

**THE EFFECT OF MINERAL FERTILIZATION  
ON ACHENES YIELD AND FUNGAL COMMUNITIES  
ISOLATED FROM THE STEMS OF MILK THISTLE  
*Silybum marianum* (L.) GAERTNER**

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**Abstract.** The achenes of milk thistle contain a variety of lipids, proteins and biologically active substance, which is why they are used in pharmaceutical and cosmetic products, as well as an ingredient of functional food and animal feed. The yield of milk thistle is determined by both agrotechnological factors (sowing date, cultivation regime, fertilization) and the health status of plants. The study was conducted over the years 2009–2011 in experimental plots located in Tomaszkowo (NE Poland). The experiment involved the following treatments: 1. N<sub>0</sub>PK, 2. N<sub>0</sub>PKMg, 3. N<sub>0</sub>PKMg+microelement B, 4. N<sub>1</sub>PK, 5. N<sub>1</sub>PKMg, 6. N<sub>1</sub>PKMg+B, 7. N<sub>2</sub>PK, 8. N<sub>2</sub>PKMg, 9. N<sub>2</sub>PKMg+B, 10. N<sub>3</sub>PK, 11. N<sub>3</sub>PKMg, 12. N<sub>3</sub>PKMg+B (where: N<sub>0</sub> – without nitrogen fertilization, N<sub>1</sub> – 40 kg · ha<sup>-1</sup> /ammonium nitrate/, N<sub>2</sub> – 80 kg · ha<sup>-1</sup>, N<sub>3</sub> – 120 kg · ha<sup>-1</sup>, P – 40 kg · ha<sup>-1</sup> /triple superphosphate/, K – 117 kg · ha<sup>-1</sup> /60% potash salt/, Mg – 20 kg · ha<sup>-1</sup> /kieserite/ before sowing, B – foliar application/Bormax/). The structure of fungal communities colonizing the stems of milk thistle was analyzed at the laboratory. Achene yield was determined after harvest. The composition of fungal communities colonizing the stems of milk thistle was affected by weather conditions, and macronutrient and B fertilization. Potential pathogens had a 50–80% share of the fungal community. The predominant species was *Alternaria alternata*, fungi of the genus *Fusarium* were identified less frequently (six species), while *Rhizoctonia solani*, *Botrytis cinerea* and *Phoma* spp. were encountered only sporadically. The abundance of *A. alternata* was lower in treatments without N fertilization and with N fertilization at 40 kg P · ha<sup>-1</sup>. In contrast to *A. alternata*, fungi of the genus *Fusarium* were less abundant in treatments with Mg and Mg+B fertilization. The yield of milk thistle achenes increased in response to increasing rates of nitrogen fertilization.

**Key words:** achenes of milk thistle, N, P, K, Mg, B fertilization, fungi, yield of seeds

## INTRODUCTION

Milk thistle *Silybum marianum* (L.) Gaertn. is an annual plant of the Aster family (*Asteraceae*), subfamily *Asteroidae* (syn. *Tubiflorae*). The species is native to Southern Europe, North Africa and Western Asia; it has been introduced to North America, South America and Australia, and it is cultivated in Argentina and North Africa [Frohne 2010]. In Poland, milk thistle is considered a weed, but it is also grown as an ornamental plant and a vegetable (young lower leaves), and it is commonly used as a medicinal plant. The vegetative organs of milk thistle plants, and mostly seeds (achenes), contain 25% essential oil (63% linoleic acid and approx. 20% oleic acid, 0.63% sterols, 0.038% tocopherols and phospholipids, 25–30% protein and minerals). The seeds of milk thistle contain the biologically active substances flavonolignans, including 1.5–3% silymarin and trace amounts of taxifolin, quercetin, dihydrocampherol and apigenin. *Flavonolignans* identified in the *silymarin complex* include silibinin (most potent), silydianin and silychristin [Kurkin et al. 2003, Kang JongSoon et al. 2004, Toklu et al. 2007]. Silymarin protects hepatocytes and the gallbladder against damage and lesions. It also helps regenerate the damaged cells by stimulating protein biosynthesis and mitotic divisions [Dvorak et al. 2003, Sridar et al. 2004]. Kang JongSoon et al. [2004] have reported the anti-inflammatory and antiseptic effects of silymarin in human patients, while Kurkin et al. [2003] and Ahmed et al. [2008] have described its antioxidant properties. The potential applications of silymarin in cancer treatment have also been investigated [Flora et al. 1998, Dhanalakshmi et al. 2004, Tyagi et al. 2004].

