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Original scientific paper

The effect of soil type on imazamox phytotoxicity to tomato

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SUMMARY

A bioassay was performed to evaluate the susceptibility of tomato to imazamox residues in loamy and sandy soils. The effects of three different levels of soil moisture (20, 50 and 70% FWC) were also examined. Imazamox was applied at rates ranging from 6.25 to 800 μg a.i./kg soil. Shoot and root fresh weight and root length were the parameters measured 21 days after treatment, as well as the content of water soluble proteins.

Imazamox caused growth delay and lower protein contents in both types of soil at all levels of soil moisture, and the degree of change depended on application rates. Inhibition was higher in plants grown in the sandy soil. The root parameters were more reliable as indicators of plant sensitivity to imazamox in soil. Soluble protein contents were lower in all trial variants but the changes did not depend on herbicide concentrations.

Keywords: Herbicides; Residues; Soils; Phytotoxicity; Tomatoes

INTRODUCTION

Imazamox is a selective herbicide in the group of imidazolinones. Its foliar absorption is fast, and somewhat slower by roots. Its translocation occurs both by the phloem and xylem. It inhibits acetolactate synthase (ALS), i.e. acetohydroxyacid syntase (AHAS) as the first conjugated enzyme in biosynthesis of the amino acids valine, leucine and isoleucine (Stidham, 1991; Stidham & Singh, 1991; Tranel & Wright, 2002). Failing synthesis of these three major amino acids prevents protein synthesis in susceptible plants and impedes translocation of photosynthetic products into meristems. Inhibited transport of photosynthetic products has a major impact on root growth as it fully depends on the energy drawn from the shoot, which makes root growth inhibition a much more sensitive indicator of harmful activity of imidazolinones than thwarted shoot growth (Shaner, 1991). Growth stops several days after treatment, while symptoms of toxic activity become visible after 1-2 weeks. Meristem tissue becomes chlorotic and expands to leaves along with necrosis (Janjić, 2005).

Imazamox is used in Serbia for treatments of broadleaf and grass weeds in soybean, pea, bean and sunflower crops tolerant to imidazolinone (Janjić & Elezović, 2010). It is applied in the post-emergence stage of crop growth that coincides with intensive weed growth, and susceptible weed species that emerge soon after treatment become affected by its extended activity. It is readily soluble in water (116-628 g/l at 25°C) and organic solvents, and weakly volatile (1.33x10^{-5} Pa at 25°C). Its half-life time (DT_{50}) varies from 12 to 207 days in soil at 20°C in the laboratory, and from 4.5 to 41 days in the field (European Commission, 2002), depending on organic carbon content (%) and pH.

Herbicide fate in a soil depends on many environmental factors, soil physicochemical properties first of all, but also on the properties of the herbicide itself. The type and content of clay, soil pH and content of organic matter are factors that significantly affect the adsorption of imidazolinones. Herbicides of that group are amphoteric compounds with acidic and basic functional groups, which is why soil pH has a significant effect on its availability and mobility. In low-pH soils its adsorption is readier (Johnson et al., 2000; Regitano et al., 2005). There is also a very strong positive correlation between the adsorptiveness of imidazolinones and contents of organic matter and clay, and a negative correlation between herbicide mobility and clay content (Undabeytia et al., 2004; Kah et al., 2007). Temperature and soil moisture also have a significant effect on imazamox persistance, so that intensive degradation processes have been detected in situations when temperature increase coincided with high soil moisture (Vischetti et al., 2002; Pannacci et al., 2006).

Imidazolinones belong to a group of herbicides with extended persistance in soil and they are therefore considered a threat to susceptible species grown in crop rotation. Significant levels of carryover damage and yield decrease have been confirmed in sugar beet and oilseed rape crops in studies of the effects of imazamox on crops in rotation (Bresnahan et al., 2002; Pannacci et al., 2006; Süzer & Büyük, 2010). Other authors have also reported wheat, cabbage, tomato, potato, spinach, fennel, green salad and cauliflower susceptibility (O´Sullivan et al., 1998; Deeds et al., 2006; Pannacci et al., 2006).

