only visible from 0.5 mM fusaric acid, directly influencing the cell differentiation process. Precocious differentiation reduces the elongation region. This explains (1) that root shortening is morphologically visible, and (2) the differentiation of a great number of lateral roots nearby the apex, which can be seen in longitudinal cuts.

Key words: fusaric acid, mycotoxin, *Zea mays* seedlings.

Resumen. Los efectos del ácido fusárico, una micotoxina producida por el hongo *Fusarium* sp, fueron investigados en relación a su acción sobre plántulas de maíz. La micotoxina inhibió el desarrollo de las plántulas de maíz, en concentración de 0,2 mM, reduciendo la longitud de la raíz. Anatómicamente, las alteraciones fueron más visibles con 0,5 mM de ácido fusárico, influyendo directamente en el proceso de diferenciación celular. La diferenciación precoz reduce la región de alargamiento. Esto explica (1) que el acortamiento de la raíz es morfológicamente visible, y (2) la diferenciación de un gran número de raíces laterales cerca del ápice, visibles en los cortes longitudinales.

Palabras clave: ácido fusárico, micotoxina, plántulas de *Zea mays*.

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INTRODUCTION

The phytotoxic effect of many secondary metabolites produced by fungi has been shown through biotests in plants (Kachlicki & Jedrycka, 1997). Among them, fusaric acid is considered the most important secondary metabolite produced by *Fusarium oxysporum*.

Fusaric acid has been characterized as a breathing inhibitor, promoting (1) electrolytes output and (2) cytological changes in corn roots (Arias, 1985; Telles-Pupulin et al., 1996).

Fusaric acid acts in the isolated mitochondrias metabolism of corn roots, inhibiting electron flux between the succinate-dehydrogenase and coenzyme Q, and the activities of the ATPase/ATP-synthase and, probably, that of the α-ketoglutarate dehydrogenase (Telles-Pupulin et al., 1996). Fusaric acid speeds up the lipid peroxidise activity in watermelon leaves, and was able to destroy the leaf cell defense system on watermelon seedlings (Wu et al., 2008).

Few studies have been produced on the effects of fusaric acid on plant tissues. For example, the growth inhibition in *Aechmea fasciata* seedlings, Bromeliaceae. Matysiak & Samyn (1996) also registered a complete inhibition of *Aechmea fasciat* seedling growth at 1 mM fusaric acid.

The objective of this study was to evaluate the morpho-anatomical alterations produced by fusaric acid on corn roots (*Zea mays* L.).

MATERIALS AND METHODS

Corn seeds were disinfected at 2.5% sodium hypochlorite in ethanol, and then washed using sterilised water. Germination was conducted in germination boxes (Gerbox), and seeds were placed within a double sheet of previously sterilised filter paper. This paper was kept moist with distilled water (control) or with a modified Hoagland solution which was supplemented with any one of the following fusaric acid concentrations: 0.1; 0.2; 0.5; 1.0; 2.0 or 5.0 mM. Seedlings obtained 8 days after seeding were photographed, and their root morphological characteristics were described. Anatomical studies were conducted on the primary roots of controls and on those seedlings exposed to the different concentrations of fusaric acid.

Roots used for anatomical studies were fixed in F.A.A. 50%. This material was maintained in ethanol 70% (Jensen, 1962). Transversal and longitudinal cuts were made in the elongation, absorption and ramification regions of the primary root. Longitudinal cuts were also made on the root tip.