Milk thistle can be used for bioenergy purposes as it produces high biomass under conditions of low-input agriculture [Sulas et al. 2008]. The high content of protein and oil with a well-balanced fatty acid profile makes milk thistle a desirable ingredient of animal feed [Langowska et al. 2002, Urbańczyk et al. 2002]. The proteins contained in the oil cake may be used to obtain antibacterial and antifungal peptides for the production of cosmetics and pharmaceuticals [Hadolin et al. 2001, Andrzejewska and Skinder 2007, Szczucińska et al. 2007]. Research results show that the yield of *Calendula officinalis* achenes [Król 2011] and milk thistle fruit [Kozera and Nowak 2004, Andrzejewska and Sadowska 2008] is determined by various agrotechnological factors, including NPK fertilization. According to the above authors, NPK fertilizers have an insignificant effect on the chemical composition of milk thistle achenes, but they increase the concentrations of silymarin and potassium. Foliar application of Basfoliar 36 Ex increases both the fruit yield and the content of K, P and fat. According to Andrzejewska and Skinder [2007], delayed sowing and crop monoculture, even short-term, lead to a decrease in achene yield. As demonstrated by Dyduch and Najda [2007], thousand achene weight is correlated with achene color – dark brown fruits have higher weight. In a study by Andrzejewska and Sadowska [2008], milk thistle yield was significantly correlated with plant height before harvest, the number of fruits per inflorescence on the main shoot and lateral shoots. The yield can also be reduced by pest infestations [Andrzejewska et al. 2006] and infections caused by pathogenic fungi, *Botrytis cinerea* and species of the genera *Alternaria*, *Fusarium*, *Rhizoctonia* and *Phoma*, commonly found in the soil environment of herbaceous and garden plants [Zimowska and Machowicz-Stefaniak 2004, Berbegal et al. 2007, Machowicz-Stefaniak and Zalewska 2007, Ortega and Pérez, 2007].

The aim of this study was to determine the effect of different mineral fertilization levels on the composition of fungal communities colonizing the stems of milk thistle and achene yield.

## MATERIALS AND METHODS

Milk thistle [*Silybum marianum* (L.) Gaertn.] from a selected local population was grown in a field experiment established in Tomaszkowo (53°73'13"N, 20°40'55"E) in 2009–2011, in a randomized block design with three replications. 16 kg seeds were sown per hectare, at row spacing of 40 cm. The experimental design involved the following treatments: 1. N<sub>0</sub>PK, 2. N<sub>0</sub>PKMg, 3. N<sub>0</sub>PKMg+microelement B, 4. N<sub>1</sub>PK, 5. N<sub>1</sub>PKMg, 6. N<sub>1</sub>PKMg+B, 7. N<sub>2</sub>PK, 8. N<sub>2</sub>PKMg, 9. N<sub>2</sub>PKMg+B, 10. N<sub>3</sub>PK, 11. N<sub>3</sub>PKMg, 12. N<sub>3</sub>PKMg+B. Nitrogen rates were as follows: N<sub>1</sub> – 40 kg · ha<sup>-1</sup>, N<sub>2</sub> – 80 kg · ha<sup>-1</sup>, N<sub>3</sub> – 120 kg · ha<sup>-1</sup>. Phosphorus was applied at 40 kg P · ha<sup>-1</sup> (triple superphosphate), potassium was applied at 117 kg K · ha<sup>-1</sup> (60% potash salt) and magnesium was applied at 20 kg Mg · ha<sup>-1</sup> (kieserite) before sowing. In treatments N<sub>1</sub> and N<sub>2</sub>, nitrogen (ammonium nitrate) was applied pre-sowing. In treatment N<sub>3</sub>, 80 kg N · ha<sup>-1</sup> was applied before sowing and 40 kg · ha<sup>-1</sup> was applied as top dressing at the rosette stage. Boron (Bormax) was applied to the leaves of milk thistle before flowering.