The present study intended to investigate the susceptibility of tomato plants to different concentrations of imazamox in loamy and sandy soils of variable moisture in laboratory bioassays.

MATERIAL AND METHODS

Imazamox, a technical grade substance of 95% purity, was obtained from BASF Serbia. Tomato seeds (Mondial F1, Enza Zaden) were used in the assay. Loamy and sandy soils were collected from areas without a history of herbicide treatments at the locations Zemun Polje and Tavankut, respectively. The loamy soil was medium calcareous, weakly alkaline and highly humic and with a good supply of total nitrogen, as well as good supply of available phorphorus and potassium. The sandy soil was medium calcareous, medium alkaline, very weakly humic and with a moderate supply of total nitrogen and good supply of available phorphorus and potassium (Table 1). The soils were dug out from 10 cm depth, cleaned from above- and underground plant remains and sifted through 3 mm sieves. Field water capacity (FWC) was determined by Richards's (1965) method using a pressure plate extractor.

Table 1. Physical and chemical properties of soil samples

Chemical properties								
Soil type	$CaCO_3$	pH		C	Humus	N	P_2O_5	K_2O
	%	H_2O	KCl	%	%	%	mg/100g	mg/100g
Loam	5.60	7.64	7.17	2.30	3.96	0.246	46.0	65.0
Sand	5.77	8.04	7.63	0.53	0.91	0.061	24.50	22.0

Soil texture					
	Sand			Silt	Clay
Soil type	Coarse (mm) 2-0.2	Fine (mm) 0.2-0.02	Total (mm) 2-0.02	0.02-0.002 (mm)	<0.002 (mm)
Loam	1.53	48.27	49.80	33.40	16.80
Sand	20.59	70.85	91.44	1.32	7.24

Bioassays were performed by treating air-dried soil (250 g) with different imazamox concentrations (6.25, 12.5, 25, 50, 100, 200, 400 and 800 μg a.i./kg soil). The soil was uniformly surface-treated and hand-stirred immediately after application, then transferred to pots, which were then planted with tomato seeds and watered up to 20, 50 or 70% FWC. The plants grew for 21 days in a growth room under 14 h daylight/10 h darkness photoperiod and 26°C/day and 21°C/night temperature. Throughout the experiment, soil moisture was constantly maintained at the defined FWC levels. Vegetative parameters – shoot and root fresh weight and root length – were measured as indicators of phytotoxicity, as well as the content of water soluble proteins.

The content of soluble proteins was determined by Bradford's (1976) method. Absorbance of the reaction mix was measured by spectrophotometry at 595 nm wavelength, and the protein contents were afterwards converted (mg/g fresh leaf weight).

The effect of imazamox concentrations on these parameters was evaluated using the F-test at 5% significance level. Statistical analysis was made in StatSoft 8.0. The data were used for a regression analysis to estimate the EC_{50} (i.e. the effective concentration of imazamox that reduced root fresh weight and root length by 50%) using the software package BIOASSAY97 (Onofri, 2005).

RESULTS AND DISCUSSION

Imazamox caused growth inhibition in tomato plants in both types of soil at all three levels of moisture, and the degree of effect depended on its concentration. In the loamy type of soil with moisture maintained at 20% FWC, the concentrations of 400 and 800 μg a.i./kg reduced the fresh weight of shoots by 44.2 and 76.7%, respectively (Figure 1). In loamy soil with 50% FWC, the same concentrations resulted in 67.2 and 76.6% reduction, while it was even more prominent (74.7-80.5%) at 70% FWC. The concentrations of ≤50 μg a.i./kg applied to sandy soil with 20% FWC caused no inhibition of fresh weight of shoots, while the highest imazamox concentration caused 38% reduction. In the same type of soil with 50% FWC, the concentrations of ≤200 μg a.i./kg reduced fresh weight of shoots to less than 26%, while stronger inhibition (43.6-53.9%) was detected at the two highest concentrations of imazamox. In the sandy soil with 70% FWC, concentrations ≤200 μg a.i./kg reduced the fresh weight of shoots by 20%, while the concentrations of 400 and 800 μg a.i./kg decreased values of this parameter by 53 and 55% (Figure 1).

