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Organic fertilization shapes the biodiversity of fungal communities associated with potato dry rot

Katarzyna Gleń-Karolczyk[a,*], Elżbieta Boligłowa[a], Jacek Antonkiewicz[b]

[a] Department of Agricultural Environment Protection, Faculty of Agriculture and Economics, University of Agriculture in Kraków, Al. Mickiewicza 21, 31-120 Kraków, Poland

[b] Department of Agricultural and Environmental Chemistry, Faculty of Agriculture and Economics, University of Agriculture in Kraków, Al. Mickiewicza 21, 31-120 Kraków, Poland

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ABSTRACT

Intensive farming based on synthetic pesticides and mineral fertilizers has led to a loss of soil biodiversity, which contributes to the suppression of plant pathogens. The key role in the restoration of biodiversity and soil aggregate stability is fulfilled by organic fertilization. Potato reacts well to this type of fertilization. On the other hand, its tubers, during both the vegetative and storage periods, are exposed to a series of infections caused by soil fungi. Of particular economic importance is dry rot, a storage disease with a complex etiology. This study presents an evaluation of the impact of different organic fertilization forms (manure, white mustard intercrop, barley stubble, barley straw and a combination of barley straw and white mustard intercrop) on losses caused by dry rot. For the first time, their role in the formation of: counts, species composition and belonging to frequency and trophic groups of fungi communities colonizing dry-rotting tubers has been specified. Furthermore, a pioneering element of this research is its evaluation of fungi community biodiversity and its influence on the development of dry rot. The main discoveries are as follows: (i) 24 fungi species (14 pathogenic, 6 saprotrophic, 4 antagonistic), which are the cause of dry rot; (ii) dry rot development is mainly determined by the share of saprotrophic fungi, where their increase reduces the percentage of infected tubers; (iii) manure and white mustard favors tuber colonization by saprotrophic and antagonistic fungi and increases biodiversity of the fungi, which results in improved healthiness; (iv) straw and a lack of fertilization increase the frequency of pathogens and reduce biodiversity, resulting in a stronger development of dry rot. In sustainable agriculture management systems, disease risk can be minimized through introduction of organic matter to soil.

1. Introduction

Dry rot in potato tubers is a storage disease of global economic importance (Mecteau et al., 2002, Recep et al., 2009, Du et al., 2012, Stefańczyk et al., 2016). It causes losses at a level of 6%–25%, up to as much as 60% in a warm climate (Sadfi et al., 2002, Cullen et al., 2005). The size of the losses is determined by the soil abundance of fungi populations infecting the tubers (Peters et al., 2008, Li et al., 2009, Fiers et al., 2012). The most common causes of dry rot are fungi of the *Fusarium* genus (Choiseul et al., 2007, Estrada et al., 2010, Du et al., 2012, Stefańczyk et al., 2016). Pathogenic fungi species composition is modified by environmental and agronomic factors. Relative soil balance between the groups of phytopathogenic, saprotrophic and antagonistic microorganisms guarantees better plant health (Doran and Zeiss, 2000, Kibblewhite et al., 2008). Its disturbance leads to the dominance of pathogens, which in the absence of resistance of the environment massively infect the plants. Synthetic fungicides have little effect on most soil pathogens and concurrently disturb soil biodiversity, which is the basis for the functioning of modern crop systems (organic and integrated) (Shafique et al., 2016). Soil organisms are most responsive to natural and organic fertilizers, since when introduced into the soil they improve its structure and quality, increase the amount of organic matter, and are the source of carbon and nutrients for microorganisms (Zaccardeli et al., 2013, Zhen et. al., 2014, Luo et al., 2015, Geisseler et al., 2017). They directly or indirectly affect the population of saprotrophic organisms with antagonistic effects on phytopathogens (Rashid et al., 2016). Organic fertilizers could be an important tool to reduce or control pathogens transferred by the soil (Boligłowa and Gleń, 2003, Lazarovits et al., 2008, Fiers et al., 2012, Zaccardeli et al., 2013). Potato requires organic fertilization, and reacts best to manure, but in its absence (no livestock) there is a need for other forms of organic fertilizers.

* Corresponding author.
E-mail address: rrglen@cyf-kr.edu.pl (K. Gleń-Karolczyk).

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The above arguments indicate that it is advisable to conduct studies on the determination of the effect of organic fertilizers (manure, barley stubble, barley straw, white mustard intercrop, and barley straw + intercrop) on potato tuber dry rot development during storage and on the degree of qualitative and quantitative diversity of the fungal populations causing this dry rot. Furthermore, the paper describes the way in which fertilization determines the biodiversity of fungi communities.

2. Materials and methods

2.1. Field experiments

The study was performed in 2012–2014 in Boczkowice (20°20′E, 50°42′N), Małopolskie Voivodship, on typical eutrophic brown soils made of loess with a granulometric texture of silt loam (SiL) (Polish Soil Classification, 2011, Soil Survey Staff, 2014, Świtoniak et al., 2016). The mean topsoil composition in a 100 g sample was –10.7 mg P_2O_5, 18.8 mg K_2O, 8.1 Mg and 1.71% humus, and pH in 1 N KCl was 5.6. The subject of the study was potato of the Tajfun variety cultivated after spring barley. Various organic fertilizers were applied: bovine manure, barley stubble, spring barley straw; intercrop (white mustard), and spring barley wheat + intercrop (white mustard). At those study plots with manure and stubble, phosphorus and potassium fertilization (80 kg P_2O_5f and 120 kg $K_2O{\cdot}ha^{-1}$) was used in autumn. On the remaining objects prior to the sowing of white mustard (second ten days of August), organic fertilizers were covered by pre-winter plowing. 80 kg $N{\cdot}ha^{-1}$ of nitrogen fertilizers in the amount of were used in Spring prior to planting. Urea in the amount of 1 kg per 100 kg of straw was applied in those plots fertilized with straw. Potatoes were planted in the second ten days of April with a spacing of 30 cm × 62.5 cm, the field size was 40 m^2, and the total working surface area was 800 m^2. The experiment was performed in a randomized block design in four replicates. Care and protection treatments were applied in accordance with the accepted principles of good agricultural practice. Until the period of emergence, mechanical and chemical maintenance was performed (1 herbicide procedure – s.a. linuron 2 $kg{\cdot}ha^{-1}$). Three fungicidal applications were performer against *Phytophthora infestans* and the same number of procedures with insecticides against *Leptinotarsa decemlineata*.