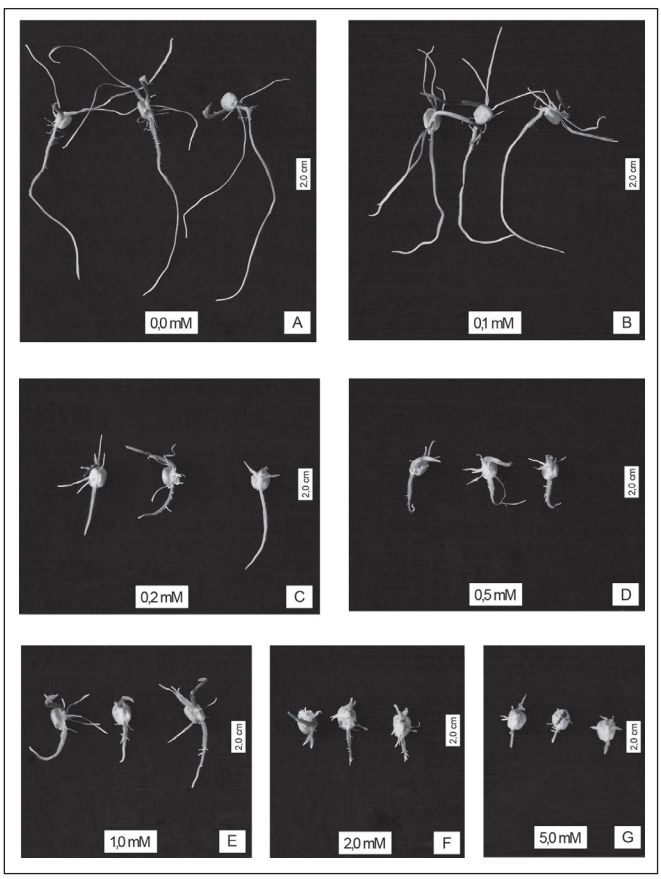
The fixed root pieces were (1) dehydrated in an ethylic sequence, and (2) in a xylolic series, (3) included in paraffin, (4) cut on the rotatory microtome (Sass, 1951), (5) dyed in safranin/Blue of Astra (Gerlach, 1969) and (6) set up in Permount, to produce permanent laminae.

Photographs were taken with a Pentax K1000 camera and the photomicrographs using an Olympus photomicroscope (coloured film ASA 100 in both cases). A micrometrical scale was used while taking the photomicrographs.

RESULTS

Effects of fusaric acid on the morphology of *Zea mays* roots start at 0.2 mM (Fig. 1). Decrease of root growth and development was even greater at 2.0 mM and 5.0 mM (Fig. 1). Morphological changes in the roots included: (1) reduction in primary root development (visible through the shortening of the elongation region such that the ramification surface area was closer to the root tip); (2) reduction in lateral roots development from the primary root, and (3) reduction in adventitial roots development which come from the hipocotyl. There was also an inhibition of shoot production.

Fig. 1. Maize seeds germinated for 8 days at 28°C, either without [control (A)] or with fusaric acid at the concentrations of 0.1 mM (B); 0.2 mM (C); 0.5 mM (D); 1.0 mM (E); 2.0 mM (F) and 10.0 mM (G).
Fig. 1. Semillas de maíz germinadas durante 8 días a 28°C, sin [control (A)] o con ácido fusárico a concentraciones de 0,1 mM (B); 0,2 mM (C); 0,5 mM (D); 1,0 mM (E); 2,0 mM (F) y 10,0 mM (G).



Anatomical changes caused by fusaric acid can be observed from Figures 2 to 8. At concentrations of 0.1 mM and 0.2 mM, there were no changes in root anatomy when compared to the control (Figs. 2(A), 4(A and B), 5(A and B), 7(A and B). At 0.5 mM, however, there was an increase in the root diameter, in both the transversal and longitudinal sections, in the different regions of the root [Figs. 2(B and C), 3(A and B), 4(C), 5(C), 6, 7(C-F)]. This occurred mainly due to the increase in the (1) number of cell layers and, (2) diameter of both the cortex and central cylinder cells.

Fig. 2. Transversal sections in the elongation region of primary roots of *Zea mays* seedlings cultivated either without [control (A)] or with fusaric acid: 0.5 mM (B) and 1.0 mM (C). ct, cortex; en, endoderm; ep, epidermis; ev, vessel element; ex, exodermis; fl, phloem; pr, pericycle, rl, secondary root.
Fig. 2. Cortes transversales de la región de crecimiento de raíces primarias de plántulas de *Zea mays*, cultivadas sin [control (A)] o con ácido fusárico: 0,5 mM (B) y 1,0 mM (C). ct, córtex; en, endodermis; ep, epidermis; ev, elementos de conducción; fl, floema; pr, periciclo; rl, raíz secundaria.