The reduction in fresh weight of tomato roots in loam containing 20% FWC was below 17% for concentrations ranging from 12.5 to 200 μg a.i./kg.

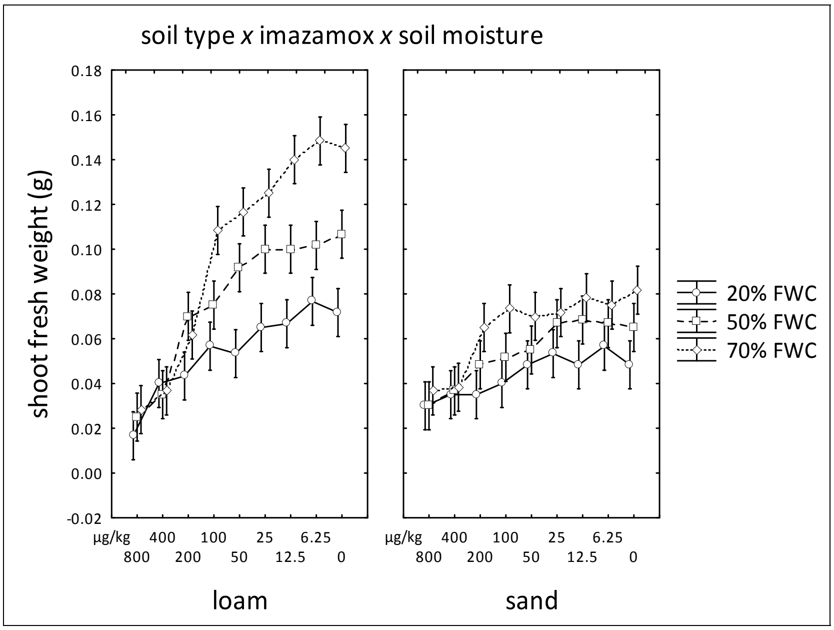

Figure 1. Changes in shoot fresh weight of tomato effected by residual activity of imazamox in two types of soil with different soil moisture percentages

The highest concentration caused a reduction of 50%, compared to control plants (Figure 2). Under conditions of higher moisture (50% FWC), inhibition was 32.5-65% for concentrations of ≥25 µg a.i./kg. Under moisture conditions of 70% FWC, the reduction in fresh root weight exceeded 50% when concentrations were higher than 100 µg a.i./kg. The concentration of 12.5 µg a.i./kg in sand with 20% FWC inhibited fresh root weight by 38.5%, while the highest concentration reduced the values of that parameter by 61.5%. In the same soil

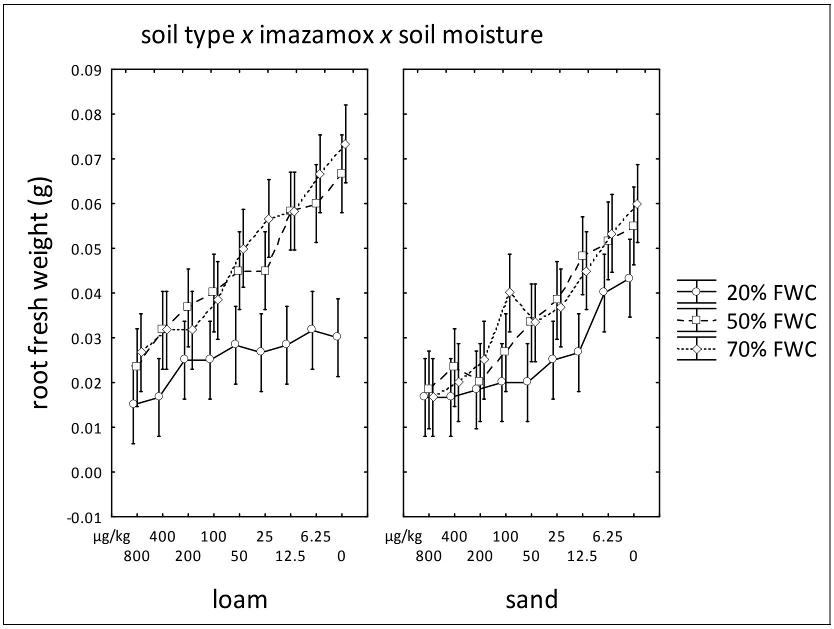

Figure 2. Changes in root fresh weight of tomato effected by residual activity of imazamox in two types of soil with different soil moisture percentages

