DOI:10.1021/jf1031263

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Effect of *Fusarium oxysporum* f. sp. *lycopersici* on the Soil-to-Root Translocation of Heavy Metals in Tomato Plants Susceptible and Resistant to the Fungus

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The purpose of this work was to gain an insight on the potential role of the phytopathogenic fungus *Fusarium oxysporum* f. sp. *lycopersici* in the translocation of metals and metalloids from soil to plant roots in tomato (*Lycopersicum esculentum*). Two varieties of tomato (one susceptible and another resistant to infection by *Fusarium oxysporum* f. sp. *lycopersici*) were challenged with the fungus for different periods of time, and several elements (V, Cr, Mn, Co, Cu, Zn, As, Se, Mo, Ag, Cd, Pb) were determined in roots and in soil substrate. Additionally, phenolic plant products were also analyzed for the evaluation of the plant response to biotic stress. In order to obtain representative results for plants cultivated in noncontaminated environments, the infected and control plants were grown in commercial soil with natural, relatively low metal concentrations, partly associated with humic substances. Using such an experimental design, a specific role of the fungus could be observed, while possible effects of plant exposure to elevated concentrations of heavy metals were avoided. In the infected plants of two varieties, the root concentrations of several metals/metalloids were increased compared to control plants; however, the results obtained for elements and for phenolic compounds were significantly different in the two plant varieties. It is proposed that both *Lycopersicum esculentum* colonization by *Fusarium oxysporum* f. sp. *lycopersici* and the increase of metal bioavailability due to fungus-assisted solubilization of soil humic substances contribute to element traffic from soil to roots in tomato plant.

KEYWORDS: ***Lycopersicum esculentum*; plant − pathogen interaction; trace elements**

INTRODUCTION

Actual status of metals/metalloids in plants is of interest in various research areas related to human nutrition and possible health hazards and also in the context of phytoremediation (*1*). It is well-established that the soil−plant traffic of chemical elements depends on plant genotype, total element concentrations and their physicochemical forms in soil, and several soil parameters such as organic matter composition, pH conditions, and microbial activity (*2*−*5*). In particular, the uptake of heavy metals by tomato plants has been studied in different contaminated environments, or while using certain soil fertilizers (*6*−*9*). The molecular mechanisms underlying element transport, their potential toxicity and defensive plant response have also been approached (*2*, *10*, *11*). It should be stressed, however, that the great majority of studies were performed in the presence of elevated metal concentrations. It is also relevant that tomato plant was reported as a relatively salt tolerant and heavy metal resistant crop (*10*, *12*, *13*).

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With regard to microbial activity, several studies pursued the role of rhizobacteria and mycorrhizal fungi in different soil−crop systems (*14*−*16*), yet the relationship between colonization by pathogens and metal status in plants has rarely been considered (*17*).

F. oxysporum is a common fungal species occurring in soil environments. Several formae speciales are known as pathogens of economically important crops (*18*). In particular, *F. oxysporum* f. sp. *lycopersici* causes a tomato plant disease called *Fusarium* wilt (*19*). The mycelium invades the plant through the roots, either by direct incursion of penetration hyphae or via wounds and cracks formed at the emerging lateral roots (*20*). During 10 to 14 days after infection, the fungus penetrates the plant through the cortex until it reaches the vascular tissue, and then it uses xylem vessels to colonize the entire plant. Typical wilt symptoms occur later, as a result of aggressive fungal growth and destruction of the infected vessels (*21*). The *F. oxysporum* colonization mechanisms as well as the plant response have been the focus of intensive research (*22*−*25*); however, to the best of our knowledge, possible effect of fungus on heavy metals translocation from soil to the infected plants has not been approached.

 Published on Web 11/05/2010

In addition to plant colonization, soil fungi are known as major decomposer organisms, and they play an important role in biogeochemical cycles of elements (*26*−*28*). In this regard, we have recently demonstrated that *F. oxysporum* f. sp. *lycopersici* presents the capability for degradation of humic substances with subsequent solubilization of metal ions associated with this soil fraction, and it was postulated that the *F. oxysporum* activity in soil might increase the actual bioaccessibility of metals to plants (*29*).

The purpose of the present study was to evaluate the effect of *F. oxysporum* f. sp. *lycopersici* on the metal/metalloid status in tomato roots. In order to examine whether plant colonization and/or increased metal bioavailability would contribute to element translocation, the susceptible and resistant varieties of *L. esculentum* were challenged with the fungus. The infected and control plants were grown in commercial soil containing natural, relatively low metal concentration levels; this was considered as an important point, since the results obtained were representative for noncontaminated environments, preferentially selected for tomato plantations. It should be stressed that such an experimental design enabled observing the specific role of the fungus, while possible effects of plant exposure to elevated concentrations of heavy metals were avoided. At different postinfection time periods, several elements were determined in the infected and in the control plants. Additionally, phenolic plant products were also analyzed for evaluation of plant response to the stress imposed by the fungus. This study provides complementary data useful for understanding the interaction of pathogen with the host tomato plant that might potentially affect nutritional value of its edible parts.