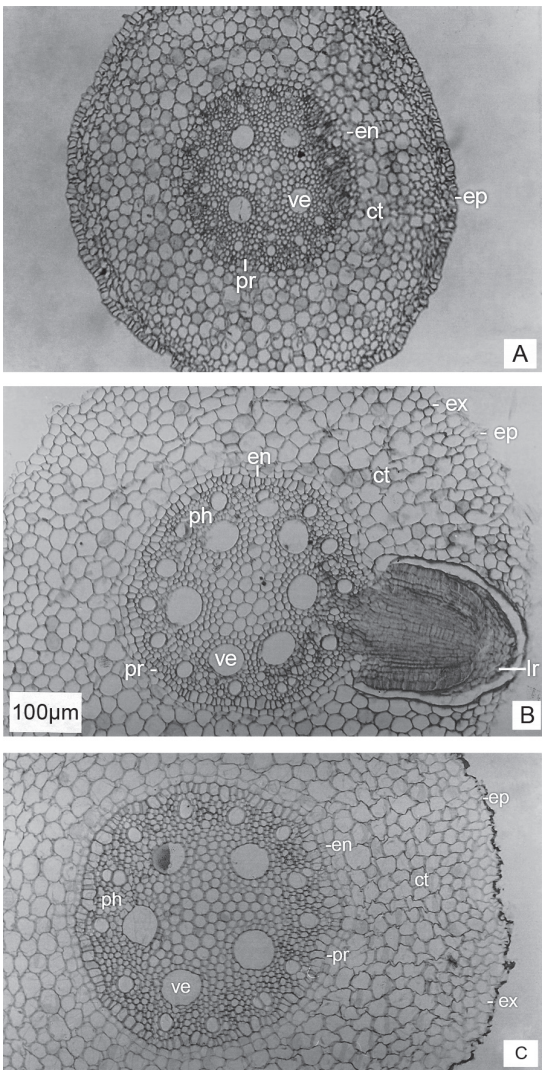
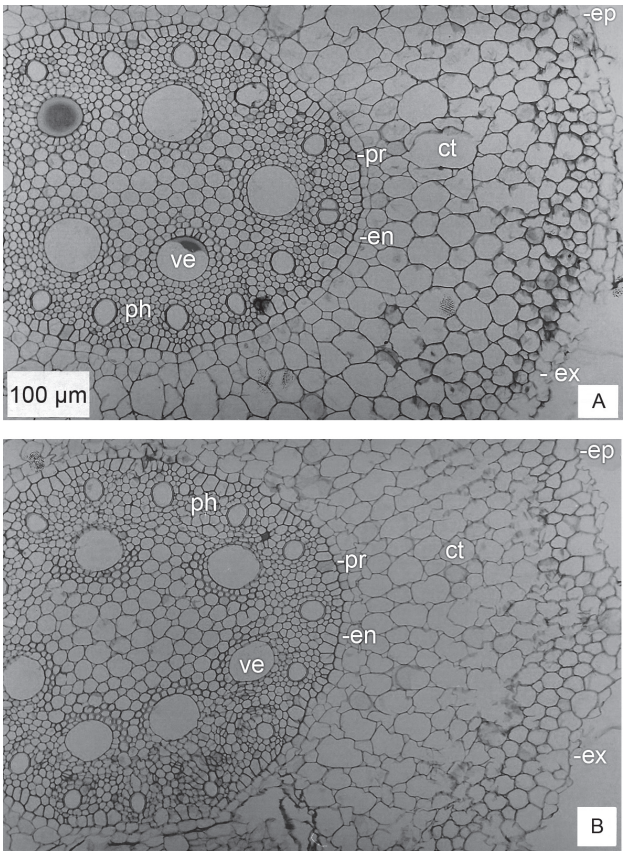


Fig. 3. Transversal sections in the elongation region of primary roots of *Zea mays* cultivated in the presence of fusaric acid: 2.0 mM (A) and 5.0 mM (B). ct, cortex, en, endoderm; ep, epidermis; ev, vessel element; ex, exodermis; fl, phloem; pr, pericycle.
Fig. 3. Cortes transversales de la región de crecimiento de raíces primarias de plántulas de *Zea mays*, cultivadas con uso de ácido fusárico: 2,0 mM (A) y 5,0 mM (B). ct, córtex; en, endodermis; ep, epidermis; ev, elementos de conducción; ex, exodermis; fl, floema; pr, periciclo.



