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Interference of three herbicides on iron acquisition in maize plants

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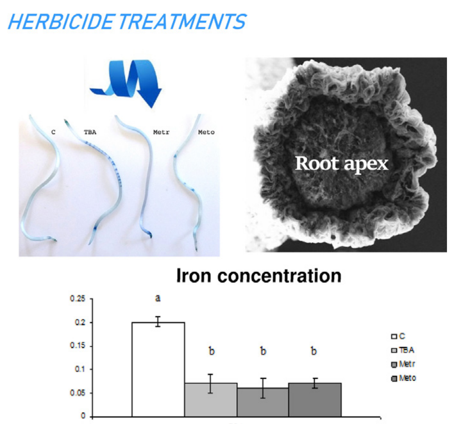
HIGHLIGHTS

- Herbicides can be hazardous to the environment and non-target organisms.
- Terbuthylazine, metribuzin and metolachlor reduced Fe content in maize roots.
- Herbicides damaged root extremities as evidenced by Evans Blue Test.
- Phytosiderophores rate release was decreases by herbicides' treatments.
- SEM-EDX showed as treatments reduced Fe, Cu, Zn and Mn contents in root apices.

GRAPHICAL ABSTRACT



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ABSTRACT

The use of herbicides to control weed species could lead to environmental threats due to their persistence and accumulation in the ecosystems and cultivated fields. Nonetheless, the effect of these compounds on plant mineral nutrition in crops has been barely investigated. This study aimed at ascertaining the effect of three herbicides (*S*-metolachlor, metribuzin and terbuthylazine) on the capacity of maize to acquire iron (Fe). Interferences on plant growth and reductions on the Fe contents were found in the plants treated. Furthermore, root cell viability and functionality losses were ascertained following the treatments, which, in turn, decreased the amount of phytosiderophores (PSs) released by the roots. An investigation carried out in greater depth on root apices of treated plants using an FE-SEM (Scanning Electron Microscope) coupled with EDX (Energy Dispersive X-ray) indicated that the reductions on Fe content started in this part of the roots. Lastly, decreases were found also in copper (Cu^{+2}), zinc (Zn^{+2}) and manganese (Mn^{+2}) content in root apices.

1. Introduction

Modern agriculture needs high crop productivity in order to cope with the increasing food demand, and this in order to meet the necessities of the growing world population (Del Buono et al., 2016). In cultivated fields, weeds compete with crops for

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nutrients and other resources (Gaba et al., 2017). In order to manage this situation, herbicides are used worldwide for crop protection against weeds (Mimmo et al., 2015). However, the massive use of these chemicals can cause resistance in weeds and affect the environment (Gaba et al., 2017). In particular, they can impact on non-target organisms and bring about hazards to human and animal health (Boily et al., 2013; van der Meulen and Singh Chauhan, 2017). Some of these chemicals can also accumulate and persist in soils (Arias-Estévez et al., 2008; Magne et al., 2006), and thus could represent a risk for crops in the following cultural cycle (Del Buono et al., 2015).

Despite their selectivity in targeting weeds, some herbicides can be phytotoxic to crop species at morphological, physiological and biological levels (Vercampt et al., 2017). Recently, some authors found significant interferences of herbicides on plant mineral nutrition. For example, glyphosate was shown to be able to reduce the uptake of certain micronutrients in soybeans (Zobiole et al., 2010) and sunflower (Eker et al., 2006). In particular, some of these studies showed reductions on iron (Fe) assimilation and distribution in various crop species (Bartucca et al., 2017; Bellaloui et al., 2009; Del Buono et al., 2015; Eker et al., 2006; Ozturk et al., 2008). In general, interferences on the capacity of plants to acquire Fe can result in Fe-chlorosis. This disorder is characterised by interveinal to complete yellowing and browning of leaves which could, in extreme situations, lead to plant death (Singh and Dayal, 1992). Fe has a fundamental role in photosynthesis, DNA and chlorophyll biosynthesis, mitochondrial respiration and nitrogen fixation (Nagajyoti et al., 2010). This element is also a key constituent of many important proteins such as ferredoxin, catalase, peroxidase and superoxide dismutase (Nagajyoti et al., 2010). Therefore Fe is a limiting factor for plant biomass production and quality (Briat et al., 2015).

Fe is acquired by plants through root-uptake mechanisms which take place in the rhizosphere. For instance, in graminaceous plants (*Strategy II* plants), Fe uptake is based on the biosynthesis of Fe^{3+} chelating compounds, called phytosiderophores (PSs) (Hell and Stephan, 2003). These compounds, which belong to the mugineic acids (MAs) family, are released by roots directly into the rhizosphere, where they form chelates with the nutrient (Colombo et al., 2014). The chelates are then imported into roots by specific transporters (Ma et al., 1995). In graminaceous plants, PS biosynthesis and secretion into the rhizosphere is markedly increased in Fe-deficiency conditions, (Nagasaka et al., 2009). The release of PSs is also reported to be enhanced by Cu^{+2}, Zn^{+2} and Mn^{+2} deficiency (Römheld, 1991). It has been hypothesized that PSs are involved in the acquisition of the abovementioned cations with which they form stable chelates (Römheld, 1991).