Stem segments collected from the lower part of milk thistle plants (30 samples per treatment) were cut into 1 cm pieces, disinfected with 50% ethylene and 1% sodium hypochlorite, and rinsed three times with sterile water. Fungi were cultured on the PDA medium, at 22°C for seven days. Fungal colonies were inoculated onto agar slants for microscopic identification [Arx 1970, Ellis 1971, Nelson et al. 1983]. Achene yield was determined immediately after harvest. The results were verified statistically (STATISTICA 2010). Weather conditions during the experimental period are presented in Table 1. May was rather cold in the growing season of 2009. Warmer weather lasted from the end of June to mid-August. Precipitation totals in the growing season of 2009 were within the normal range, but their distribution was uneven – half of the rainfall occurred in June. In the growing season of 2010, May was both cold and wet. Moderate temperatures were recorded in June, while July and August were very warm. Precipitation totals ranged from 80 mm to 95 mm in each of the months. In 2011, temperatures were moderate from the end of May until the end of the growing season. Rainfall totals were comparable with the long-term average, only in July the average rainfall was more than two times higher than normal (200 mm).

## RESULTS AND DISCUSSION

The fungal community colonizing the stems of milk thistle was diverse, and it consisted of 31 species of filamentous fungi, non-sporulating cultures and yeast-like fungi (tab. 2). Among saprotrophic fungi, species of the order *Mucorales* (*Mortierella alpina*, *M. isabelina*, *Mucor circinelloides*, *M. hiemalis*, *Rhizopus nigricans* and *Zygorhynchus* spp.) were abundant, accounting for 12.8% of all isolates. Fungi of the genus *Penicil-*

Table 1. Weather conditions (Meteorological Station in Tomaszkowo)  
Tabela 1. Warunki pogodowe (Stacja Meteorologiczna w Tomaszkowie)

