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Monitoring of herbicide effect in maize based on electrical measurements

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A b s t r a c t. The effect of the herbicide acetochlor on root growth was studied by a non-destructive electrical impedance and capacitance method in pot experiments on maize. Acetochlor was applied both as single active ingredient and mixed with safener AD-67 in two dosages. Without safener addition, acetochlor had a permanent inhibiting effect on plant root expansion. The safener AD-67 was capable of providing protective effect against herbicide application. High correlations between root electrical impedance or capacitance and the root dry mass or surface area under our laboratory conditions were confirmed by plant harvest method. Root electrical impedance and capacitance measurements proved to be valid for monitoring the effect of the herbicide influencing root development and for distinguishing plant groups subjected to different stress conditions.

K e y w o r d s: acetochlor, electrical capacitance, electrical impedance, maize, root growth

INTRODUCTION

Water and nutrient uptake is a key function of plant roots. Conventional root investigation methods *ie* soil cores, monoliths, in-growth cores are inefficient and inherently destructive, thus unadapted for continuous monitoring of root growth. Non-destructive techniques, such as minirhizotron, isotopes, magnetic resonance imaging (MRI), X-ray imaging or ground penetrating radar also have their own limitations (Cao *et al*., 2010).

Electrical impedance (EI) and capacitance (EC) measurements are capable to provide an assessment about *in situ* root status without plant damaging. The EC method was first developed by Chloupek (1972) using several crop plants under greenhouse and field conditions. Root EI and EC proved to be directly correlated with various root properties, such as fresh or dry weight, root surface area and root volume. Dalton (1995) presented a conceptual model in which root surface was considered to be the surface area of a group of cylindrical condensers. Since electrical attributes are sensitive to edaphic factors, the effect of soil type, soil water saturation and ionic status, as well as plant electrode placement can be considered at using electrical measurements (Dalton, 1995; Ozier-Lafontaine and Bajazet, 2005).

The EI and EC method have been used to demonstrate the response of plants to different abiotic and biotic stress factors: cold acclimation, freeze-thaw injury, drought stress, nutrient deficiency or pathogen infection can be detected by electrical measurement (Greenham *et al*., 1982; Higgins *et al*., 1977; Räisänen *et al*., 2007; Repo *et al*., 1994). Since root EI and EC depend on root mass or on active root surface, the method appears to be valid for monitoring the effect of chemicals exerting influence on root expansion. Acetochlor is a herbicide active ingredient of the chloroacetanilide family, used as a pre-emergent and early post-emergent agent for the control of annual grasses and dicotyledonous weeds on maize fields. It acts as a growth inhibitor by suppressing the anabolic pathways of fatty acids, proteins and gibberellic acids (Abu-Qare and Duncan, 2002). The inhibition prevails not only over the target weeds, but also over the crop plant, resistance of which may need to be facilitated by safener addition. Herbicide safeners selectively protect crop plant from herbicide toxicity without reducing activity in target weed species. Safeners might alter herbicide metabolism directly by acting as chemicals activators of particular functional group(s) in the herbicide, or by affecting biological systems (enzymes) involved in herbicide metabolism. Via the activation of glutathione-S-transferase and cytochrome P450 monooxygenase enzymes, safener AD-67 increases the metabolism rate of the herbicide into non-toxic residues (Ekler *et al*., 1993).

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The present work was designed to:

- test whether EI and EC measurement was adequate for discriminating between herbicide-treated and control plants, and
- examine the herbicide effect on root development both with pesticidal preparations containing acetochlor as a single active ingredient and mixed with safener AD-67.