In this study the effect of three herbicides on the capacity of maize (*Zea mays*, L. a *Strategy II* plant) to acquire Fe was investigated. The experiments were carried out with plants grown in Fe-sufficiency conditions. The herbicides selected were terbuthylazine (TBA), metribuzin (Metr) and *S*-metolachlor (Meto). They are applied to maize fields all over the world to control a wide range of annual and perennial grasses and broadleaf weeds. They were selected for this study also for their long persistence in the environment, which can result in their accumulation in the soil. The excessive or inappropriate use of these chemicals has in fact resulted in their frequent detection in the environment at concentrations above the European limits (Del Buono et al., 2016; Jaikaew et al., 2015; Lopez-Piñero et al., 2014). TBA and Metr, which belong respectively to the *s*-triazine and triazinone families of herbicides, act by interrupting the photosynthetic electron transport chain at the level of photosystem II (Godinho de Almeida et al., 2017; Mimmo et al., 2015). Meto, a member of the chloroacetanilide chemical family, inhibits plant growth by disturbing long-chain fatty acid biosynthesis (Copin et al., 2016).

Therefore, in order to reach the aim proposed, maize plants were treated with TBA, Metr and Meto, and plant biomass, chlorophyll concentration, root health status and functionality, Fe accumulation in roots and shoots and PS secretion in roots were assessed. As stated in the literature, the apex is the most active part of the root in nutrient absorption, as well as in the excretion of exudates (Uren and Reisenauer, 1988). For this reason Fe was quantified on the sub-apical cross sections of maize roots using a Scanning Electron Microscope (SEM) equipped with EDX (Energy Dispersive X-rays). Cu, Zn and Mn were also quantified in treated samples. To our knowledge, this is the first study aimed at investigating the effect of these herbicides on the Fe acquisition process in maize plants.

2. Materials and methods

2.1. Plant material and growth conditions

Maize (*Zea mays* L.) seeds were soaked in ultrapure water and left in continuous agitation for 8 h. Then they were placed in Petri dishes, with ultrapure water added, and kept in the dark. After 4 days, the seedlings were transferred into a growth chamber (12/12 h of light/dark, 23/19 °C) and placed in tanks containing a continuously aerated hydroponic solution composed as follows: 2 mM $Ca(NO_3)_2$ $4H_2O$, 0.5 mM $MgSO_4$ $7H_2O$, 0.7 mM K_2SO_4, 0.1 mM KCl, 0.1 mM KH_2PO_4, 1 μM H_3BO_3, 0.5 μM $MnSO_4$ H_2O, 0.5 μM $CuSO_4$, 0.5 μM $ZnSO_4$ $7H_2O$, 0.01 μM $(NH_4)_6Mo_7O_{24}$ $4H_2O$ and 100 μM Fe-EDTA. Two weeks after sowing, plants were treated with 2.0 mg L^{-1} of TBA or Metr or Meto (Sigma Aldrich, St. Louis, MO, USA), while other samples were untreated and left as controls. The applied concentration of the herbicides (2.0 mg L^{-1}) was chosen to reach high field rates (usually employed to manage problematic weeds), and/or simulate situations of herbicide accumulation in soils. Plants were collected at 24, 48 and 72 h after the treatment, and length and weight of shoots and roots were assessed. SPAD index (SPAD-502 Plus, Konica Minolta, Japan) was measured to estimate the chlorophyll concentration in leaves. SPAD measurements were taken for the third leaf of each plant, 5–10 cm from the bottom, midway between the midrib and the leaf margin.

2.2. Determination of shoot and root Fe concentration

Plants were harvested at 24, 48 and 72 h after the treatments. Roots were separated from shoots and washed with water. Both the roots and shoots were then oven dried at 60 °C and microwave digested with 8.0 mL nitric acid (65% v/v, Carlo Erba) and 2.0 mL hydrogen peroxide (30% v/v) using a microwave system (ETHOS One, High-Performance Microwave Digestion System, Milestone Inc, Sorisole, Bergamo, Italy). Iron concentration was determined by Atomic Absorption Spectroscopy (AA-680 Series, Shimadzu, Kyoto, Japan).

2.3. Evans Blue Test

Cell death in roots of controls and herbicide-treated samples was evaluated using the Evans Blue Test. 72 h after the treatments, collected roots were washed with water for 10 min, separated from leaves and placed into Petri dishes; roots were then stained for 1 min with 0.5% (w/v) Evans blue (Sigma, USA) at room temperature and under stirring. These samples were then washed several times with ultrapure water until the solution became colourless. Images were captured using a digital camera.