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Effect of cropping system on development dynamics of cellulolytic microorganisms in soil

Wpływ systemu uprawy na dynamikę rozwoju drobnoustrojów celulolitycznych w glebie

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Słowa kluczowe: drobnoustroje celulolityczne, gleba, ryzosfera, system uprawy, ziemniak

Abstract
The effect of ecological and conventional cropping systems on the population of cellulolytic microorganisms under potato cultivation was evaluated in this study. The 3-year experiment aimed at investigating the development dynamics of microorganisms capable of decomposing cellulose in the soil beyond rhizosphere, in the rhizosphere and rhizoplane. The study indicated that in most cases larger populations of microorganisms occurred under potato cultivation in the ecological system in comparison with the conventional system. Only during the period of flowering, a smaller number of them were isolated from the soil cultivated in the ecological system. More favourable conditions for the development of cellulolytic microorganisms were found both in the rhizosphere and rhizoplane of potato than in the soil beyond the roots, irrespective of the cropping system. Moreover, the date of sampling, corresponding to the potato developmental stages, turned out to be a significant factor affecting the count level. A significant increase in their population was observed during tuber maturity at harvest.

Streszczenie
W pracy oceniono wpływ systemu uprawy ekologicznego i konwencjonalnego na populację drobnoustrojów celulolitycznych pod uprawą ziemniaka. W trzyletnim doświadczeniu badano dynamikę rozwoju mikroorganizmów zdolnych do rozkładu celulozy w glebie pozaryzosferowej, ryzosferze i ryzoplanie. Przeprowadzone badania wykazały, że na ogół większe populacje drobnoustrojów występowały pod uprawą ziemniaka w systemie ekologicznym w porównaniu z konwencjonalnym. Tylko w jednym terminie (w okresie kwitnienia) izolowano ich mniej z gleby uprawianej systemem ekologicznym. Zarówno w ryzosferze jak i ryzoplanie ziemniaka stwierdzono korzystniejsze warunki do rozwoju drobnoustrojów celulolitycznych niż w glebie oddalonej od korzeni niezależnie od systemu uprawy. Ponadto istotnym czynnikiem wpływającym na poziom liczebności okazał się termin pobierania próbek, odpowiadający fazom rozwoju ziemniaka. Istotny wzrost ich populacji stwierdzono w czasie dojrzałości bulw do zbioru.

1. INTRODUCTION

Plants produce about 4×10^9 tons of cellulose annually, in which its large amounts remain in the soil with died plant tissues. Its crystal structure and insoluble nature pose a big challenge for enzymatic hydrolysis (Irfan et al. 2012). Microorganisms play a significant role in cellulose decomposition both in soil and in water ecosystems. This process proceeds with the participation of enzymes from a group of cellulases. This complex is composed of three enzymes: cellulase (endo-β-1,4-glucanase), exo-β-1,4-glucanase and celobiase (β-glucosidase) (Górska, Russel & Łabętowicz 1999; Russel, Górska & Wyczółkowski 2005). Different systematic and physiological groups of both bacteria – actinomyces – and fungi show the ability to decompose cellulose (Wirth & Ulrich 2002).

The number of cellulolytic microorganisms and the intensity of processes catalysed by them depend on physical and chemical soil properties, particularly on the content of assimilable compounds of carbon and nitrogen. Complex substances containing cellulose remaining in the soil undergo further transformations (hydrolysis, mineralisation) into simple compounds and become the available source of food for all soil heterotrophs. For this reason, the rate of fibre decomposition is regarded as the significant parameter indicating the biological activity of soil. There is a strong relationship between the activity of microorganisms in soil and its fertility (Mijangos et al. 2006; Ulrich, Klimke & Wirth 2008).

The nature and the intensity of undergoing microbiological changes also depend on the vegetation and cultivation practices. The way the field crop fertilisation and the crop protection preparations are applied forms the main criterion for dividing the cropping system into conventional and ecological system. The ecological system eliminates the use of mineral fertilisers and chemical crop protection preparations (Sawicka & Kuś 2000).

Maintaining arable soils in their optimal state of balance should be the objective of modern production systems. Improperly conducted cultivation practices may lead to a decrease in the number and diversity of microorganisms and their enzymatic activity, and consequently, to a decrease in plant yield (Acosta-Martinez, Mikha & Vigil 2007). In spite of the common opinion about the positive aspects of ecological cropping, constant monitoring should be performed for changes that occur in the soil environment.