MATERIALS AND METHODS

At experiment day 1, three-day-old maize seedlings (*Zea mays* L., DK-440 hybrid) were transplanted from germination beds into 1.25 dm^3 plastic pots containing 1.8 kg of oven-dry soil. The medium used was an Arenosol (FAO-UNESCO, 86.3% – sand, 8.3% – silt, 5.4% – clay) with pH_{H_2O} of 6.14 and pH_{KCl} of 5.95, CEC of 7.50 $mmol_c$ 100 g^{-1}, 0% – lime content, 1.18% – humus content, 1.53 kg dm^{-3} – bulk density and 0.19 cm^3 cm^{-3} – water content at field capacity. At planting, KNO_3, KH_2PO_4 and NH_4NO_3 was added to soil for plant nutrition, equivalent to 200, 120 and 200 mg N, P and K per kg of dry soil, respectively. Plant cultivation was carried out in a growth chamber with the following conditions: 24/18°C and 16/8 h for day/night temperature and photoperiod, respectively, irradiance of 25 000 lx and relative humidity of 50-80%. Pots were irrigated daily after weighing to maintain the soil moisture content near field capacity.

Over the control group of plants ('CON'), two herbicide dose treatments ('1HC' and '2HC') were executed in compliance with the minimum and maximum labeled application rate (1200 and 2000 g ha^{-1}) used in agricultural practice. Herbicide treatments were carried out both with acetochlor as a single active ingredient ('HC-N' plants) and with a mixture of acetochlor and safener AD-67 ('HC-A' plants) in either dosage. At 'HC-N' plants, product Harness (Monsanto Eur. S.A., Antwerpen, Belgium), containing 900 g dm^{-3} acetochlor [2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide] was applied, while to 'HC-A' plants Acenit A 880 EC (Agan Chem. Man. Ltd., Ashdod, Israel), containing 800 g dm^{-3} acetochlor and 80 g dm^{-3} safener AD-67 [N-dichloro-acetyl-11-oxo-azaspiro-(4,5)-decane] was added. 100 cm^3 aqueous solution of herbicides was poured over the soil surface on experiment day 9.

EI, EC and phase angle (Φ; strength of capacitive character) was measured with a HP 4284A LCR-bridge (Agilent Techn., Santa Clara, USA) with a spectrum of 54 frequencies from 100 to 10 000 Hz with 1 V terminal voltage. One terminal of the LCR-bridge was connected to the ground electrode consist of a stainless steel metal rod (6.3 mm diameter, 15 cm long) inserted to a depth of 10 cm into the potting soil 6 cm away from the stem base. The second terminal was attached to the plant stem with a spring tension clamp (Kendall *et al.*, 1982; Van Beem *et al.*, 1998; Rajkai *et al.*, 2005). Since the position of a stem electrode has a strong effect on the electrical measurements, a distance of 20 mm was precisely maintained between the lower edge of the clamp and the surface of rooting media (root neck). A thin layer of electrocardiograph paste smeared around the stem kept up electric connection, and dry leaves that did not conduct current were carefully removed from the basal part. Root development was monitored eight times between experiment day 15 and 40. Two hours before the measurement the pots were brought into the laboratory (22°C) and were irrigated to field capacity for avoiding the moisture content effect of the soil medium. Percentage water content of soil was determined by Trime-FM3 TDR instrument (IMKO GmbH, Ettlingen, Germany) prior to the measurement. Electrode polarization, cable inductance and the effect of herbicides on the electric properties of soil were also determined for precluding the possibilities of systematic errors in EI and EC reading.

Correlations between root EI or EC and root expansion (root dry mass and surface area) were confirmed also destructively: through the growing season, altogether 18 maize plants were harvested right after the electrical measurements. Root systems were washed thoroughly off the potting soil then were oven-dried at 60°C for 72 h and reweighed. For assessing root surface area, root systems were subjected to scanning and image analysis.

Changes in acetochlor content in soil were measured six times between experiment days 10 and 40 by gas chromatography coupled with mass spectrometry (GC-MS), using a Varian Saturn 2000 workstation, equipped with a Varian 8200 autosampler. The GC-MS parameters and soil extraction procedure were carried out according to the published method (Oldal *et al.*, 2006). Degradation rate and half-life of the ingredient were calculated from these results.

Statistical evaluation of EI and EC results was performed by analysis of variance. SD values of the compared groups were examined by Bartlett test, and Tukey-Kramer procedure was applied in multiple comparisons. Simple regression analysis was carried out in order to relate root EI and EC to both root dry mass and surface area. Degradation rate of acetochlor was also determined by standard regression method. Statistical significance was assessed at $P<0.05$ in each case, and only the regression equations with the greatest R^2 were accepted.