In the elongation region, there was a thickness and lignification (1) on the walls of the protoxylem tracheal elements and (2) of the cells opposite to the metaxylem vessels. This characterises an early cell differentiation (Figs. 2-7), which is also supported by the lateral root emergence, in the elongation region at 1.0 mM (Fig.6). Shortening of the primary root was confirmed anatomically by length reduction of the elongation region. This explains the presence of a great number of lateral roots at the biggest/largest acid concentrations (Figs. 6 and 7). At 0.5 mM, thickness of the exodermis cells was greater (Figs. 2C, 4C and 5C).

In the longitudinal sections made on the root tip, the early cell difference was made clearer. This was because of the element difference in the xylem vessels. These were already well elongated at 0.5 mM (Fig. 8C), when compared to 0.0 (control) and 0.1 mM (Figs. 8 A and B). On the tip, there was necrosis and destruction of root apical meristems (Fig. 8 D-F).

DISCUSSION

Tissues and plant organs arise from the division, elongation and differentiation of cells derived from the apical and lateral meristems. This sequence, which ends up with plant growth, can be regulated through genetic or metabolic processes. The observed responses to fusaric acid in corn root tissues are the result of the effects of this acid upon the basic cell metabolism. This was emphasised by Arias (1985) and Telles-Pupulin et al. (1996) in this same plant species.

Toxicity effects of fusaric acid were evident in the morphoanatomical changes which it induced in corn seedlings. These changes occurred at fusaric acid concentrations similar

Fig. 4. Transversal sections in the absorption region of primary roots of *Zea mays* seedlings cultivated in absence [control (A)] and presence of fusaric acid: 0.1 mM (B); 0.5 mM (C). ct, cortex; en, endoderm; ep, epidermis; ev, vessel element; ex, exodermis; fl, phloem; pr, pericycle.
Fig. 4. Cortes transversales de la región de absorción de raíces primarias de plántulas de *Zea mays*, cultivadas sin uso [control (A)] y con uso de ácido fusárico: 0,1 mM (B) y 0,5 mM (C). ct, córtex; en, endodermis; ep, epidermis; ev, elementos de conducción; ex, exodermis; fl, floema; pr, periciclo.