MATERIALS AND METHODS

Microorganism and *L. esculentum* Varieties. *F. oxysporum* f. sp. *lycopersici* strain 4287 (race 2) was generously provided by Professor M. I. G. Roncero (Departamento de Genética, Universidad de Córdoba, España) and stored as a microconidial suspension in 30% glycerol at −80 °C. Tomato seeds (*L. esculentum*, cv. Monika, Novartis) susceptible to infection by the strain 4287 race 2 were obtained from Instituto Nacional de Semillas y Plantas de Vivero (Madrid, España) and provided by Professor M. I. G. Roncero. The seeds of Yaki tomato plant variety, resistant to *F. oxysporum* f. sp. *lycopersici* strain 4287 (race 2), were a kind gift from Professor M. Acosta (Departamento de Parasitología Agrícola, Universidad Autónoma de Chapingo, Mexico).

Infection of Tomato Plant with *F. oxysporum*. Tomato seeds were sterilized by soaking in 1.4% sodium hypochlorite solution for 30 min with agitation and, then, rinsing with a sterilized deionized water. The seedlings were obtained as described elsewhere (*30*). At the first true leaf stage (10 days), 30 seedlings of each variety were removed from the substrate and the roots washed with sterilized deionized water and then soaked in *F. oxysporum* f. sp. *lycopersici* strain 4287 conidial suspension (5×10^6 microconidia mL^{-1}) for 30 min. An additional 30 seedlings of each variety (Yaki and Monika) were used as controls; their roots were submerged in sterilized deionized water for 30 min. The infected and control seedlings were planted in sterilized commercial soil (Sushine Premium Potting Soil, Sun Gro Horticulture, Inc.) and grown in a greenhouse (20−25 °C, 70 ± 5% humidity, being watered each three days with tap water (pH 7.33 ± 0.04). Trace elements in tap water were screened by ICP-MS, showing low concentration levels: Cr, Co, Se, Ag, Cd not detected; 0.91 ± 0.05 $\mu g\ L^{-1}$ V; 4.2 ± 0.1 $\mu g\ L^{-1}$ Cu; 0.87 ± 0.03 $\mu g\ L^{-1}$ Pb; 0.29 ± 0.05 $\mu g\ L^{-1}$ As; 0.90 ± 0.04 $\mu g\ L^{-1}$ Mo; 3.2 ± 0.1 $\mu g\ L^{-1}$ Mn; 63.2 ± 0.5 $\mu g\ L^{-1}$ Zn. At different time periods after inoculation, severity of disease symptoms was recorded, using an index 1 (healthy plant) to 5 (dead plant) as described elsewhere (*21*, *30*). For chemical analyses, 15 plants of each group were harvested at 14 days after inoculation and the other 15 plants were analyzed 21 days postinfection. The roots were separated from aerial parts manually, by cutting each plant with a plastic knife at a site corresponding to the soil−air boundary, then they were washed with deionized water and with 0.05 mol L^{-1} $CaCl_2$ (ultrasonic bath, 10 min) and then pooled separately for the infected and controls of this same plant variety.

ICP-MS Determination of Total Metal Concentrations in Tomato Plants and in Commercial Soil. A portion of each pooled root samples was dried, and wet digestion with concentrated nitric acid and hydrogen peroxide was performed as described elsewhere (*2*). The extraction of metals from the commercial soil samples was carried out using this same wet digestion procedure (*2*).

A model 7500ce inductively coupled plasma mass spectrometer (Agilent Technologies, Tokyo, Japan) with a Meinhard nebulizer and Peltier-cooled spray chamber (2 °C) was used. For total element determination the instrumental operating conditions were as follows: forward power 1500 W, plasma gas flow rate 15 L min^{-1}, carrier gas flow rate 0.89 L min^{-1}, makeup gas flow rate 0.15 L min^{-1}, sampling depth 10 mm, nickel sampling and skimmer cones, dwell time 100 ms per isotope, collision/reaction cell gas He, 4 mL min^{-1}. The isotopes ^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{65}Cu, ^{68}Zn, ^{75}As, ^{82}Se, ^{95}Mo, ^{107}Ag, ^{111}Cd, ^{208}Pb were monitored (^{115}In as internal standard, IS).