RESULTS AND DISCUSSION

According to our preliminary trials, the electrode polarization proved to be negligible at the used current frequency interval, and cable inductance was not detectable. EI and EC measurement directly aimed at the planting medium did not reveal any effect of herbicide application on the electric properties of soil.

Both the root EI and EC showed a downward trending curvilinear relationship with increasing current frequency. The strength of capacitive character also had a dependence

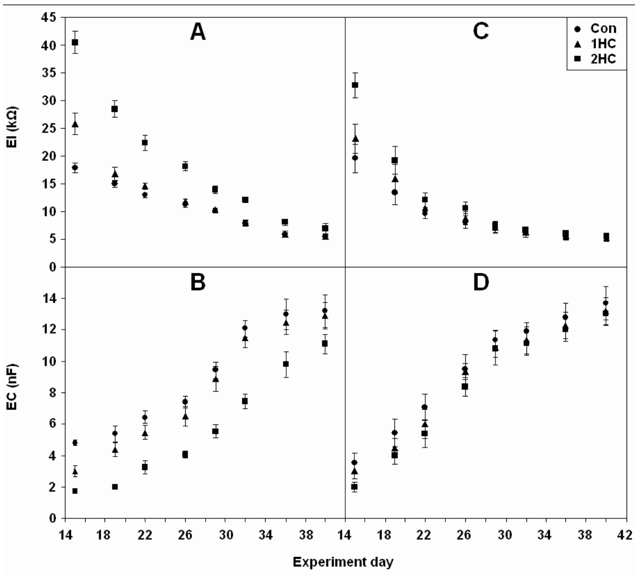

Fig. 1. Signal intensity in root EI (kΩ) and EC (nF) measured at 1 000 Hz related to time in herbicide treatments with application of acetochlor as single active ingredient (A and B) and acetochlor mixed with safener AD-67 (C and D).

of frequency: the maximal value of phase angle ($\Phi = -32.7°$) was observable at the range from 1 000 to 1 200 Hz, therefore statistical evaluation (Tukey-Kramer multiple comparison test) was carried out at the same current frequency of 1 000 Hz. In relation to plant root expansion through the experiment time, EI and EC showed a continuous decrease and increase, respectively.

Without safener addition, the ingredient of acetochlor had a considerable inhibiting effect on plant root expansion, clearly appeared in EI and EC values were obtained. '2HC-N' plants tended to have significantly higher EI and lower EC than either the 'CON' or the '1HC-N' groups throughout the whole experiment, indicating the permanently impeded root growth caused by the high dosage of herbicide (Fig. 1a, b). Influence of the low-dose herbicide application was temporary: root development of '1HC-N' plants has been reduced for 13 days after herbicide treatment (until experiment day 22), thereafter the measured EI and EC proved to be equal with values of 'CON' plants. Significance levels and q-values of the Tukey-Kramer tests are summarized in Table 1. The safening activity of AD-67 was expressly demonstrated by the electrical properties of plant root: no significant differences were found between the EI or

T a b l e 1. q-values and significance levels of the Tukey-Kramer test performed for data evaluation of different herbicide treatments