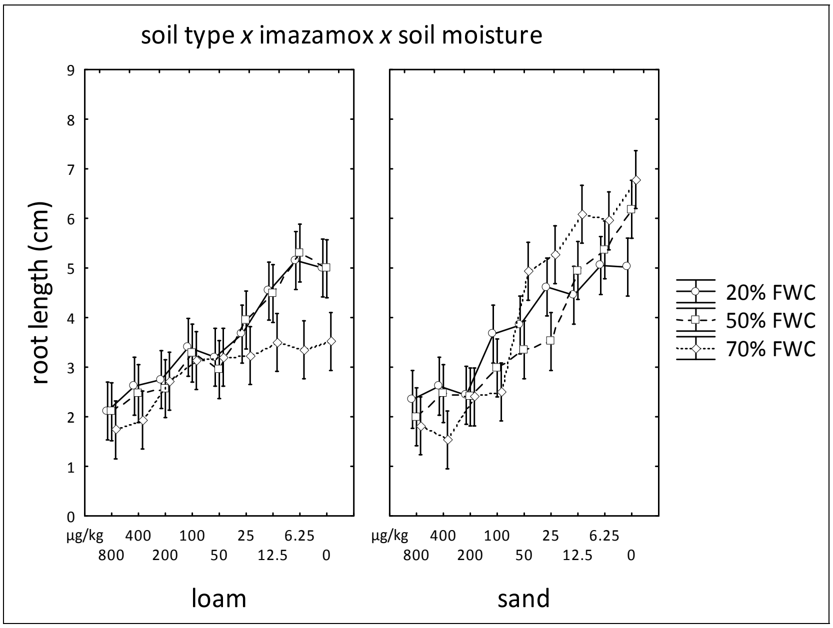

Figure 3. Changes in root length of tomato effected by residual activity of imazamox in two types of soil with different soil moisture percentages

containing 50% FWC, concentrations of 25-800 µg a.i./kg inhibited the fresh weight of tomato roots by 30.3-66.7%, while the reduction in sand of 70% FWC was more significant (58.3-72.2%) for concentrations of 200-800 µg a.s./kg.

Imazamox applied at concentrations higher than 50 µg a.i./kg in loam with moisture maintained at 20 and 50% FWC reduced root length 32-58% (Figure 3). On the other hand, concentrations of ≤200 µg a.i./kg applied under the highest moisture (70% FWC) caused root lenght inhibition below 20%, while the two highest concentrations caused 45 and 51% reduction. A greater reduction in root length (47.8 – 53.2%) in sandy soil with 20% FWC was achieved by the concentration rates of 200-800 µg a.i./kg, while 50% FWC caused inhibition exceeding 43% already at the rate of 25 µg a.s./kg. An even greater reduction in tomato root length was found in sandy soil of 70% FWC as the concentrations ≥100 µg a.i./kg resulted in 63-77.4% reduction.

A full factorial analysis of variance showed that all of its three factors and their interactions had significant impact on the fresh weight of tomato shoots (Table 2). However, the interaction of soil type and different imazamox concentrations, and interactions among the three factors had no influence on the fresh weight of tomato roots at 95% significance. Statistical analysis of our data concerning the lenght of tomato roots showed that soil moisture had no influence ($p<0.05$), while herbicide concentration, soil type and interactions between the factors had impact on that parameter (Table 2).