External calibration was performed using multielement standard solution (0.1% nitric acid (v/v)) at element concentration levels 0, 0.2, 0.4, 1.0, 2.0, 5.0 and 10 $\mu g\ L^{-1}$ with addition of the internal standard (5.0 $\mu g\ L^{-1}$ In). The analytical accuracy was demonstrated by analyzing two certified reference materials NIST 1643d and NIST 1572 (the results obtained are presented in Table 1S in the Supporting Information). All analyses were run in triplicate.

Extraction of Phenolic Compounds. The procedure reported elsewhere was adopted (*31*). In brief, an aliquot of the pooled roots from each group (Monika, Yaki infected, and Monika, Yaki controls) was ground in liquid nitrogen and freeze-dried. To each sample (30−40 mg), 20 ng of *o*-anisic acid was added as internal standard (*32*), and the mixture was homogenized (polytron) with 1.5 mL of 70% (v/v) methanol and centrifuged at 10000*g* for 10 min. The pellet was resuspended in 90% (v/v) methanol, vortexed for 1 min, and centrifuged. The two supernatants were combined and evaporated at 40 °C in the nitrogen stream. Then, 200 μL of trichloroacetic acid 5% (m/v) was added and the free phenolic fraction was extracted with two 500 μL portions of ethyl acetate:cyclohexane (1:1). The organic phases were combined and evaporated to dryness. For glycoside bound soluble fraction, the aqueous phase remaining after acetate:cyclohexane extraction was acidified with 12 mol L^{-1} HCl (final HCL concentration 4 mol L^{-1}) and incubated for 1 h at 80 °C. After hydrolysis, the reaction mixture was extracted two times with 500 μL of ethyl acetate: cyclohexane (1:1) and the combined extract was evaporated to dryness. Similarly as for the free fraction, 20 ng of *o*-anisic acid was added as IS. The fraction of phenolics bound to cell walls was extracted from the pellet: 4 mol L^{-1} sodium hydroxide was added, and the mixture was incubated at 96 °C for 1 h to release the ester bound compounds. Then, the sample was acidified to pH 2 with HCl and centrifuged at 10000*g* for 10 min, the supernatant was extracted two times with 500 μL of ethyl acetate:cyclohexane (1:1) and the combined extract was evaporated to dryness. For cell wall glycoside bound fraction, the second pellet was hydrolyzed with 12 mol L^{-1} HCl, the released phenolics were extracted to ethyl acetate: cyclohexane (1:1) and the extracts were evaporated to dryness.

HPLC Analysis of Phenolics. Each plant extract was redissolved in 200 μL of the initial mobile phase (H_2O:ACN:MeOH: 30 mmol L^{-1} phosphate buffer, pH 2.5 (5:4:1)), and 40 μL was introduced to the HPLC system. An Agilent series 1200 liquid chromatograph equipped with an autosampler, a diode array detector, a spectrofluorimetric detector and Chemstation (Agilent Technologies, Santa Clara, CA, USA) was used. The chromatographic column was Luna C18(2) (250 × 4.6 mm, 5 μm) with an extended guard column, both from Phenomenex. Gradient elution with three mobile phases (A, deionized water; B, acetonitrile:methanol (1:1); C, 30 mmol L^{-1} phosphate buffer pH 2.5) was as follows: 0 min 20% B, 10% C; 0−2.0 min 40% B, 10% C; 2.0−14.2 min 80% B, 10% C; 14.2−14.5 min 20% B, 10% C . The column temperature was kept at 35 °C, and the total flow rate was 1.0 mL min^{-1}. For DAD detection, the analytical wavelengths were 280, 254 and 300 nm (reference 380 nm), and for FLD detection, excitation was at 305 nm and fluorescence was measured at 365, 407 and 436 nm. Phenolics were identified by their retention times and UV−vis spectra, using the following standards: salicylic, vanillic, caffeic, benzoic, 2,3-dihydroxybenzoic, 2,5-dihydroxybenzoic,

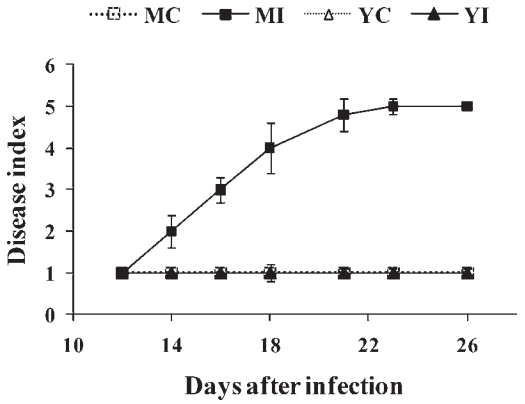

Figure 1. Wilt disease index in tomato plants susceptible and resistant to *F. oxysporum* f. sp. *lycopersici*. Disease severity was scored at different days after infection, using an index from 1 to 5: 1, healthy plant; 2, one wilted leaf; 3, two wilted leaves; 4, three or more wilted leaves; 5, dead plant (MC, Monica control; MI, Monika infected; YC, Yaki control; YI, Yaki infected.) The mean values obtained for 15 plants are presented with the respective standard deviation.

p-coumaric, *trans-o*-hydroxycinnamic, ferulic, cinnamic and *p*-hydroxybenzoic acids.