2.2. The characteristics of the organic matter and hydrothermal conditions of the experiment

In the tested organic matter the following macroelements were determined: total nitrogen – Kjeldahl method (FOSS2200), phosphorus – colorimetry, Egner – Rriehm method (SPEKOL 21), potassium – using the FLAPHO40 flame spectrophotometry emission method. The examined organic fertilizers differed in dry matter content and chemical composition (Table 1). The analyzed years were characterized by an irregular distribution of precipitation (Table 1). In 2013 and 2014 the total precipitation (421.0, 448.5) per vegetative period was at the optimum level for potato. In contrast, precipitation deficiencies and higher temperatures were determined in 2012. The Sielianinov indices (K) for the year 2013 clearly indicate interchanging extremely humid and humid periods (June, September) and extremely dry and dry (August, July). The most favorable hydrothermal conditions for the growth and development of potato were observed in 2014, which is demonstrated by the values of the index remaining in the range from 1.22 to 2.55.

2.3. Collection and storage of samples for the evaluation of the occurrence of tuber dry rot

During the harvest (1st/2nd ten days of September), 10 kg samples were collected from each field. The potato tubers were equal in terms of size (5–6 cm diameter), not mechanically damaged and healthy – without any signs of rot. Samples were stored in plastic openwork chests for 7 months at a temperature of 6 °C and 90% relative air humidity. Following the storage period, the percentage share of tubers with dry rot symptoms – sunken, necrotic tuber tissue change without liquid secretion (Fig. 1a). Batches of infected tubers were weighed, and their weight was compared with the sample weight.

2.4. Isolation and identification of fungi colonizing dry-rotting tubers

Isolation of the fungi from potato tubers infected by dry rot were performed after each storage period. In accordance with the generally assumed phytopathological procedure, from each experimental combination the same amount of tubers with clear dry rot symptoms was selected. The infected tubers were washed under running water, cut with a sterile scalpel into 3–4 parts in the lesion site. Sections of 2–3 mm were cut on the borderline of healthy and diseased tissue, and these were disinfected for 30 s in 50% ethanol, and rinsed twice in sterile distilled water. Dried sections were placed on PDA medium (Potato Dextrose Agar), 10 pieces on each, with 0.05 g/L chloramphenicol in Petri dishes. 200 pieces were taken from each combination. The plates were incubated at a temperature of 23 °C for 5–7 days under a 12 h lighting cycle. Fungal cultures were passed to PDA medium and water agar.

Fungi species were identified using the MALDI-TOF MS (Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) technique, utilizing mass spectroscopy for the determination of protein ribosome profiles. Reference spectra for these are available in the database of the Bruker Daltonik GmbH library.

The culture and preparation of filamentous fungi samples were performed in accordance with the standard procedure developed by Bruker Daltonik GmbH (De Carolis et al., 2012). Samples from 8 ml modified liquid medium, catalog No.: 221014 (Becton Dickinson) were inoculated with a small amount of three-day fungi filaments and closed with a cap. Subsequently, they were placed in a SB2 rotator (cat. No.: Y552, Carl Roth GmbH & Co KG) and, with top mixing, they were incubated until a sufficient amount of biological material was determined.

A solution of alpha-cyano-4-hydroxycinnamic acid was prepared - HCCA matrix (cat. No.: 8255344). To the tube containing HCCA, 250 µl of standard solvent was admeasured (acetonitrile 50%, water 47.5% and trifluoroacetic acid 2.5%, cat. No.: 19182, Sigma-Aldrich) and this was shaken on a vortex at room temperature, until the solution became clear. The culture tubes were transferred from the rotator onto a table. After approx. 10 min, 1.5 ml of precipitate was collected from the bottom of the tubes to an Eppendorf tube and this was then centrifuged for 2 min at 13,000 rpm (table centrifuge – Biofuge fresco: cat. No. 75005510, Scientific).

Then, the supernatant was removed, the tube was supplemented with 1 ml deionized water and centrifuged again. These procedures were repeated to rinse the precipitate lump, to which 300 µl of water and 900 µl of absolute ethanol (EtOH) were added. The whole mixture was shaken on a vortex, and then centrifuged at maximum rate (13,000 rpm).

With the use of a pipette, the supernatant was carefully removed, then centrifuged for a few seconds and the remaining ethanol was entirely removed. The precipitate lump was left for several minutes until it dried up completely. Appropriately to the amount of sediment, from 10 µl to 20 µl of 70% formic acid was added to the sediment (a maximum of 100 µl in the case of a large lump). The tube was supplemented with the same amount of acetonitrile and carefully mixed, and centrifuged for 2 min at approx. 13,000 rpm.

1 µl of the supernatant (raw protein extract) was applied on a steel MALDI plate and left to dry at room temperature. Then, 1 µl of the HCCA solution was applied and also left to dry at room temperature.

The plate was placed into a MALDI Biotyper microflex apparatus, connected via computer to the database of reference spectra for fungi