| Month – Miesiąc   | 2009                            |                                   |                                 | 2010                              |                                 |                                   | 2011                            |                                   |                               | Mean for Srednia dla lat 1960–1990 |                               |                                 |                               |                                 |
|---|---------------------------------|-----------------------------------|---------------------------------|-----------------------------------|---------------------------------|-----------------------------------|---------------------------------|-----------------------------------|-------------------------------|------------------------------------|-------------------------------|---------------------------------|-------------------------------|---------------------------------|
|   | monthly mean średnia miesięczna | mean for 10 days średnia dekadowa | monthly mean średnia miesięczna | mean for 10 days średnia dekadowa | monthly mean średnia miesięczna | mean for 10 days średnia dekadowa | monthly mean średnia miesięczna | mean for 10 days średnia dekadowa |                               |                                    |                               |                                 |                               |                                 |
| May   | 12.4                            | 12.0                              | 12.0                            | 11.1                              | 14.0                            | 12.0                              | 10.8                            | 12.2                              | 12.9                          | 13.1                               | 8.7                           | 14.2                            | 16.2                          |                                 |
| June  | 14.9                            | 12.2                              | 13.8                            | 18.8                              | 16.4                            | 16.4                              | 18.2                            | 15.5                              | 15.6                          | 17.1                               | 18.8                          | 15.4                            | 16.9                          |                                 |
| July  | 20.4                            | 18.8                              | 23.1                            | 19.3                              | 21.1                            | 21.1                              | 20.0                            | 23.3                              | 20.1                          | 17.9                               | 17.1                          | 19.2                            | 17.6                          |                                 |
| August  | 17.6                            | 18.9                              | 17.1                            | 16.9                              | 19.3                            | 19.3                              | 20.7                            | 21.0                              | 16.6                          | 17.6                               | 18.2                          | 16.9                            | 17.7                          |                                 |
| mean for the growth season średnia dla sezonu wegetacji | 16.3                            |                                   | 17.2                            |                                   |                                 | 16.4                              |                                 |                                   |                               | 15.3                               |                               |                                 |                               |                                 |
| Month Miesiąc   | monthly total suma miesięczna   | total for 10 days suma dekadowa   | monthly total suma miesięczna   | total for 10 days suma dekadowa   | monthly total suma miesięczna   | total for 10 days suma dekadowa   | monthly total suma miesięczna   | total for 10 days suma dekadowa   | monthly total suma miesięczna | total for 10 days suma dekadowa    | monthly total suma miesięczna | total for 10 days suma dekadowa | monthly total suma miesięczna | total for 10 days suma dekadowa |
| May   | 52.9                            | 1.3                               | 6.8                             | 44.8                              | 131.9                           | 25.4                              | 46.1                            | 0.4                               | 51.1                          | 10.8                               | 32.5                          | 7.8                             |                               |                                 |
| June  | 136.9                           | 73.8                              | 32.8                            | 30.3                              | 84.8                            | 25.3                              | 36.0                            | 23.5                              | 81.7                          | 39.9                               | 30.7                          | 11.1                            |                               |                                 |
| July  | 48.3                            | 28.3                              | 9.8                             | 10.2                              | 80.4                            | 31.3                              | 6.2                             | 42.9                              | 202.8                         | 124.8                              | 36.4                          | 41.6                            |                               |                                 |
| August  | 19.3                            | 3.9                               | 12.8                            | 2.6                               | 95.3                            | 26.6                              | 17.4                            | 51.3                              | 82.1                          | 20.4                               | 44.9                          | 16.8                            |                               |                                 |
| ∑ for the growth season ∑ dla sezonu wegetacji          | 257.4                           |                                   | 392.4                           |                                   | 417.7                           |                                   | 284.4                           |                                   |                               |                                    |                               |                                 |                               |                                 |

*lium* had a low share (3.0%) of the community. The above fungi, except for species of the genus *Zygorhynchus*, were most often isolated from the stems of milk thistle in the last year of the study. Fungi of the order *Mucorales* and the genus *Penicillium*, as well as pathogens of the genera *Alternaria* and *Fusarium*, are frequently isolated from the aboveground parts of plants [Kulikov et al. 2006].

Table 2. Fungi isolated from the stems of milk thistle (mean for 2009–2011)

Tabela 2. Grzyby wyizolowane z łodyg ostropestu plamistego (średnia z lat 2009–2011)