The present study aimed at determining the effect of the ecological and conventional cropping systems on the occurrence of cellulolytic microorganisms in soil beyond the rhizosphere and on potato roots. Moreover, attention was focused on their development

dynamics depending on the date of the study corresponding to the plant development stages.

2. MATERIALS AND METHODS

Soil samples and plant roots were collected from fields of different cropping systems: ecological (E) and conventional (K). The fields were located at Kiełpin (53°37'32"N, 17°53'08"E), in the north part of the Kuyavian-Pomeranian Voivodeship. Potato (cv Aster) was cultivated in luvisols with the grain size composition of heavy loamy sand. The content of humus in the soil samples ranged from 13.4 to 19.6 g·kg^{-1} and the total nitrogen varied from 0.64 to 0.94 g·kg^{-1} in the soil (Table 1). The amount of assimilable forms of phosphorus, potassium and magnesium in the arable layer amounted to 0.008–0.056 g·kg^{-1} P_2O_5, 0.167–0.246 g·kg^{-1} K_2O and 0.03–0.066 g·kg^{-1} of soil MgO.

Different rotations of crops and technologies of production were used in compared systems. The ecological farm from which the material for the study was collected was cultivated according to the rules of ecological farming, not applying mineral fertilisation and chemical crop protection preparations. The potato plantation was fertilised with compost in the single dose of 20 t·ha^{-1}, ground basalt 300 kg·ha^{-1} and peat (5–15 t·ha^{-1}). The pesticides used included only a biological preparation against Colorado potato beetle – Novodor (4 l·ha^{-1}). The conventional farming included organic-and-mineral fertilisation with manure (25 t·ha^{-1}) and mineral fertilisers NPK: ammonium nitrate (100 kg·ha^{-1}), ordinary superphosphate (200 kg·ha^{-1}), potassium salt 40% (200 kg·ha^{-1}) and Ekolist – microelement concentrate with N, K, Mg + chelacid (5 l·ha^{-1}). On the conventional farm, the following pesticides were used: Sandofan Manco 64 WP (active substance – oxadixyl + mancozeb) and Curzate M 72.5 WP (active substance – cymoxanil + mancozeb) in the doses of 2 kg·ha^{-1} and preparation Bancol 50 WP (active substance – bensultap) – 0.4 kg·ha^{-1}.

Soil samples beyond rhizosphere for analyses were collected for three consecutive years from the topsoil 0–20 cm: the first time immediately before planting (except for the first year of the study) and three times during the vegetation period. In the collected samples, the pH in H_2O and in 1M KCl was determined (once every year), as well as moisture (three times over the growth). The rhizosphere soil and plant roots were analysed three times over the vegetation period: during emergence (BBCH 13), flowering (BBCH 65) and tuber harvest maturity (BBCH 91). The rhizosphere soil was assumed to be the several millimetres long layer of soil adjacent to roots, and rhizoplane – the root surface deprived of soil particles.

To determine the count of cellulolytic microorganisms, the plate method following Koch was applied, using tenfold dilutions of soil and plant roots prepared in Ringer fluid. The microorganisms were isolated on the agar medium with CMC-Na (0.1% sodium carboxymethyl cellulose) according to Strzelczyk and Szpotański (1989). All the analyses were done in four replications.

The obtained results of the number of microorganisms expressed in cfu (colony forming units) were variance analysed with the Student's t-test (p = 0.95) using STATISTICA.

3. RESULTS AND DISCUSSION

The results of microbiological analyses from each year of the study are presented in Table 2, whereas the syntheses from 3 years of the study are compared in Figures 1 and 2. Based on the obtained results, a significant difference in the quantitative composition of cellulolytic microorganisms was observed depending on the cropping system applied as well as the plant developmental stage. The smallest amounts of microorganisms able to decompose cellulose were determined in the soil beyond rhizosphere. In the ecological system, their count increased in the beginning of vegetation, with a small decrease during the plant flowering, and then again an increase in their population occurred. By contrast, in the soil cultivated with the conventional system, an upward tendency was recorded to the flowering stage inclusive, whereas a decrease in their count occurred at the last date, at the end of vegetation (Figure 1). Substantially higher numerical values of microorganisms hydrolysing cellulose were obtained in the rhizosphere and rhizoplane of potato (Figure 2). The date of collecting samples, corresponding to the stages of potato development, turned out to be a significant factor influencing the count (Table 1). More cellulolytic microorganisms were isolated both from rhizosphere and rhizoplane of potato cultivated in the ecological system than from cultivation in the conventional system. Especially, a rapid growth in their population was observed during the stage of tuber harvest maturity. The obtained numerical values were more than five times higher for microorganisms colonising the rhizosphere and two times higher for microorganism in the rhizoplane of potato cultivated in the ecological system, as compared with the conventional system.

