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EFFECT OF INFECTION OF VIRGINIA MALLOW SHOOTS BY FUNGUS *Sclerotinia sclerotiorum* ON THE CONTENTS AND DISTRIBUTION OF ALUMINIUM, MANGANESE AND IRON

Dorota Kalembasa[1], Dawid Jaremko[1], Beata Bik[1], Joanna Kaczmarek[2], Małgorzata Jędryczka[2]

[1] Siedlce University of Natural Sciences and Humanities
[2] Institute of Plant Genetics of Polish Academy of Sciences

Abstract. The aim of the study was to determine the effect of inoculation of Virginia mallow (*Sida hermaphrodita* [L.] Rusby) with fungus *Sclerotinia sclerotiorum* [Lib.] de Bary, which causes stem rot, on the contents and distribution of aluminium, manganese and iron in the shoots of this plant. Field study was carried out during three years (2011--2013) in the soil and weather conditions of central-west Poland. Inoculation was carried out with isolates obtained from rapeseed and mallow. It consisted in a slight damage of stem epidermis and inserting wheat grain overgrown with mycelium of a given isolate. During plant growth, strong infection of mallow shoots was observed, which caused inhibition in the growth and development of the plant. At the stage of technological maturity, shoots were harvested from both the control and inoculated plots for chemical analyses. Contents of the studied elements were determinated in analytical solutions obtained from biomass ash after its "dry combustion" method, using ICP-OES spectrometer. Contents and distribution of aluminium, manganese and iron in mallow shoots depended upon plant parts and placement of pathogens on the stem. The highest amount of aluminium was found in the inoculated part, and the lowest in the apices part, regardless of isolate origin. In the case of iron and manganese distribution, statistically significant effect of the applied pathogen was found. Isolate from the mallow caused greater manganese accumulation and lower iron accumulation. The conducted study demonstrates a significant effect of infection with fungus *S. sclerotiorum* on the physiological processes that occur in Virginia mallow plants.

Key words*:* Al, Fe, Mn, *Sclerotinia sclerotiorum*, *Sida hermaphrodita*, stem rot

* Corresponding author: prof. dr hab. Małgorzata Jędryczka, Institute of Plant Genetics of Polish Academy of Sciences in Poznań, Strzeszyńska 34, 60-479 Poznań, e-mail: mjed@igr.poznan.pl

INTRODUCTION

Plants grown for biomass, including some grass species, willow, and Victoria mallow, play an increasingly important role on the energy market as a source of renewable energy [Kościk 2003, Dudkiewicz and Bolibok 2011, Niedziółka 2012]. High amount of formed biomass is an advantage of those plants [Borjesson 1996, Borkowska 1996, Kalembasa 2006, Kalembasa and Wiśniewska 2006, Kuś and Matyka 2009, Stolarski *et al*. 2011, Borkowska *et al*. 2015], as well as the effect they have on limiting CO_2 emission to the atmosphere. During plant biomass combustion, the excreted CO_2 may be taken up by plants during photosynthesis, in so called sequestration process which causes that the greenhouse gas enter a closed circulation. According to Directive 2009/28/EC in the European Union countries, until the year 2020, 20% of electric energy ought to come from renewable energy sources (RES), and for transportation means: 10%. In Poland, RES share in the final energy consumption until 2020 should amount to 15%, and in fuels: 10% [Niedziółka 2012]. The above settlement results from the decisions of the climate and energy panel from 2008, which oblige the EU member countries to, by the year 2020: 1) reduce CO_2 emission by 20% in relation to the year 1990, 2) increase the participation of RES energy to 20%, 3) decrease energy consumption by 20% in relation to the foreseen amount, through increasing energy effectiveness.

In order to fully cover the demand for biomass intended for solid fuel, energy plant plantation area in Poland, in the year 2020, ought to reach 660 000 ha [Kuś and Matyka 2009], taking into account the economic side of the problem and environment protection. Considering the demand of local electrical power and heating plants for constant and systematic biomass supply, it was first assumed that, in the future, the majority of the materials would originate from perennial energy plant plantations. However, since the year 2009, there has been no significant increase in the cultivation areas due to the lack of demand in industrial energy production for this kind of material. In March 2015, President of Poland signed a bill on renewable energy sources (RES). The bill was ratified after nearly five years of workings. Its aim is to guarantee continued economy growth with a simultaneous increase in energy security and environment protection. Significant part of the regulations in the bill concerns new forms of support for RES energy producers. It is expected that, with the bill, feed-in tariffs will be introduced, which will ensure that the producers sell electric energy produced in small, home RES installations, at feed-in tariffs for 15 years. Introduction of the above regulations and the necessity to carry out the decisions included in new regulations caused a development in studies related to, among others, agrotechnics problems (fertilization, harvest date, or equipment), and also further dealing with biomass, in order to obtain as best energy acquiring parameters as possible.