| Species – Gatunek                                    | Experimental treatments – Obiekty doświadczenia |     |     |     |     |     |     |     |     |     |     |     |
|--|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|  | 1*  | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  |
| <i>Acremonium strictum</i> W. Gams                   |   |     |     |     | 6   |     | 7   | 5   |     | 21  | 8   | 4   |
| <i>Alternaria alternata</i> (Fr.) Keissler           | 53**  | 53  | 67  | 58  | 59  | 91  | 40  | 74  | 64  | 59  | 67  | 49  |
| <i>Arthrinium sphaerospermum</i> Fuckel              |   |     |     | 7   |     |     |     | 2   |     |     | 2   |     |
| <i>Botrytis cinerea</i> Pers.                        |   | 2   | 5   | 3   | 1   | 6   | 2   | 7   | 2   | 4   | 5   | 3   |
| <i>Cladosporium cladosporioides</i> (Fres.) de Vries | 2   |     | 2   |     |     |     | 6   |     | 6   |     | 10  | 3   |
| <i>Epicoccum</i> spp.                                | 4   | 6   | 14  |     | 5   |     | 5   |     |     |     | 7   |     |
| <i>Fusarium avenaceum</i> (Fr.) Sacc.                | 21  |     | 7   | 17  | 10  | 6   | 24  | 7   |     | 12  | 7   | 17  |
| <i>Fusarium culmorum</i> W.G.Sm.) Sacc.              | 13  | 13  | 10  | 45  | 12  | 4   | 7   | 5   | 1   | 4   | 6   | 6   |
| <i>Fusarium equiseti</i> (Corda) Sacc.               | 7   | 3   | 3   | 3   | 3   | 1   |     |     | 2   | 1   | 1   | 8   |
| <i>Fusarium fusarioides</i> Frag. & Cif.) Booth.     |   | 2   |     |     |     |     | 5   | 3   | 7   |     |     | 5   |
| <i>Fusarium oxysporum</i> Schlecht.                  | 12  | 5   |     |     | 2   |     | 2   |     |     | 2   |     |     |
| <i>Fusarium poae</i> Peck.) Wollenweber              | 3   |     |     | 2   | 2   |     |     | 3   |     | 3   | 5   | 2   |
| <i>Gliomastix murorum</i> (Corda) Hughes             |   |     | 2   | 2   |     |     |     | 2   |     |     | 2   | 3   |
| <i>Humicola brevis</i> Gilman & Abbott               |   |     | 6   |     |     |     |     |     |     |     |     |     |
| <i>Mortierella alpina</i> Peyronel                   | 1   |     |     |     |     |     |     |     |     |     | 2   | 10  |
| <i>Mortierella isabelina</i> Oudemans                |   | 1   |     |     |     |     |     |     |     |     | 5   | 3   |
| <i>Mucor circinelloides</i> von Tieghem              |   |     |     |     | 8   |     |     | 2   |     | 5   | 8   |     |
| <i>Mucor hiemalis</i> Wehmer                         | 5   | 5   | 8   | 14  | 20  | 6   | 9   | 22  | 3   | 22  | 20  | 6   |
| <i>Paecilomyces roseum</i> (Thom) Samson             |   |     |     | 2   |     |     | 2   | 4   |     |     | 3   |     |
| <i>Papulaspora irregularis</i> Hotson                |   |     |     | 4   |     |     |     | 7   | 18  | 6   |     | 8   |
| <i>Penicillium</i> spp.                              |   |     | 2   | 17  | 5   | 3   | 5   | 10  | 5   | 5   | 3   |     |
| <i>Phialophora</i> spp.                              |   |     |     |     |     |     | 8   | 1   | 2   | 3   |     | 4   |
| <i>Phoma</i> spp.                                    |   |     |     |     |     |     | 5   |     |     |     |     |     |
| <i>Rhizoctonia</i> spp.                              | 1   | 31  | 1   | 2   | 8   |     | 16  | 1   | 2   |     | 12  | 9   |
| <i>Rhizopus nigricans</i> Ehrenb.                    |   | 4   | 4   |     | 20  |     | 1   | 2   | 2   | 1   | 3   | 1   |
| <i>Sporotrichum olivaceum</i> Fries                  | 2   |     |     | 3   |     |     |     |     |     |     |     | 3   |
| <i>Stagonospora</i> spp.                             | 2   |     | 2   | 1   |     |     |     |     |     |     |     |     |
| <i>Trichoderma hamatum</i> (Bon) Bain                |   | 3   |     |     |     |     |     |     |     |     |     |     |
| <i>Trichoderma harzianum</i> Rifai                   |   |     |     |     |     |     | 1   |     | 1   |     | 2   |     |
| <i>Trichoderma koningii</i> Oudemans                 |   |     |     |     |     |     |     |     |     |     | 2   |     |
| <i>Zygorhynchus</i> spp.                             | 21  |     |     |     |     |     |     |     |     |     |     |     |
| Yeast-like fungi – Drożdżopodobne                    | 1   |     |     |     |     |     |     |     | 17  |     |     |     |
| Non-sporulating cultures<br>Kultury niezarodnikujące |   | 1   |     |     | 6   | 10  | 4   | 6   | 7   | 6   | 2   | 5   |
| Total – Razem  | 148   | 129 | 133 | 180 | 167 | 127 | 149 | 163 | 141 | 154 | 182 | 149 |