Variable data showing the susceptibility of particular plant species have been reported from studies investigating the residual effects of imidazolinone herbicides on the growth and development of vegetable crops. O´Sullivan et al. (1998), as well as Greenland (2003) reported leaf chlorosis and thwarted growth of tomato plants in trials in which soybean pre-crops had been treated with imazamox in the previous year, but visualized symptoms did not exceed 10%, so that they had no effect on yield. On the other hand, Colquhoun et al. (2003) recommended to make an 18-month intermission after imazamox treatment before sowing tomato, pepper or cucumber crops in the same plots. Alister and Kogan (2005) detected a significant reduction in growth and yield of tomato and pepper when the crops were sown one year after treatment with the herbicide combinations imazapyr with imazapic, and imazapyr with imazethapyr.

However, most authors agree that the physicochemical properties of soil and weather conditions are crucial for phytotoxicity occurring both during the year in which imidazolinones are being used and the year of sowing potentially sensitive species. Imazamox behaves as a weak acid, and the presence of both acid and base functional groups in the molecules of that compound results in soil pH exerting a significant influence on its availability and mobility, so that its adsorption in soil increases with decreasing pH. Some authors have found important a very notable correlation between the adsorptiveness and content of organic matter and clay (Undabeytia et al., 2004; Kah & Brown, 2006; Kah et al., 2007). Temperature and soil moisture also have a significant impact on imazamox as intensive degradation has been detected to occur under coinciding rise in temperature and soil moisture (Vischetti et al., 2002; Pannacci et al., 2006).

Table 2. Three-way ANOVA for determining the effects of imazamox, soil type and soil moisture on shoot and root fresh weight and root length of tomato plants

Factor	Shoot fresh weight		Root fresh weight		Root lenght	
	F	p	F	p	F	p
Soil (type)	249.637	0.000000	36.119	0.000000	39.549	0.000000
Imazamox (concentration)	98.509	0.000000	45.598	0.000000	99.422	0.000000
Soil moisture (%FWC)	177.478	0.000000	80.662	0.000000	1.344	0.262524
Soil type *x* imazamox	18.017	0.000000	0.659	0.727415	7.153	0.000000
Soil type *x* soil moisture	23.808	0.000000	9.127	0.000146	19.355	0.000000
Imazamox *x* soil moisture	6.445	0.000000	2.319	0.003233	2.478	0.001547
Soil type *x* imazamox *x* soil moisture	3.005	0.000122	36.119	0.000000	4.197	0.000000

Both soils used in our experiments were weakly alkaline (pH>7.1), which probably reduced adsorption initially and enhanced the availability of imazamox in soil solution, and resulted in high inhibition values of the parameters measured, especially in the sandy soil containing a low percentage of organic matter. Based on the calculated EC_{50} values, considering fresh root weight, the highest degree of susceptibility was detected in plants grown on the sandy soil with 20% FWC, while the same effect on loam required ten times more herbicide (Table 3). Considering the length of roots as a measure of susceptibility, the highest difference was revealed for soil moisture at 70% FWC as the values were four-fold higher in loam than in sand.

Table 3. The EC_{50} values for root fresh weight and root length of tomato

Soil type	FWC (%)	EC_{50} ±SD (μg a.i./kg)	
		root fresh weight	root lenght
Loam	20	133.71 ±37.22	42.55 ±8.89
	50	48.40 ±8.30	35.62 ±6.84
	70	38.62 ±5.29	185.10 ±25.51
Sand	20	13.17 ±1.86	65.28 ±10.61
	50	34.87 ±3.18	22.73 ±2.85
	70	34.85 ±9.30	47.75 ±7.25

The effect of different imazamox concentrations on contents of soluble proteins was examined in this trial (Figure 4). In loamy soil with 50% FWC, the highest degreee of inhibition (24.7%) was found for the concentration of 100 μg a.i./kg. When moisture of that soil was set to 20 and 70% FWC, the greatest reduction in protein contents of 31.6-41.7%, and 41.7-49.5%, respectively, was found within the concentration range of 25-100 μg a.i./kg. In the sandy soil with 50% FWC, none of the imazamox concentrations caused significant reduction (≤28%) in contents of soluble proteins in tomato plants. Concentrations ranging from 200-800 μg a.i./kg in sandy soil with 20% FWC caused inhibition of this parameter from 23.7 to 30.9%, while 32.5-42.9% inhibition was found in soil with 70% FWC containing imazamox at 50-800 μg a.i./kg.