The obtained results indicate that the ecological cropping system under conditions of proper cultivation technology created a more favourable condition for the development of cellulolytic bacteria than conventional system. In the study on the occurrence of other groups of microorganisms taking part in C and N transformations (amylolytic, proteolytic) under potato cultivation in compared cropping systems, higher amounts of microorganisms were also determined in the soil cultivated in the ecological system (Breza-Boruta & Paluszak 2006). Also, studies by Martyniuk et al. (2001, 2007) showed a favourable effect of ecological cultivation on the enzymatic and biological activity of soil.

The study carried out by Frąc et al. (2011) indicated that a higher activity of soil microorganisms in the soil under wheat grown in the ecological system was connected with a greater supply of organic

Table 1. Selected physical and chemical properties of the soils in ecological (E) and conventional (K) systems

Year	Soil	pH		C-org	N- total	Moisture %		
		H_2O	KCl	g·kg^{-1}		Emergence phase (BBCH 13)	Flowering phase (BBCH 65)	Maturity phase (BBCH 91)
I	E	5.9	5.0	18.2	0.801	10.7	9.3	11.0
	K	5.7	4.9	19.6	0.941	7.2	9.3	12.3
II	E	6.9	6.3	16.9	0.700	12.8	7.3	14.1
	K	6.5	5.9	19.1	0.862	12.4	9.1	13.8
III	E	6.7	6.0	18.2	0.638	12.4	11.7	12.4
	K	5.6	4.9	13.4	0.857	12.0	11.2	12.0

Table 2. Number of cellulolytic microorganisms in the non-rhizosphere soil, rhizosphere and rhizoplane of potato cultivated in ecological (E) and conventional (K) systems

Number of microorganisms	Date of analyses	Cultivation system/year					
		E	K	E	K	E	K
		I		II		III	
Non-rhizosphere soil (10^6 cfu · g^{-1} soil d.m.)	1	–	–	2.2	2.2	4.5	3.0
	2	2.2	7.5	6.9	5.4	11.1	10.8
	3	8.8	8.8	6.2	6.6	9.2*	15.8*
	4	4.2	8.6	8.5	5.8	15.4*	10.2*
	Mean	5.1	8.3	6.0	5.0	10.1	9.9
Rhizosphere (10^6 cfu · g^{-1} soil d.m.)	2	29.1*	10.6*	18.4	9.1	24.0	8.5
	3	8.1	12.1	21.6	14.9	29.1	13.5
	4	550.4*	68.8*	331.7*	46.4*	148.3*	79.5*
	Mean	195.9	30.5	123.9	23.5	67.1	33.9
Rhizoplane (10^6 cfu · g^{-1} fresh roots)	2	31.0*	5.0*	41.0	13.0	44.0	37.3
	3	8.0	20.3	25.5	16.5	35.3	32.3
	4	320.0*	140.0*	240.0*	175.0*	305.0*	85.0*
	Mean	119.7	55.1	102.2	68.2	128.1	51.5

$LSD_{T, p<0,05}$ cultivation system × date.
[1]Date of microbiological analyses: 1 – before planting, 2 – BBCH 13, 3 – BBCH 65, 4 – BBCH 91.
[2]No analyses were made in the date I year of study.
*Marked values in the same line for the year are significantly different (p<0.05)

matter and the soil pH value more favourable for the development of microorganisms. Also, in the present study the soil pH under potato cultivation in the organic cropping system was more optimal for the development of cellulolytic microorganisms. Lower pH values were determined in soil from the conventional farm (Table 1). According to the literature data, the decomposition of fibre occurs faster in soils with neutral or slightly acidic pH than in acidified soils. However, the optimal pH for cellulolytic bacteria is usually about 7 (Hatami et al. 2008; Irfan et al. 2012). It is notable that a higher soil moisture in the ecological system during the plant growth also favoured a more intensive development of microorganisms (Table 1). The population level of microorganisms colonising the rhizosphere and rhizoplane indicates favourable effect of potato root excretions, particularly at the end of growth. This effect could be connected with the composition of excreted substances and an increased plant root mass. The results of means from 3-year analyses presented in Figure 2 confirmed a significant development of cellulolytic microorganisms in the period of tuber harvest maturity. At that time, the most significant differences in the size of microorganism population between the compared cropping systems were the most noticeable. The age and development of plants affect the character of root excretions, which is reflected in changes in the microorganisms population. Many microorganisms accumulate mainly around the plant roots, where they find an abundant source of nutrients and a suitable place for fast multiplication (Wielgosz & Szember 2006).