Explanations – objaśnienia: \*1 – N<sub>0</sub>PK, 2 – N<sub>0</sub>PKMg, 3 – N<sub>0</sub>PKMg + microelements – N<sub>0</sub>PKMg + mikroelementy, 4 – N<sub>1</sub>PK, 5 – N<sub>1</sub>PKMg, 6 – N<sub>1</sub>PKMg + microelements – N<sub>1</sub>PKMg + mikroelementy, 7 – N<sub>2</sub>PK, 8 – N<sub>2</sub>PKMg, 9 – N<sub>2</sub>PKMg + microelements – N<sub>2</sub>PKMg + mikroelementy, 10 – N<sub>3</sub>PK, 11 – N<sub>3</sub>PKMg, 12 – N<sub>3</sub>PKMg + microelements – N<sub>3</sub>PKMg + mikroelementy

\*\* number of isolates – liczba izolatów

Fungi of the genera *Gliocladium*, *Paecilomyces* and *Trichoderma*, showing antagonistic activity against pathogens, are a desirable component of soil fungi. In the present study, only single isolates of *Paecilomyces roseum*, *Trichoderma hamatum*, *T. harzianum* and *T. koningii* were obtained from the stems of milk thistle. Fungi of the genus *Trichoderma* (*T. hamatum*, *T. koningii*) were isolated by Kita [1988] from the phyllosphere of sunflowers, and by Moszczyńska et al. [2011] from *Echinacea purpurea*. The latter authors reported that the above fungi suppressed the growth of *Fusarium* pathogens.

The most diverse fungal community, including the highest percentage share of potential pathogens (76.7%), was isolated in the last year of the experiment. In 2009 and 2010, pathogenic fungi accounted for 72.5% and 51.8% of all isolates, respectively. The predominant species was *Alternaria alternata*, which, due to favorable weather conditions, had an approximately 60% of total fungal community in 2009 compared to 38.3% and 34.4% in 2010 and 2011, respectively. Gannibal [2010] added two new species to the 32 *Alternaria* species known from *Asteraceae* plants, *A. silybi* described from *Silybum marianum* and *A. simmonsii* from *Sonchus* sp. In a study by Moszczyńska et al. [2011], *A. alternata* and *Fusarium* spp. (*F. avenaceum*, *F. culmorum*, *F. equiseti* and *F. oxysporum*) occurred in great abundance in the phyllosphere of *Echinacea purpurea*. Similarly as in our experiment, high rainfalls contributed to the growth of *Fusarium* spp. In the growing season of 2011, characterized by heavy precipitation and moderate temperatures, fungi of the genus *Fusarium*, represented by six species, had the highest share (over 30%) of the isolated community. In the first two years of the study, the abundance of those fungi was lower by more than half. As demonstrated by Kita [1988], the above fungi are permanent components of communities isolated from the aboveground parts of sunflowers. Fungi of the genera *Alternaria* and *Fusarium*, as well as *Phoma*, *Rhizoctonia* and *Botrytis cinerea*, are commonly found on the green tops of vegetables and herbs [Machowicz-Stefaniak and Zalewska 2002, Machowicz-Stefaniak and Zimowska 2007]. Weather conditions in the growing season of 2011 promoted the growth of *Rhizoctonia* fungi (11.6%). In 2009, the group of potentially pathogenic fungi was included of single isolates of *Phoma*, and in 2010 and 2011 – *Botrytis cinerea*. Batra et al. [1981] reported that milk thistle plants were infected by *Puccinia* sp. and *Ustilago cardui*, whereas Moscow and Lindow [1989] observed infection caused by *Septoria silybi*.