Gaston et al. (2002) examined the influence of imazethapyr on pea growth and found the herbicide to inhibit ALS activation in leaves by 45% as early as the first day after treatement, while the activity fully stopped three days later. They reported that imazethapyr caused a slight decrease in concentrations of soluble proteins and a high increase in free amino acids in the root, as well as the shoot. Testing the activity on maize shoots, Shaner and Raider (1986) similarly noted that low concentrations of that herbicide caused decreases in water soluble proteins up to 40%. Our experiments also revealed a decrease in water soluble proteins but we detected no correlation between

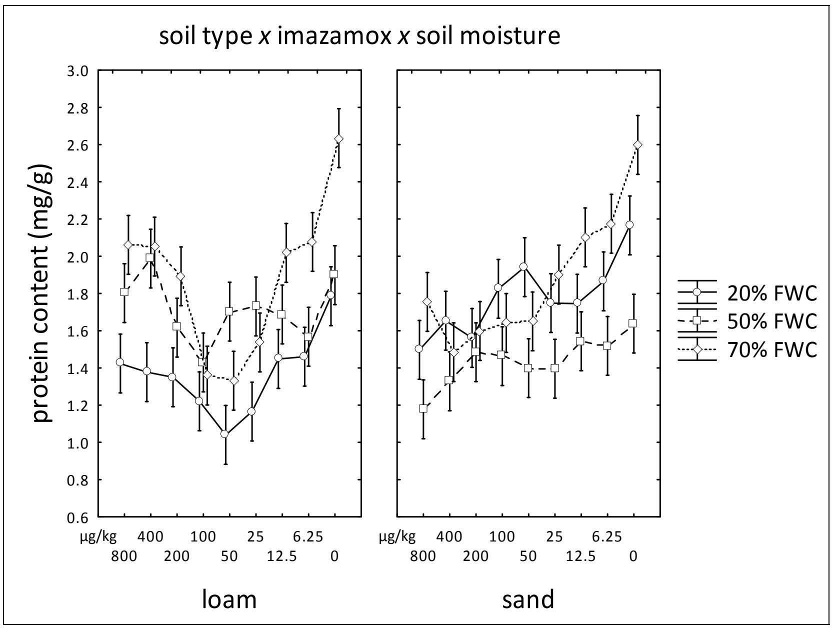

Figure 4. Changes in soluble protein contents in tomato plants effected by residual activity of imazamox in two types of soil with different soil moisture percentages

changes in that parameter and imazamox concentrations. As their inhibition was not consistent with inhibition of plant growth, we inferred that this biochemical parameter should not be considered as relevant in assessing plant susceptibility in bioassays.

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Uticaj ostataka imazamoksa u različitim zemljištima na usev paradajza

REZIME

Metodom biotesta u laboratorijskim uslovima je ispitivana osetljivost paradajza na rezidualno delovanje imazamoksa u zemljištima tipa ilovača i peskuša. Praćen je i uticaj tri različita nivoa vlažnosti zemljišta (20, 50 i 70% PVK). Imazamoks je primenjen u seriji koncentracija od 6,25 do 800 µg a.s./kg zemljišta. Biljke su rasle 21 dan, a nakon tog perioda mereni su: sveža masa izdanka i korena, dužina korena i sadržaj proteina rastvorljivih u vodi.

Imazamoks je izazvao zaostajanje u porastu i smanjenje sadržaja proteina u oba tipa i na svim nivoima vlažnosti zemljišta, a stepen ispoljenih promena je zavisio od koncentracije herbicida. Veći stepen osetljivosti ispoljile su biljke koje su gajene u peskovitom zemljištu. Utvrđeno je da su parametri korena (sveža masa i dužina) osetljiviji i pouzdaniji pokazatelj osetljivosti na prisustvo imazamoksa u zemljištu. U svim varijantama ogleda konstatovano je i smanjenje sadržaja proteina rastvorljivih u vodi, ali je utvrđeno da ne postoji zavisnost promene ovog parametra sa promenom koncentracije herbicida.

Ključne reči: Herbicidi; Ostaci; Zemljište; Fitotoksičnost; Paradajz