Experimental day	CON vs. 1HC	CON vs. 2HC	1HC vs. 2HC	CON vs. 1HC	CON vs. 2HC	1HC vs. 2HC
Treatment with acetochlor as single active ingredient						
15	11.443 ***	32.887 ***	21.444 ***	19.143 ***	32.951 ***	13.808 ***
19	3.905 *	27.991 ***	24.085 ***	5.333 **	11.667 ***	6.333 ***
22	4.357 *	25.869 ***	21.513 ***	5.749 **	18.776 ***	13.027 ***
26	2.769 NS	30.393 ***	27.625 ***	3.085 NS	18.755 ***	13.670 ***
29	2.111 NS	22.979 ***	20.867 ***	2.269 NS	16.031 ***	13.762 ***
32	0.354 NS	18.560 ***	18.206 ***	2.750 NS	20.939 ***	18.189 ***
36	0.238 NS	9.933 ***	9.694 ***	1.418 NS	9.018 ***	7.600 ***
40	0.074 NS	5.615 **	5.541 **	0.907 NS	5.933 **	5.086 **
Treatment with acetochlor + safener AD-67						
15	3.357 NS	13.272 ***	9.615 ***	2.530 NS	7.747 ***	5.218 **
19	2.544 NS	5.932 **	3.388 NS	3.491 NS	5.091 **	1.600 NS
22	1.735 NS	4.451 *	2.716 NS	3.057 NS	4.697 *	1.640 NS
26	1.619 NS	4.014 *	3.109 NS	0.463 NS	3.767 *	3.304 NS
29	0.465 NS	2.100 NS	1.635 NS	1.562 NS	1.869 NS	0.307 NS
32	0.165 NS	1.430 NS	1.595 NS	1.867 NS	2.576 NS	0.709 NS
36	0.554 NS	2.106 NS	1.552 NS	1.547 NS	2.380 NS	0.833 NS
40	0.659 NS	2.131 NS	1.472 NS	1.269 NS	1.773 NS	0.503 NS

*0.05, **0.01, ***P<0.001; NS – non significant.

EC means of 'CON' and '1HC-A' groups at any measurements (Fig. 1c, d; Table 1), and root growth inhibition in '2HC-A' group was detectable only for 17 days after treatment (until experiment day 26). Thus, the herbicide toxicity was totally prevented at the low-dose acetochlor treatment, while the reduced root growth was detectable only provisionally even at maize plants treated with the high dosage of herbicide.

In the plant harvest experiment, highly significant correlations (P<0.001) were found between electrical root properties and root system size (Fig. 2). Simple regression analysis showed negative curvilinear relationships between the measured root EI and both the root dry mass ($R^2 = 0.9225$, Fig. 2a) and the root surface area ($R^2 = 0.9138$, Fig. 2c), whereas in the case of root EC, positive linear correlations were revealed with the root dry mass ($R^2 = 0.9373$, Fig. 2b) and the root surface area ($R^2 = 0.8930$, Fig. 2d).

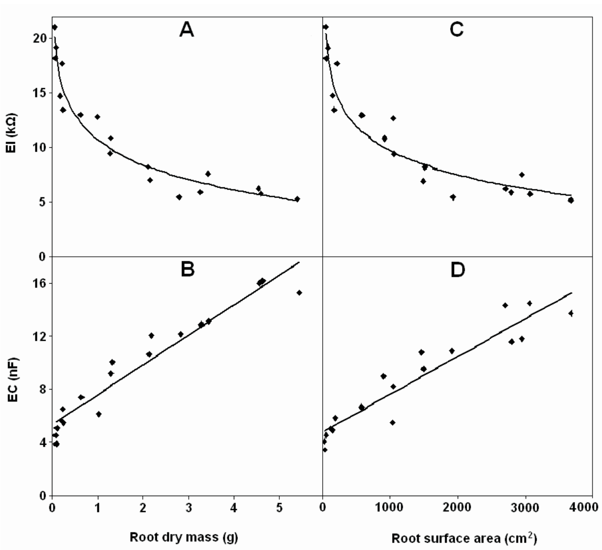

Fig. 2. Relation of root EI (A, C) and EC (B, D) measured at 1 000 Hz with the root dry mass (g) and root surface area (cm^2) of maize.

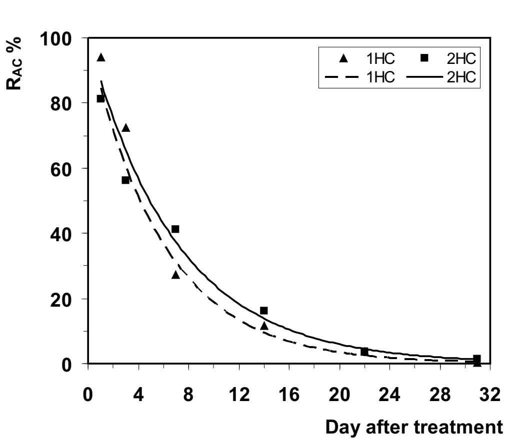

Fig. 3. Changing in the percentage rate of acetochlor residue (R_{AC}) in the low- and high-dose herbicide treatments.