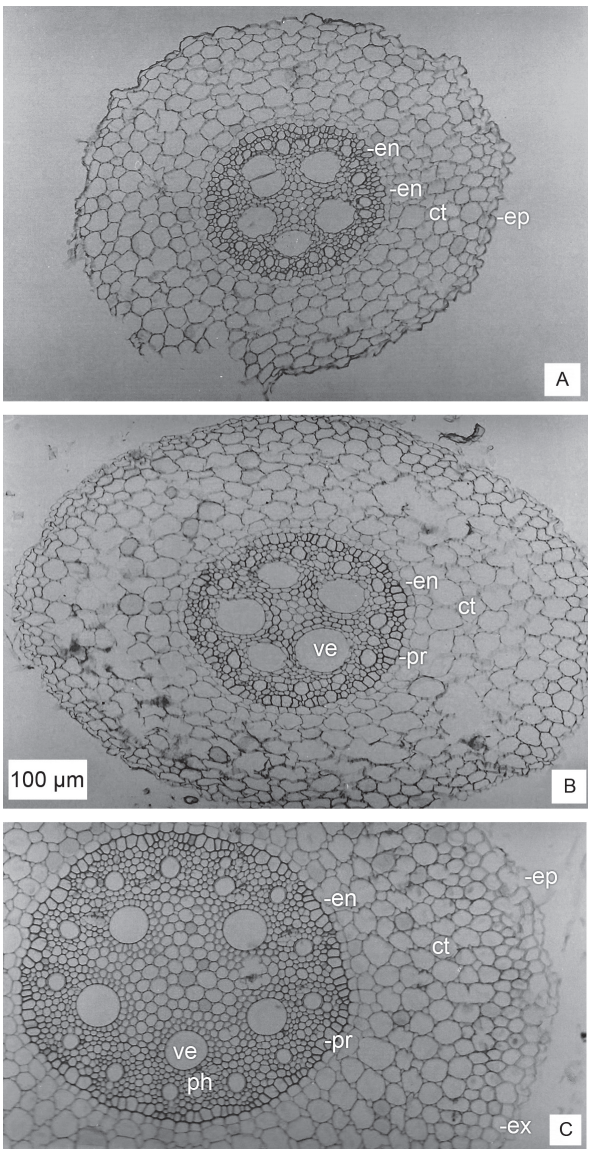
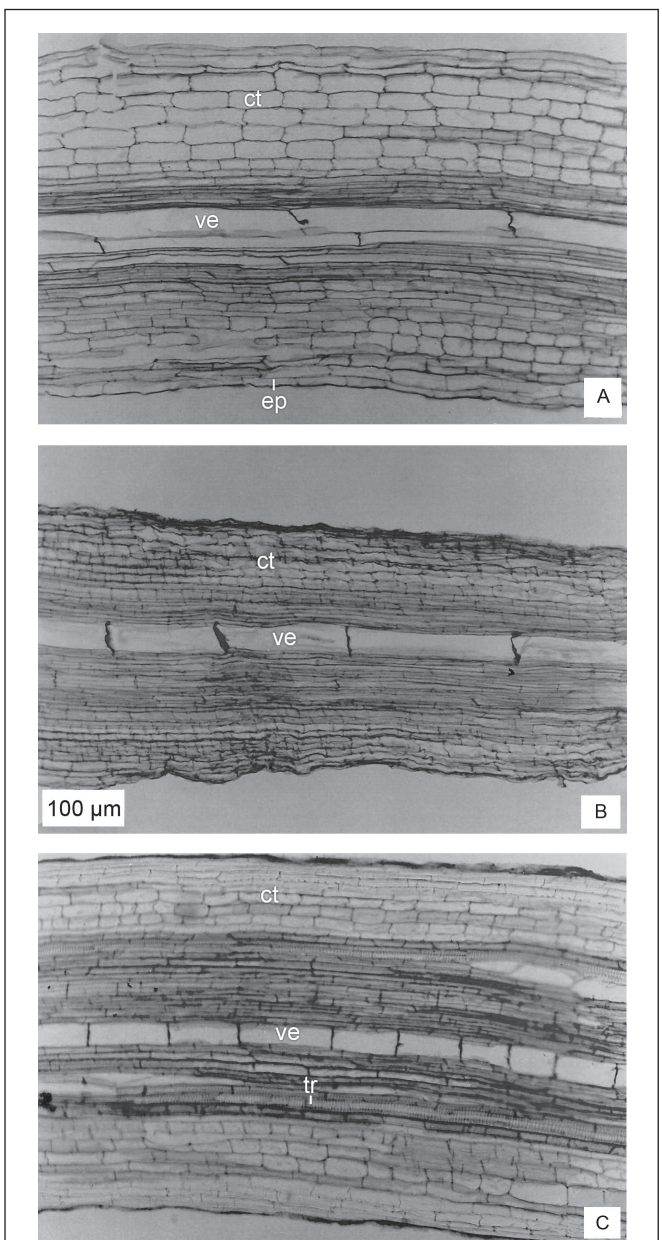


Fig. 5. Longitudinal sections in the elongation region of primary roots of *Zea mays* seedlings cultivated in absence [control (A)] and presence of fusaric acid: 0.1 mM (B) and 0.5 mM (C). ct, cortex; ep, epidermis; ev, vessel element; tr, tracheid.
Fig. 5. Cortes longitudinales de la región de crecimiento de raíces primarias de plántulas de *Zea mays*, cultivadas sin uso [control (A)] y con uso de ácido fusárico: 0,1 mM (B) y 0,5 mM (C), ct, córtex ; ep, epidermis; ev, elementos de conducción; tr, traqueida.



to those which affect the energetic metabolism in root cell mitochondria of the study plant species (Telles-Pupulin et al., 1996). Changes caused by fusaric acid are supported by the alterations that the toxin produces in the plant cell biochemistry, since root development depends on ATP supply (Tamari & Kaji, 1953; Gaumann, 1958; Arias, 1985).