In our experiment, mineral fertilizers had variable effects on the population size of potential fungal pathogens colonizing the stems of milk thistle in 2009 and 2011. In 2010, the share of pathogenic fungi in the community was lower in all treatments fertilized with 80 and 120 kg N·ha<sup>-1</sup> (21.3% to 52.0%) than in treatments without N fertilization and fertilized with 40 kg N·ha<sup>-1</sup> (57% to 87%, Fig. 1a, b, c). In the latter treatments, milk thistle plants were frequently colonized by the potential pathogen *A. alternata*. Throughout the experiment, this species was more abundant (except in a few cases) in fungal communities isolated from plants that received supplemental Mg and Mg+B fertilization, compared to those fertilized with NPK. The occurrence frequency of *Fusarium* fungi followed a certain pattern. In the first year of the study, with normal precipitation, *Fusarium* species were more frequently encountered in treatments without N fertilization and fertilized with 40 kg N·ha<sup>-1</sup> (up to 28%) than in treatments with

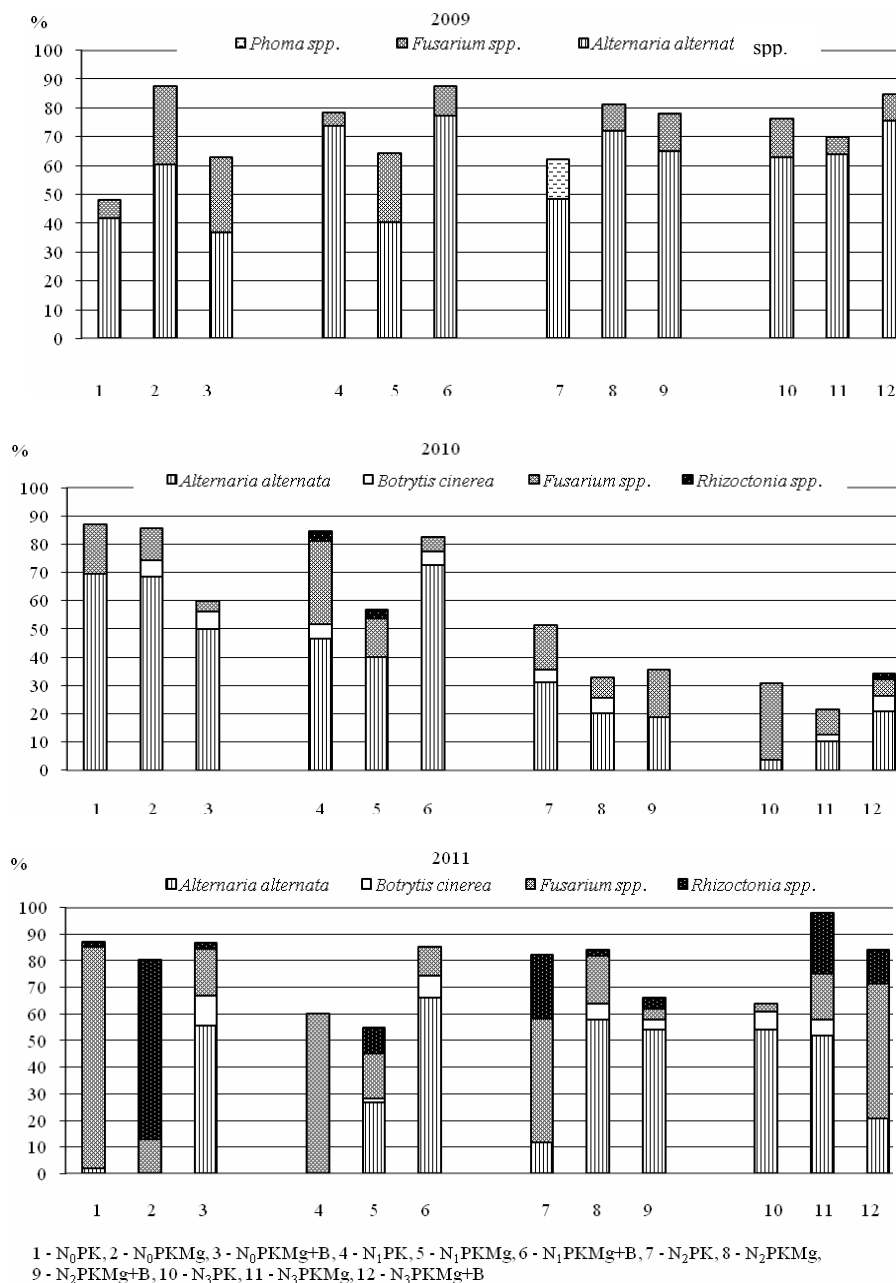


Fig. 1. Pathogenic fungi isolated from the stems of milk thistle in the experimental period  
 Rys. 1. Grzyby patogeniczne wyizolowane z podstawy łodyg ostropestu plamistego w okresie badań

higher rates of N fertilization (up to 14%). In the wet growing seasons of 2010 and 2011, *Fusarium* species were isolated in great abundance from treatments fertilized with PK and NPK, and this trend was particularly noticeable in the last year of the experiment: N<sub>0</sub>PK (83.3%), N<sub>1</sub>PK (60.0%) and N<sub>2</sub>PK (46.3%). Fungi of the genus *Fusarium* were less abundant in treatments with Mg and Mg+B fertilization, with the exception of N<sub>3</sub>PKMg+B. According to literature data [Cwalina-Ambroziak and Sienkiewicz 2008], increasing rates of mineral fertilizers contributed to the growth of *Fusarium* species and reduced the populations of *Epicoccum*, *Penicillium* and *Mucorales*. In a study by Moszczyńska et al. [2011], N fertilization had variable effects on the abundance of *Fusarium* fungi isolated from the leaves of *Echinacea purpurea*.