Statistical Analysis. Data were tested for statistical significance using the unpaired *t* test included in Microsoft Excel 2007. The significance was established at $p < 0.05$.

RESULTS AND DISCUSSION

The goal of this study was to evaluate possible role of *F. oxysporum* f. sp. *lycopersici* in the soil-to-root translocation of metals/metalloids. The important interrogation was whether the plant colonization by fungus, the potential increase of metal bioavailability due to fungus-assisted solubilization of humic substances, or both processes would contribute to actual element concentrations in plant roots. In the first approach, the substrate used for the plant growth was characterized. Based on three independent determinations of elements in soil, the mean concentrations with respective values of standard deviations were as follows: 7.38 ± 0.91 $\mu g\ g^{-1}$ vanadium, 7.24 ± 1.15 $\mu g\ g^{-1}$ chromium, 55.5 ± 1.7 $\mu g\ g^{-1}$ manganese, 3.14 ± 0.10 $\mu g\ g^{-1}$ cobalt, 3.55 ± 0.06 $\mu g\ g^{-1}$ copper, 11.0 ± 0.3 $\mu g\ g^{-1}$ zinc, 0.45 ± 0.15 $\mu g\ g^{-1}$ arsenic, 0.30 ± 0.04 $\mu g\ g^{-1}$ selenium, 0.16 ± 0.02 $\mu g\ g^{-1}$ molybdenum, 0.05 ± 0.02 $\mu g\ g^{-1}$ silver, 0.07 ± 0.01 $\mu g\ g^{-1}$ cadmium, 1.45 ± 0.03 $\mu g\ g^{-1}$ lead. The above values fall within the ranges reported as background levels in different geographical regions (*33*−*35*), thus assuring that the soil conditions were representative for noncontaminated environments. The soil pH was 6.8 ± 0.1, and it contained 324 ± 15 mg g^{-1} of humic substances (HS), as assayed by UV/vis spectrophotometry (*36*). According to the previous reports (*2*, *29*), a fraction of total soil element content was associated with humic substances. Relatively low levels of metal/metalloids, the presence of humic substances and partial association of metals with the soil fractions (*2*, *29*) indicated the usefulness of the commercial soil for the purpose of the study.

Plant Response to the Stress Imposed by *F. oxysporum* f. sp. *lycopersici*. In order to distinguish between effects related to plant colonization and those potentially caused by fungus-assisted increase of metal/metalloid bioavailability, susceptible (Monika) and resistant (Yaki) varieties of tomato plant were used. The disease index for the two types of plant challenged with the fungus as well as for the respective control plants was evaluated (*21*, *30*), and the results obtained are shown in **Figure 1** (several plant photos taken at different times after the infection are presented in the Supporting Information, Figure 1S). The disease symptoms were observed only in the Monika plants exposed to the fungus. In agreement with earlier reports, the first wilted leaves in the infected Monika plant appeared around 14 days after infection, indicating the collapse of infected vessels and clogging due to mycelium and host-derived production of gums and tyloses (*21*). Three weeks postinfection, the infected Monika plants were dead, while no disease symptoms were observed in the other plant groups. Furthermore, control Monika, control Yaki and infected Yaki plants presented similar growth during three weeks, considering the number of leaves, their size and coloration (Supporting Information, Figure 1S). The results obtained confirm that only susceptible Monika variety was colonized by the fungus.

Phenolic plant products are important in the pathogenic interactions between plants and fungi (*24*, *31*, *37*, *38*). These compounds are distributed between their free forms and a fraction bound as esters and glycosides. It is generally accepted that binding to cell-wall materials implies the increased resistance to fungal invasion and contributes to the formation of physical barrier against fungal penetration. To get an insight on the response of Monika and Yaki plants to *F. oxysporum* infection, free and bound phenolic compounds were extracted from roots at 14 days postinfection and analyzed by reversed phase liquid chromatography.