Fig. 6. Longitudinal sections in the elongation region of primary roots of *Zea mays* seedlings cultivated in presence of fusaric acid: 1.0 mM (A); 2.0 mM (B) and 5.0 mM (C). ct, cortex; ev, vessel element; tr, tracheid; rl, secondary root.
Fig. 6. Cortes longitudinales de la región de crecimiento de raíces primarias de plántulas de *Zea mays*, cultivadas con uso de ácido fusárico: 1,0 mM (A); 2,0 mM (B) y 5,0 mM (C). ct, córtex; ev, elementos de conducción; tr, traqueida; rl, raíz secundaria.

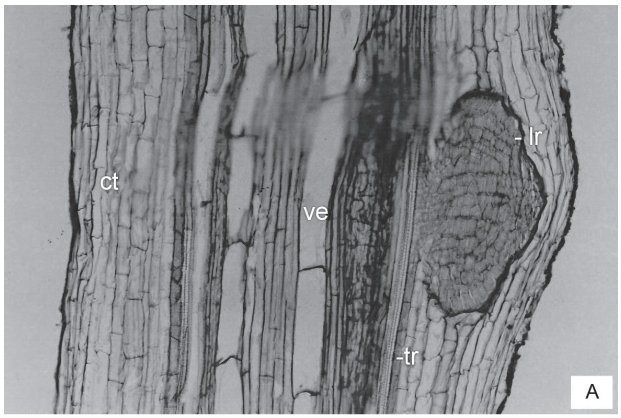
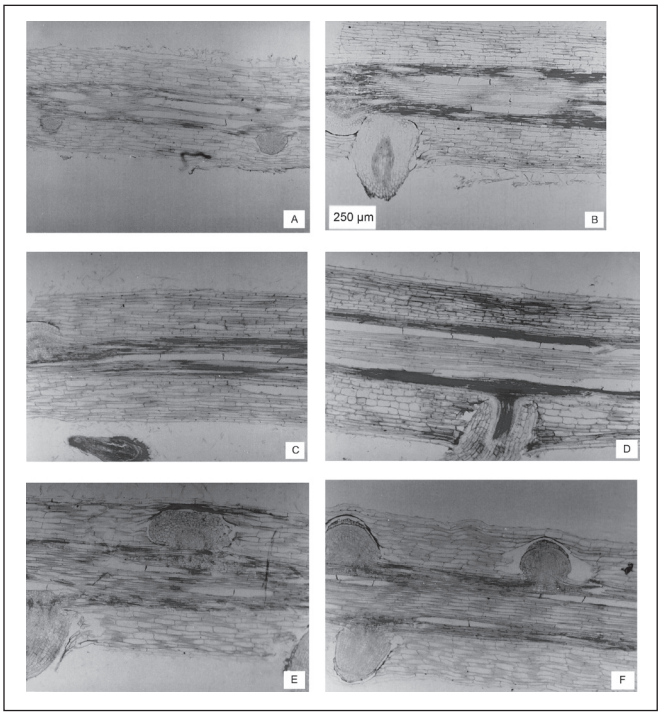


Fig. 7. Longitudinal sections in the ramification region of primary roots of *Zea mays* seedlings cultivated in presence of fusaric acid: 0.1 mM (A); 0.2 mM (B); 0.5 mM (C); 1.0 mM (D); 2.0 mM (E); 5.0 mM (F).
Fig. 7. Cortes longitudinales de la región de ramificación de raíces primarias de plántulas de *Zea mays* cultivadas con aplicación de ácido fusárico: 0,1 mM (A); 0,2 mM (B); 0,5 mM (C); 1,0 mM (D); 2,0 mM (E); 5,0 mM (F).