A higher amount of differentiated organic matter remain in the soil of that system, and no mineral fertilisation applied probably created favourable conditions for better development and activity of soil microflora in the ecological system. Maintaining balance in functioning

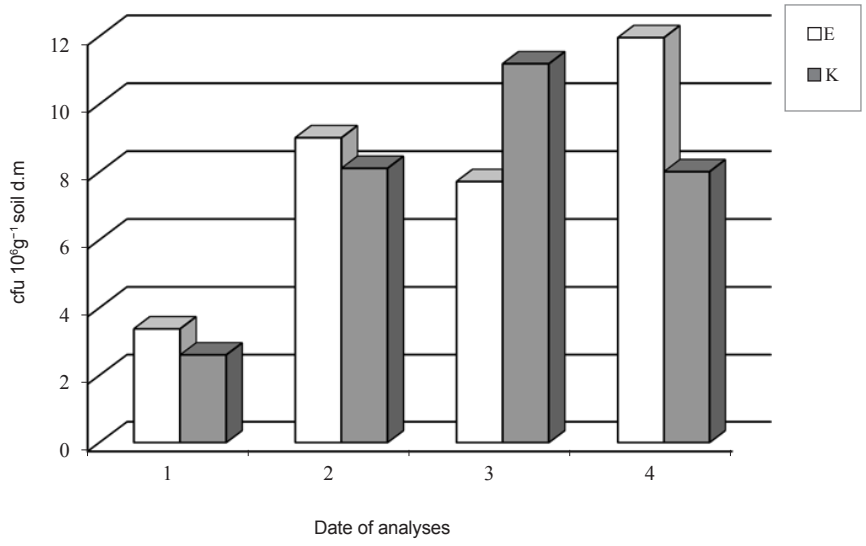

Figure 1. Population dynamics of cellulolytic microorganisms in soil under potato grown in ecological (E) and conventional (K) systems. Results of means from II–III years analyses.
Date of analyses: 1 – before planting, 2 – BBCH 13, 3 – BBCH 65, 4 – BBCH 91.

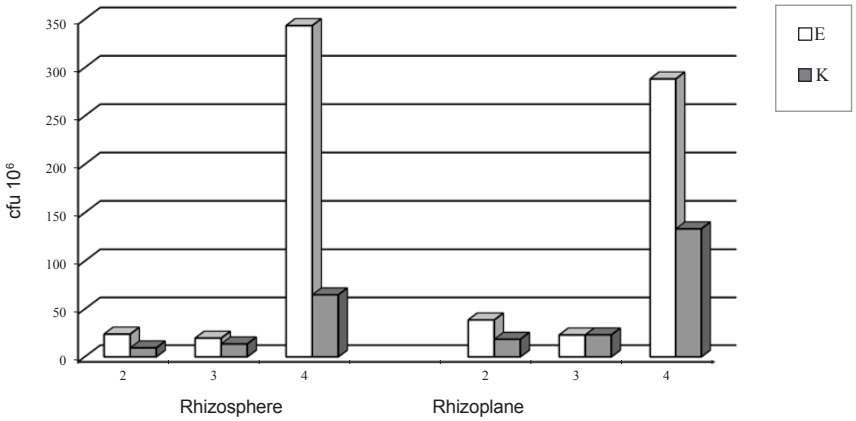

Figure 2. Population dynamics of cellulolytic microorganisms in rhizosphere and rhizoplane of potato cultivated in ecological (E) and conventional(K) systems. Results of means from 3-year analyses. See Figure 1.

of agroecosystem and providing the proper level of agricultural production require constant monitoring of the changes that occur in biological, chemical and physical properties of the soil.

4. CONCLUSIONS

1. The number of microorganisms hydrolysing cellulose was higher in potato cultivation in the organic cropping system than conventional system, which may indicate a more intensive activity of soils cultivated with this farming technique.
2. Significant differences in the development of cellulolytic microorganisms between the compared cropping systems were the most noticeable in the rhizosphere and rhizoplane at the end of the plant vegetation, where two and five times larger amounts, respectively, were found in the organic farming.
3. A considerable increase in the number of studied microorganisms in the rhizosphere zone and rhizoplane of potato was observed, as compared with soil beyond the roots. This may result from the favourable effect of potato root excretions on the cellulolytic activity of soil microflora.

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