Three phenolic fluorescent compounds were detected in methanol root extracts, namely, salicylic, 2,5-dihydroxybenzoic and 2,3-dihydroxybenzoic acids (typical chromatograms of standards and free and glycoside bound phenolics in methanolic root extracts are shown in the Supporting Information, Figure 2S). In **Figure 2a**, quantitative results obtained for salicylic acid are presented. As can be observed, the concentration of free compound was increased in the infected compared to the control tomato plants ($p < 0.01$), indicating the activated plant defense response both in the susceptible and in the resistant varieties (*38*, *39*). Apparently, the fractions of bound salicylic acid in Yaki and in Monika were not affected by the fungus ($p > 0.05$). For 2,3-dihydroxybenzoic and 2,5-dihydroxybenzoic acids (oxidation products of salicylic acid), relatively lower concentration levels of free compounds were found as compared to salicylic acid with no significant differences between the infected and control plants ($p > 0.05$, **Figure 2b** and **2c**). On the contrary, the bound fractions of these two acids were significantly increased in both plant varieties challenged with fungus ($p < 0.01$, **Figure 2b** and **2c**). It should also be noted that the concentration levels of bound fractions of salicylic, 2,3-dihydroxybenzoic and 2,5-dihydroxybenzoic acids in the Yaki extracts were always higher than in Monika ($p < 0.01$), confirming that salicylic acid pathway is involved in the resistance of Yaki variety to the fungus (*38*).

The overall elution profiles obtained for root hydrolysates (alkaline and acid hydrolysis) indicate drastic changes in the concentration levels of cell wall bound phenolics in Monika variety after infection. For fungus resistant Yaki variety, the plant extracts contained generally higher levels of these compounds and less marked changes between plants grown in the presence and in the absence of *F. oxysporum* were observed (typical chromatograms shown in Figure 3S in the Supporting Information). The quantitative results obtained for glycoside bound, ester bound and total bound ferulic acid are presented in **Figure 3a**. For glycoside bound acid, no important differences were observed between infected and control Yaki and Monika. On the other hand, the ester bound and total bound fractions were significantly decreased in the infected Monika plants with respect to the controls ($p < 0.01$). Furthermore, higher levels of bound ferulic acid were observed for the fungus resistant variety ($p < 0.01$), and the difference between infected and control plants

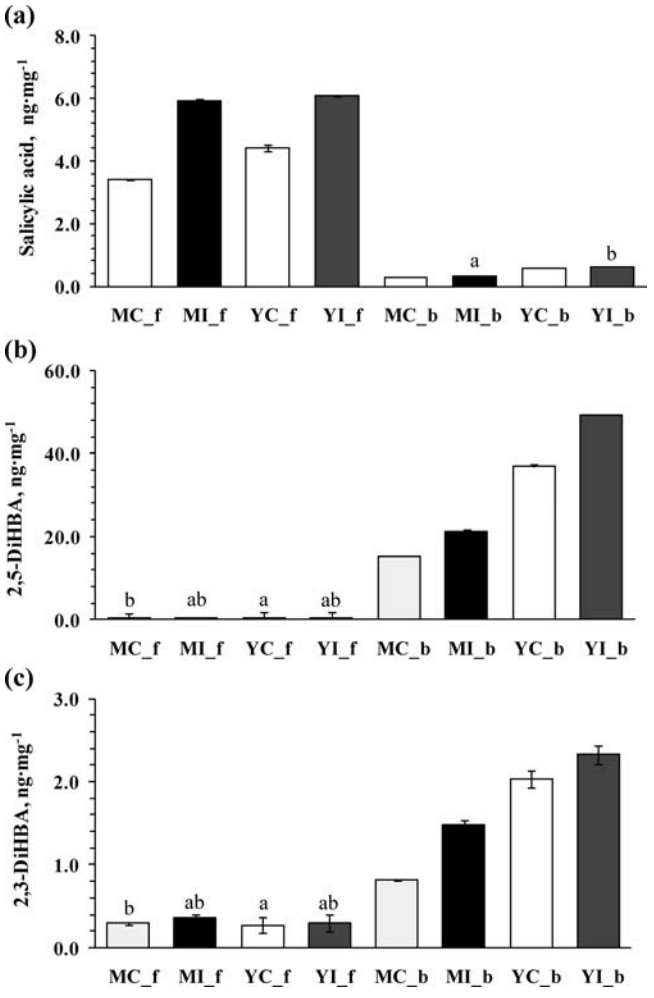

Figure 2. Concentration levels of free and glycoside bound salicylic acid (**a**); 2,5-dihydroxybenzoic acid (**b**); 2,3-dihydroxybenzoic acid (**c**) in methanol extracts of tomato roots 14 days postinfection: MI_f, MI_b, free and bound fraction in the infected Monika plants; MC_f, MC_b, control Monika; YI_f, YI_b, infected Yaki; YC_f, YC_b, control Yaki plants, the concentrations given as ng mg^{-1} of freeze-dried root mass. The bars indicate mean values from three independent experiments with respective standard deviation, and the letters are used as follows: a, not statistically different from MC; b, not statistically different from YC.