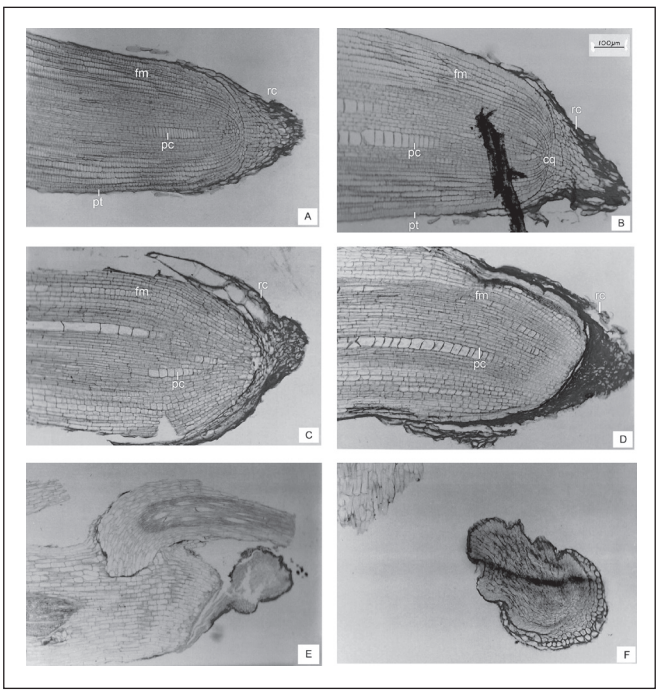
Results similar to those found in this study were observed by Rodella (1991) in *Sorghum bicolor* (L.) Moench seedlings, on the 10th day after progressive doses of 2,4-D, atrazine and alachlor herbicide were applied. The worst effect on seedlings was caused by the last herbicide. Therefore, fusaric acid leads to uncoupling of the oxidative phosphorylation, involving the ATP synthesis (Arias, 1985). Trifluralin caused a decrease in the meristem tissue region of developing cotton roots (*Gossypium hirsutum* L.).

Figures 2 to 4 in transversal cut have shown that there was no difference in the cell number of the cortex at increasing concentrations of fusaric acid. However, there was an increase in the cortex cell diameter. Similar results occurred with the vascular cylinder cells (central), and specifically with the parenchymatic cells. There was a gradual increase of vascular elements as a result of relative increases of fitotoxin concentrations. At the same time, a gradual lignification of vase elements stood out in the perimedular region.

When making a longitudinal cut in the root extension region (Fig. 5), we notice a decrease in vase element cell length and in length of the cortical parenchymal cells at increasing concentrations of fusaric acid.

As shown in figure 6, longitudinal cuts of the root extension region revealed a reverse effect at 1.0 mM than at lower concentrations of fusaric acid.

Fig. 8. Longitudinal sections on the tip of primary roots of *Zea mays* seedlings cultivated in absence [control (A)] and presence of fusaric acid: 0.1 mM (B); 0.5 mM (C); 1.0 mM (D); 2.0 mM (E); 5.0 mM (F). cf, root cap; cq, quiescent centre; mf, fundamental meristem; pc, procambium; pt, protoderm.
Fig. 8. Cortes longitudinales de la región apical –region meristemática de raíces primarias de plántulas de *Zea mays* cultivadas sin uso [control (A)] y con uso de ácido fusárico: 0,1 mM (B); 1,0 mM (D); 2,0 mM (E); 5,0 mM (F). cf, punta de la raíz, Cq, centro quiescente; mf, meristema fundamental; pc, procambio; pt, protoderma.



The branching region in longitudinal cut (Fig. 7) shows an increase in the number of lateral roots, suggesting an acceleration of the root aging process as a result of the increase in fusaric acid concentrations. Cells at 2.0 and 5.0 mM fusaric acid concentrations also appeared more extended.

A longitudinal cut of the apex or meristematic region (Fig. 8) showed a progressive decrease of cellular division at 1.0 mM fusaric acid concentration. At 2.0 and 5.0 mM fusaric acid, the apex region was completely destroyed.

Fusaric acid synthesized by pathogens inside the host tissue can provide the same injury symptoms than *in vitro*. Production of fusaric acid inside the host tissue was investigated with *Fusarium vasinfectum* in tomatoes (Gaumann, 1958) and in cotton, and with *Fusarium oxysporum* in lettuce (Matheron & Koike, 2003).

Alterations of fusaric acid in the range of milimoles are apparently higher than those normally found in biological systems. This may be explained by the different distribution rate and accumulation of fusaric acid in some parts of the plant, and by the plant's own sensitivity to the toxin. In addition, sensitivity to fusaric acid varies in different affected tissues. Different sensitivity in different tissues can be partly attributed to cell's vitality, which depends on energy metabolism and on tissue water absorption (Gaumann, 1958; Arias, 1985; Telles-Pupulin et al., 1996).

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