Energy plants, depending on the species, may be cultivated on soils of different quality, but their higher yield is obtained on soils with optimum humidity, rich in available nutrients [Borkowska and Lipiński 2008, Kuś and Matyka 2009].

Virginia mallow (*Sida hermaphrodita* L. Rusby) is one of the energy plants the shoots of which are infected by *Sclerotinia sclerotiorum* [Lib.] de Bary, which causes stem rot. Those fungi belong to the Ascomycota type in the subkingdom of Dikarya. Infection is conditioned by the presence of inoculum, including mainly the ascospore of that fungus and the hydrothermal characteristics of given climate, chiefly air humidity [Purdy 1979]. With conditions favourable to disease development, the fungus causes

heavy symptoms on plants and may contribute to significant yield losses in many species, including Virginia mallow.

Research was carried out on the effect of infection by *S. sclerotiorum* on the contents and distribution of chosen elements (Al, Mn, and Fe) in the shoots of Virginia mallow.

MATERIAL AND METHODS

Study material was uptaken from the filed experiment carried out at the Experimental Station of the Institute of Plant Genetics of the Polish Academy of Sciences in Cerekwica near Szamotuły (52º31' N; 16º41' E), in the years 2011-2013, on light typical lessives soil with the granulometric composition of loamy sand, with slightly acid reaction (pH_{KCl} = 6.0). Experiment was conducted on a Virginia mallow plantation with yearly maintenance and biomass harvest like on a production plantation (fertilization with; N- 90, P- 54 and K- 100 $kg \cdot ha^{-1}$). Detailed studies started at a four-year-old plantation (first study year) with settled lowland meadow structure, and in the subsequent years on five-year-old and six-year-old plantations, respectively, (second and third study year), (study years were the third study factor: C). In order to obtain strong, steady mallow shoot infection with *S. sclerotiorum,* inoculation was carried out with the use of isolate Sc-8 obtained from winter rapeseed and Sc-1-68 from Virginia mallow (first factor: A), cultivated for 10 days at room temperature on wheat seeds. Inoculation consisted in a slight damage of stem epidermis at the height of about 50 cm from the soil surface and putting infected wheat grain on the same spot. In every variant, 15 plants were inoculated, choosing healthy mallow plants of medium height and thickness, typical structure and shoot number. Inoculation was carried out in mid-June. Control group was made of plants treated in the same way as inoculated plants, but the applied wheat grains were not infected with *S. sclerotiorum*. After the end of growth and when mallow reached technological maturity, 10 plant shoots were chosen from every plot. The experiment was carried out in four replication

The plant samples from each objects (control and infected plants), mallow shoots were divided into four parts (second study factor: B): 1 – lower shoot part (below the inoculation spot), 2-inoculated part at the high of 30 – 60 cm, 3 – part above the inoculation spot with no visible symptoms of stem rot infection, 4 – apices part of the plant. The collected fragments of the particular mallow shoot parts were used for chemical analysis. Plant material was fragmented to parts below 0.25 mm and oxidized by "dry combustion " method at the temperature of 450ºC . The obtained crude ash was dissolved with aqueous solution of hydrochloric acid (1:1) in order to decompose carbonates. The excess of HCl was evaporated to dryness at sand bath. The obtained proper ash was again dissolved with 10% HCl in order to obtained analytical solution, which was infiltrated through hard Whatman filter paper in order to separate silica and then it was transferred to a volumetric flask and filled up with water to strictly specific volume. In thus prepared solution, total contents of aluminium, manganese and iron were determined using an atomic emission spectroscope ICP-AES Optima 3200RL made by Perkin Elmer. The obtained study results were statistically processed with the analysis of variance, and in the case of significance, $LSD_{0.05}$ and $LSD_{0.01}$ values with the Tukey's test were calculated.

RESULTS AND DISCUSSION

In the dry matter of Virginia mallow shoots (its four parts), inoculated with two fungus *S. sclerotiorum* isolates, grown in the soil and weather conditions of central-west Poland, during three years, diversified contents of aluminium, manganese and iron were found.