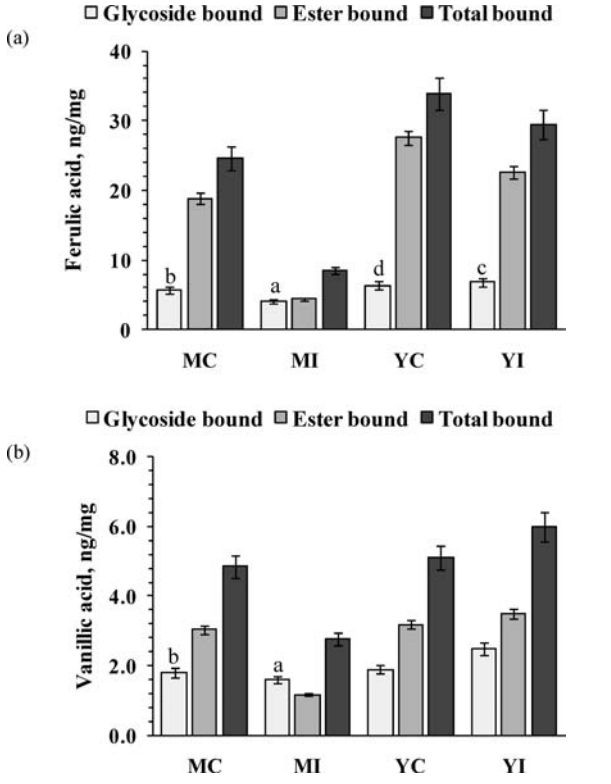

Figure 3. Concentration levels of glycoside bound, ester bound and total bound ferulic acid (**a**) and vanillic acid (**b**) in tomato root hydrolysates 14 days postinfection: MI, MC, Monika infected and control plants; YI, YC, Yaki infected and control plants, the concentrations given as ng mg^{-1} of freeze-dried root mass. The bars show mean values from three independent experiments with respective standard deviation, and the letters are used as follows: a, not statistically different from MC; b, not statistically different from MI; c, not statistically different from YC; d, not statistically different from YI.

was less pronounced, yet still statistically significant ($p < 0.01$). The second compound identified in the root hydrolysates was vanillic acid. As shown in **Figure 3b**, a significant decrease of ester bound and total bound fractions occurred in Monika infected plants with respect to the controls ($p < 0.01$). For Yaki variety, the plant infection caused the increase of glycoside bound, total ($p < 0.01$) and ester bound ($p < 0.05$) vanillic acid.

In summary, the results obtained for plant phenolic compounds reveal the activation of salicylic acid signaling pathway and the deterioration of root cell walls in susceptible Monika plants 14 days postinfection with *F. oxysporum* f. sp. *lycopersici*. On the contrary, no wilt symptoms were observed for the resistant Yaki plants and, apparently, the integrity of cell walls was conserved in the presence of the fungus. Hence, it was considered that the two varieties can be used as a simple model to evaluate the potential role of *F. oxysporum* f. sp. *lycopersici* in the translocation of metals/metalloids from soil to tomato roots. In particular, the time-related evolution of element contents in susceptible plants would provide information relevant to plant colonization by the fungus, while modifications of metal/metalloid profiles in the resistant plants should be ascribed to fungus-assisted changes in their bioavailability.

Concentration Levels of Metals/Metalloids in the Sensitive and Resistant Varieties of Tomato Plant. The results of total element determination in soil substrate and in roots at two weeks postinfection are presented in **Figure 4**. For control Monika plants, the values of bioconcentration factor (BCF) evaluated as metal concentration ratio of plant roots to soil (*40*) were in the range 0.5−1.7 for V, Cr, Co, As, Se, Mo and Ag, which confirms data reported elsewhere (*10*, *12*, *13*). Relatively higher concentrations of Mn, Zn and Cu in roots with respect to soil (BCF 3.4−3.6) should be ascribed to specific uptake of essential nutrients. Even though the absolute concentrations of Cd and Pb in roots were low, for these two elements the most marked differences were observed between soil and root (BCF 7.5 and 4.3 respectively), which suggests the ability of tomato plants for increased uptake of these two common heavy metals even at their low concentration levels in soil, in agreement with some recent reports (*41*, *42*).