The decrease of acetochlor level in potting soil during the days after treatment is presented in Fig. 3. The regression curve equation for the percentage rate of acetochlor residue (R_{AC}) related to time (*t;* day) was:

$$R_{AC} = 100e^{-0.1677t} \; (R^2 = 0.9807)\,, \qquad (1)$$

and

$$R_{AC} = 100e^{-0.1411t} \; (R^2 = 0.9918)\,, \qquad (2)$$

for the low- and high-dose herbicide treatment, respectively, with 4.13 and 4.91 days half-life (DT_{50}) of the herbicide in soil. These results clearly show the relatively fast decline of acetochlor ingredient in the planting medium.

Our results definitely show both the significant growth-impeding influence of acetochlor on maize seedlings, and the capability of safener AD-67 of providing protective effect against post-emergence herbicide application. These experiences corroborate previous observations achieved by conventional root investigation techniques (Kazinczi *et al.*, 2003). Dissipation rate of pesticides are strongly influenced by various physicochemical and biological soil properties (pH, organic carbon and clay content, activity and distribution of microorganisms) as well as environmental conditions, such as temperature and moisture content (Hu *et al.*, 2011). The reported half-life of acetochlor ranges from 4 to 29 days, with a mean value of 13 days. The steadily high temperature and water content existed in our pot experiment certainly promoted the microbial decomposition processes that generally played important role in acetochlor degradation. Besides the abundant water supply, the relatively low organic carbon and clay content also contributed to keeping the herbicide in the soil solution (reduced the sorption capacity of soil; Hiller *et al.*, 2009), leading to an increased uptake and detoxification rate by plant organism. These conditions jointly resulted in the high dissipation and short half-life of acetochlor.

The present study indicated that root EI and EC measurement is an acceptable and very useful non-destructive method for monitoring the effect of herbicide influenced on root development and for distinguishing plant groups subjected to different stress conditions. The root-soil system behaved as a capacitor mainly at the current frequency range around 1000 Hz, the applicability of this frequency has been confirmed by several former studies (Chloupek *et al.*, 2006; Kendall *et al.*, 1982; Rajkai *et al.*, 2005). The main limitation to the generalization of the method, that it may only suitable for relative comparisons of root mass because of edaphic factors affecting electrical conductance (Dalton 1995; Ozier-Lafontaine and Bajazet, 2005; Van Beem *et al.*, 1998). Conversely, under well-defined laboratory and field conditions the method can provide a good estimation about root system size as was demonstrated by the regression analysis of our plant harvest experiment data.

Electrical root properties measurements may be of importance to diverse scope of agricultural researches. The technique can facilitate the quantification of the optimal and toxic herbicide dosage, development of efficient safeners, detection of herbicide resistant crops and weeds, or selection for desirable root characteristics in genotypes adapted to different stress conditions (Chloupek *et al*., 2006; Preston *et al*., 2004).

Investigation of acetochlor has a great importance in environmental protection, as its high leaching potential often leads to widespread surface and groundwater pollution (Oldal *et al*., 2006). Furthermore, the ingredient destroys the original composition and structure of soil microorganism communities, resulting in a decline of functional diversities and metabolic activities (Su *et al.*, 2007). This effect is generally associated with soil degradation processes. In consequence, excessive acetochlor application poses immediate and long-term risks for ecosystems and should be considered to be a serious threat to the quality of the environment and human life.

CONCLUSIONS

1. Without safener addition, acetochlor has a significant root-growth inhibiting effect in maize plants both with the minimum and maximum labeled herbicide application rate used in agriculture.
2. The herbicide toxicity can be prevented or reduced by addition of safener AD-67.
3. Root electrical impedance and electrical capacitance measurements give a good estimation about root system size under well-defined laboratory conditions.
4. Electrical impedance and electrical capacitance methods are an adequate non-destructive technique for monitoring the effect of stress factors having influence on root development.

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