Aluminium content in the analyzed mallow parts, average from the studied plants, amounted to 60.5 mg·kg^{-1} (Table 1).

Table 1. Aluminium content in stems of Virginia mallow (*Sida hermaphrodita* [L.] Rusby), mg·kg^{-1} of d.m.

Factor		C			Mean
A	B	1st year	2nd year	3rd year	
A1	1	95.6	62.1	58.3	**72.0**
	2	76.5	68.4	59.0	**68.0**
	3	73.8	37.9	29.5	**47.1**
	4	55.8	29.4	26.7	**37.3**
Mean		**75.4**	**49.5**	**43.4**	**56.1**
A2	1	93.9	42.5	47.7	**61.4**
	2	95.3	49.6	38.4	**61.1**
	3	84.2	47.8	51.0	**61.0**
	4	78.9	68.2	11.6	**52.9**
Mean		**88.1** [a]	**52.0** [b]	**37.2** [b]	**59.1**
A3	1	105.4	51.9	36.7	**64.7**
	2	115.5	80.9	56.7	**84.4**
	3	96.5	31.2	49.7	**59.1**
	4	90.3	37.7	45.1	**57.7**
Mean		**101.9** [a]	**50.4** [b]	**47.1** [b]	**66.5**
A	1	**98.3**	**52.2**	**47.6**	**66.0**[ab]
	2	**95.8**	**66.3**	**51.4**	**71.1**[a]
	3	**84.8**	**39.0**	**43.4**	**55.7**[ab]
	4	**75.0**	**45.1**	**27.8**	**49.3** [b]
Mean		**88.5** [a]	**50.6** [b]	**42.5** [b]	**60.5**
$LSD_{0.05}$* and $LSD_{0.01}$**					
part of stem		17.1			
year of experiment		13.0*; 17.5**			

A – inoculation; A1 – control group, A2 – isolate from winter rapeseed Sc-8, A3 – isolate from Virginia mallow Sc-1-68

B – part of stem: 1 – bottom part, 2 – inoculation spot, 3 – part above inoculation, 4 – apices part

C – year of experiment 1st, 2nd, 3rd

The amount of this element was significantly diversified in the particular shoot parts and study years. On average, the highest amount of aluminium (71.1 mg·kg^{-1}) was found in the inoculated shoot part, and the lowest amount in the apices part (49.3 mg·kg^{-1}). This fact may denote limited aluminium transportation due to inoculation. In the first study year, mallow shoots contained the highest amount of aluminium, on average, (88.5 mg·kg^{-1}), and in the third year the lowest amount (42.5 mg·kg^{-1}), (no significant differences were found between the contents in the second and third study year). Effect of the discussed factors was also significant at the level of $LSD_{0.01}$, which indicates clear direction of the effect of a given factor. Physiological role of aluminium is not fully clear. It is assumed that small amounts of this element regulate physical properties

of plasma and permeability of cell membrane. Excess of aluminium decreases biomass growth, in particular roots, limits photosynthesis, and may cause changes in DNA [Kabata-Pendias and Szteke 2012].

Average manganese content in mallow shoots amounted to 11.6 mg·kg^{-1} and was diversified depending on plant healthiness and study year (Table 2). Borkowska and Lipiński [2007], in the shoots of Virginia mallow grown on very acid soil, in the soil and weather conditions of central-east Poland, stated 25.6 mg Mn·kg^{-1} dry matter. The highest amount of manganese was found in mallow shoots collected in the first study year (18.0 mg·kg^{-1}), and significantly less in the second and third year (8.25 and 8.68 mg·kg^{-1}, respectively). In the described experiment, the inoculation of mallow shoots with isolate Sc-1-68 caused a significant increase in manganese content (14.3 mg·kg^{-1} on average), in relation to its amount in non-inoculated shoots (9.75 mg·kg^{-1} on average). Inoculation with isolate Sc-8 only to a small degree affected the increase in manganese content (10.8 mg·kg^{-1} on average), in comparison with the shoots of the control plants. Different response of plants was probably caused by differences between the studied pathogen isolates. It results from the studies conducted by Ziman *et al*. [1999] that between the isolates of the same fungus species, significant differences may occur in isolate aggressiveness and in them forming toxic secondary metabolites. The above results were confirmed by tests, which indicated significant differences between isolates at the genetic level [Sun *et al*. 2005].