The concentration levels found in the infected Monika roots were significantly higher compared to control plants for the majority of elements analyzed (except Se and Ag, **Figure 4**). With regard to the fungus resistant Yaki variety, generally lower element concentrations were found at 14 days both for plants grown in the presence and for plants grown in the absence of *F. oxysporum* compared to Monika (**Figure 4**). In particular, significantly lower concentrations of V, Cd ($p < 0.01$), Pb, As, Se, Mo ($p < 0.05$) were found in control Yaki compared to control Monika plants. Furthermore, the concentrations of Cu, Mn, Se, As ($p < 0.001$) and Pb, Co, V ($p < 0.05$) were lower in Yaki

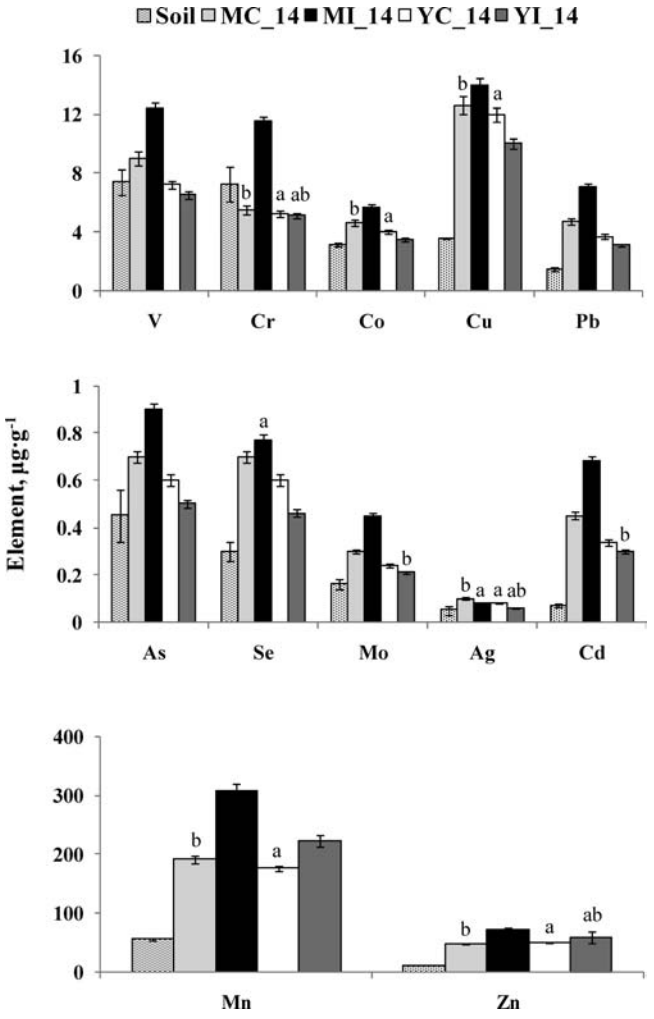

Figure 4. Concentration levels of elements found in soil substrate and in tomato roots 14 days postinfection with *F. oxysporum* f. sp. *lycopersici* and in control plants: MC_14, control Monika 14 days postinfection; MI_14, infected Monika; YC_14, control Yaki; YI_14, infected Yaki, the concentrations given as μg g^{-1} of freeze-dried root mass. The bars show mean values from three independent experiments with respective standard deviation, and the letters are used as follows: a, not statistically different from MC_14; b, not statistically different from YC_14.