Table 2. Manganese content in stems of Virginia mallow (*Sida hermaphrodita* [L.] Rusby), mg·kg^{-1} of d.m.

Factor		C			Mean
A	B	1st year	2nd year	3rd year	
A1	1	17.7	4.12	4.89	**8.90**
	2	18.2	4.99	5.92	**9.70**
	3	21.4	4.12	3.96	**9.83**
	4	13.4	9.02	9.24	**10.6**
Mean		**17.7**	**5.56**	**6.00**	**9.75^{b}**
A2	1	17.4	7.14	11.7	**12.1**
	2	17.6	7.94	5.48	**10.3**
	3	13.9	6.2	9.22	**9.77**
	4	14.4	10.1	9.06	**11.2**
Mean		**15.8**	**7.85**	**8.87**	**10.8^{ab}**
A3	1	32.7	8.71	9.18	**16.9**
	2	24.3	16.1	11.7	**17.4**
	3	10.8	12.6	12.6	**12.0**
	4	13.9	8.01	11.2	**11.0**
Mean		**20.4**	**11.4**	**11.2**	**14.3^{a}**
A	1	**22.6**	**6.66**	**8.59**	**12.6**
	2	**20.0**	**9.68**	**7.70**	**12.5**
	3	**15.4**	**7.64**	**8.59**	**10.5**
	4	**13.9**	**9.04**	**9.83**	**10.9**
Mean		**18.0^{a}**	**8.25^{b}**	**8.68^{b}**	**11.6**
$LSD_{0.05}$* and $LSD_{0.01}$**					
inoculation		4.49			
year of experiment		4.49*; 6.01**			

explanations as in Table 1

Average iron content in the dry mass of mallow shoots amounted to 70.8 mg·kg^{-1} and was diversified in regard to inoculation and study year (Table 3). Higher amounts of this element in mallow shoots (183.2 mg·kg^{-1}) were found by Borkowska and Lipiński [2008], which may be explained by higher uptake of Mn and Fe from the soil of very acid pH than from less acid soil. The authors also noted an increase in the iron content with higher nitrogen and phosphorus fertilization doses. Inoculation with isolate Sc-1--68 caused a significant increase in the iron content in mallow shoots (98.6 mg·kg^{-1} on average), in comparison with isolate Sc-8 (54.5 mg·kg^{-1} on average). Significantly higher iron content was found in mallow shoots collected in the first study year (112.3 mg·kg^{-1} on average), in comparison with the second year (54.5 mg·kg^{-1} on average) and the third year (45.8 mg·kg^{-1} on average). Relations for iron were similar to the ones for aluminium content.

Table 3. Iron content in stems of Virginia mallow (*Sida hermaphrodita* [L.] Rusby), mg·kg^{-1} of d.m.

Factor		C			Mean
A	B	1st year	2nd year	3rd year	
A1	1	74.6	62.1	73.6	**70.1**
	2	104.2	81.4	44.0	**76.5**
	3	81.5	34.2	22.3	**46.0**
	4	68.4	33.5	32	**44.6**
Mean		**82.2**	**52.8**	**43.0**	**59.3^{ab}**
A2	1	77.9	32.4	59.4	**56.6**
	2	91.3	38.6	16.2	**48.7**
	3	98.1	29.4	48.3	**58.6**
	4	86.2	52.4	24.1	**54.2**
Mean		**88.4**	**38.2**	**37.0**	**54.5^{b}**
A3	1	205.4	106.1	20.4	**110.6**
	2	238	82.3	60	**126.8**
	3	75.9	40.3	123	**79.7**
	4	145.7	60.7	25.9	**77.4**
Mean		**166.3**	**72.4**	**57.3**	**98.6^{a}**
A	1	**119.3**	**66.9**	**51.1**	**79.1**
	2	**144.5**	**67.4**	**40.1**	**84.0**
	3	**85.2**	**34.6**	**64.5**	**61.4**
	4	**100.1**	**48.9**	**27.3**	**58.8**
Mean		**112.3^{a}**	**54.5^{b}**	**45.8^{b}**	**70.8**
$LSD_{0.05}$* and $LSD_{0.01}$**					
inoculation		39.7			
year of experiment		39.7*; 53.2**			

explanations as in Table 1

Analysis of the results demonstrated that study years significantly affected aluminium, manganese and iron contents in Virginia mallow shoots. Aluminium content was also significantly affected by shoot parts, and manganese and iron contents also by plant healthiness. The same relation between Mn and Fe contents and the discussed characteristics may be explained by the similarity of chemical properties of those elements, particularly oxidation and reduction effect..

SUMMARY AND CONCLUSIONS

Inoculation of Virginia mallow shoots with two isolates of fungus *Sclerotinia sclerotiorum* diversified the contents of aluminium, manganese, and iron in four separate parts of the tested plant stems.

1. In the dry mass of Virginia mallow shoots, grown in the soil and weather conditions of central-west Poland, collected at the stage of technological maturity, in a three-year-long field experiment, average contents of the determinate elements amounted as means from all objects in $mg \cdot kg^{-1}$: aluminium: 60.5, manganese: 11.6, and iron: 70.8.
2. Contents of Al, Mn, and Fe in mallow shoots were significantly affected by the study year and plant part.
3. Aluminium content was significantly diversified in the separate mallow shoot parts. The highest amount of this element was found in the inoculated part, and the lowest in the apices part.
4. Inoculation of mallow shoots with the isolates of fungus *S. sclerotiorum* Sc-1-68 (mallow isolate) significantly increased average manganese content, in comparison with the control plants, as well as average iron content, in relation to the amount of this element in the shoots inoculated with isolate Sc-8 (winter rapeseed isolate).
5. Infection of Virginia mallow plants significantly affected the contents and distribution of aluminium, manganese, and iron in the plants.

It results from the conducted research that intrusive and polyphagous fungus *S. sclerotiorum* to a high degree changes the physiology of the plants it attacks, which then affects the contents and distribution of elements, and therefore also the composition of ash obtained during biomass combustion. Further research in this matter is necessary in order to check what the occurrence and distribution of other elements in mallow plants is. The most recent studies point out to the possibility of using plants grown for biomass for other purposes than only obtaining energy, for example for the production of more easily biodegraded materials, such as PET with the addition of 40% lignocellulosic mass [Paukszta *et al*. 2013] .

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WPŁYW PORAŻENIA PĘDÓW ŚLAZOWCA PENSYLWAŃSKIEGO GRZYBEM *Sclerotinia sclerotiorum* NA ZAWARTOŚĆ I ROZMIESZCZENIE GLINU, MANGANU I ŻELAZA

Streszczenie. Celem badań było oznaczenie wpływu inokulacji ślazowca pensylwańskiego (*Sida hermaphrodita* [L.] Rusby) grzybem *Sclerotinia sclerotiorum* [Lib.] de Bary, wywołującym zgniliznę twardzikową, na zawartość i rozmieszczenie glinu, manganu i żelaza w pędach tej rośliny. Badania polowe prowadzono przez trzy lata (2011-2013) w warunkach glebowo-klimatycznych środkowozachodniej Polski. Inokulację przeprowadzono izolatami uzyskanymi z rzepaku i ślazowca; polegała ona na lekkim uszkodzeniu epidermy na łodydze i umieszczeniu w tym miejscu ziarna pszenicy przerośniętego grzybnią danego izolatu. W trakcie wegetacji roślin stwierdzano silne porażenie pędów ślazowca, powodujące zahamowanie wzrostu i rozwoju roślin. W fazie dojrzałości technologicznej z obiektów kontrolnych i inokulowanych pobrano pędy do analiz chemicznych. Zawartość badanych pierwiastków oznaczono w roztworach analitycznych uzyskanych z popiołu biomasy po jej mineralizacji „na sucho", na spektrometrze ICP--OES. Zawartość i rozmieszczenie glinu, manganu i żelaza w pędach ślazowca była uzależniona od zdrowotności roślin oraz miejsca na łodydze. Najwięcej glinu stwierdzano

w części inokulowanej, a najmniej w części wierzchołkowej, niezależnie od pochodzenia izolatu. W przypadku rozmieszczenia żelaza i manganu stwierdzono statystycznie istotny wpływ zastosowanego patogena; izolat ze ślazowca spowodował większe nagromadzenie manganu, ale mniejsze – żelaza. Wykonane badania wskazują na znaczny wpływ porażenia grzybem *S. sclerotiorum* na procesy fizjologiczne przebiegające w roślinach ślazowca pensylwańskiego. Osobniki z objawami zgnilizny twardzikowej tworzą biomasę o innych parametrach jakościowych.

Słowa kluczowe: Al, Fe, Mn, *Sida hermaphrodita, Sclerotinia sclerotiorum*, zgnilizna twardzikowa

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