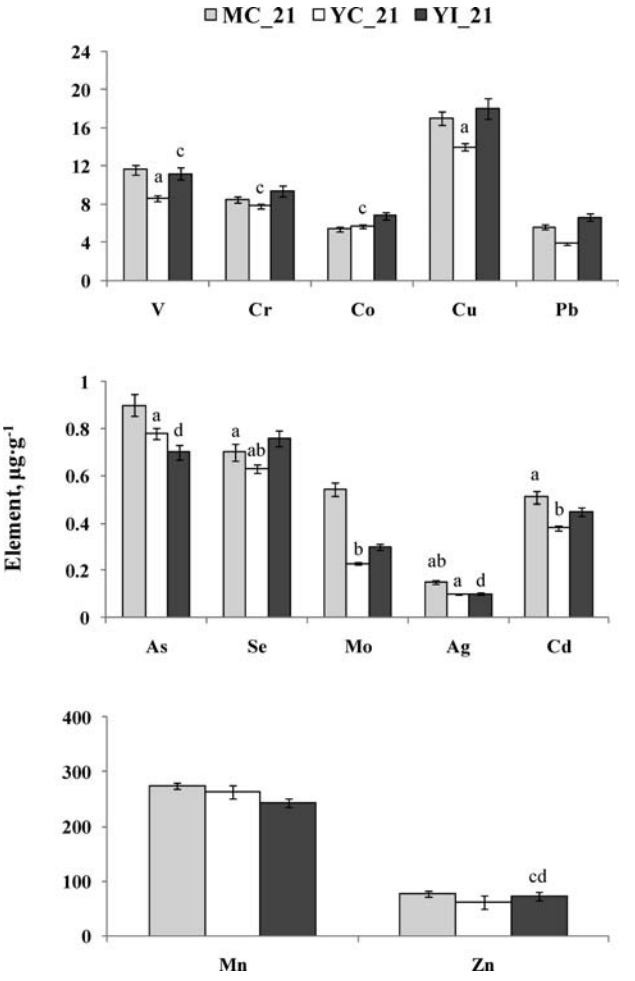

Figure 5. Concentration levels of elements found in tomato roots 21 days postinfection with *F. oxysporum* f. sp. *lycopersici* and in control plants: MC_21, control Monika 21 days postinfection; YC_21, control Yaki; YI_21, infected Yaki, the concentrations given as μg g^{-1} of freeze-dried root mass. The bars indicate mean values from three independent experiments with respective standard deviation, and the letters are used as follows: a, not statistically different from MC_14; b, not statistically different from YC_14; c, not statistically different from MC_21; d, not statistically different from YC_21.

plants that grew in the presence of *F. oxysporum* as referred to the nonexposed plants of this same variety (**Figure 4**). These results, together with information provided by the analysis of phenolic compounds and plant disease index, supply the evidence that the increased concentration levels of elements in the infected susceptible plants were caused by the damage of cell walls and alteration of membrane functions during fungal invasion. On the contrary, the reinforcement of cell walls, intrinsic to the resistance mechanism, was apparently responsible for lower capabilities to metal uptake observed for Yaki variety. A tendency toward lower element concentrations in Yaki plants exposed to *F. oxysporum* versus controls (**Figure 4**) points to the activated defensive response discussed in the first part of this work.

In **Figure 5**, the concentration levels determined in control roots of two varieties and in the exposed Yaki plants at 21 days postinfection are presented. The infected Monika roots were not included, since at this stage the susceptible plants were dead (**Figure 1**). As expected, in the control Monika plants further increase was observed for the majority of elements analyzed (except Se, Ag and Cd, **Figure 5**). More interestingly, in the resistant Yaki variety grown in the presence of *F. oxysporum* the concentrations of V, Co, Cu, Pb ($p < 001$) and Cr, As, Se, Mo, Ag, Cd ($p < 0.05$) were elevated compared to those found in control resistant plants (**Figure 5**). Additionally, several elements presented higher concentrations in the infected Yaki plants after 21 days with respect to those harvested 14 days postinfection ($p < 0.01$ for Cr, Mn, Cu, As, Mo, Cd and $p < 0.05$ and for V, Co, Pb), which cannot be related to the impairment of root integrity by fungus. In the view of our earlier results (*29*), the observed effect should be ascribed to the fungus-mediated degradation of humic substances with subsequent solubilization of elements associated with this soil fraction. The affinity of metal ions to humic substances decreases in the following order: Cu(II) > Ni(II) > Co(II) > Pb(II) > Cd(II) > Cr(III) ≫ Mn(II), Mo(VI), Zn (*36*), and indeed, higher concentration levels of elements associated with humic substances (also V, Se and Ag) were found in Yaki infected plants 21 days postinfection compared to respective controls and plants analyzed 14 days after infection (**Figure 4**, **Figure 5**).

In conclusion, it was demonstrated that the presence of fungus in soil causes the increased concentration levels of several metals/metalloids in tomato roots, however different plant responses to

F. oxysporum challenging and different element profiles were observed in susceptible versus resistant plant varieties. The results obtained in this work and in the previous study (*29*) provide evidence on the two different courses of action by which *F. oxysporum* f. sp. *lycopersici* influences soil-to-root metal traffic in tomato plants. The first effect occurs at relatively short postinfection time and is due to the deterioration of cell walls and membrane functions during plant colonization, while the second effect is delayed and relies on the fungus-assisted degradation of soil humic substances and solubilization of associated heavy metals, which results in the increase of their bioavailability. This later effect might be visualized as an additional plant−microbe interaction mechanism, which might contribute to the deterioration of nutritional value of this important vegetable crop, even if the plant is not directly invaded by the pathogenic fungus. Further studies are needed to evaluate potential effects of *F. oxysporum* f. sp. *lycopersici* on metal/metalloid status in tomato fruits.

Supporting Information Available: Table of data for ICP-MS determination of elements in NIST 1643d and NIST 1572 and figures depicting photos of Yaki and Monika tomato plants at different times after fungus inoculation, RP chromatogram of flourescent standards, and chromatograms of free and glycoside bound fractions of methanol plant extracts, obtained with FLD detection, and of root hydrolysates, obtained with DAD detection. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received for review August 11, 2010. Revised manuscript received October 22, 2010. Accepted October 22, 2010. The financial support from CONACYT (Mexico), Project 49405, is gratefully acknowledged.