Table 3. Yield of milk thistle achenes  
Tabela 3. Plon niełupiek ostropestu plamistego

| Treatments – Obiekty              | Years of the study – Lata badań |          |          | Mean Średnia |
|-----------------------------------|---------------------------------|----------|----------|--------------|
|                                   | 2009                            | 2010     | 2011     |              |
| N <sub>0</sub> PK                 | 1.100gh                         | 0.517j   | 1.340f   | 0.986e       |
| N <sub>0</sub> PKMg               | 1.320fg                         | 0.520j   | 1.427def | 1.089de      |
| N <sub>0</sub> PKMg+microelements | 1.320fg                         | 0.787i   | 1.447c-f | 1.184d       |
| N <sub>1</sub> PK                 | 1.627a-e                        | 1.070h   | 1.513b-f | 1.403c       |
| N <sub>1</sub> PKMg,              | 1.433def                        | 0.950hi  | 1.547b-f | 1.310c       |
| N <sub>1</sub> PKMg+microelements | 1.413ef                         | 1.740ab  | 1.533b-f | 1.562b       |
| N <sub>2</sub> PK                 | 1.490b-f                        | 1.693abc | 1.713ab  | 1.632ab      |
| N <sub>2</sub> PKMg               | 1.713ab                         | 1.423ef  | 1.640a-e | 1.592ab      |
| N <sub>2</sub> PKMg+microelements | 1.613a-e                        | 1.547b-f | 1.727ab  | 1.629ab      |
| N <sub>3</sub> PK                 | 1.693abc                        | 1.740ab  | 1.680a-d | 1.704a       |
| N <sub>3</sub> PKMg               | 1.633a-e                        | 1.703ab  | 1.807a   | 1.714a       |
| N <sub>3</sub> PKMg+microelements | 1.593a-e                        | 1.550b-f | 1.620a-e | 1.588ab      |
| Mean – Średnia                    | 1.495b                          | 1.270c   | 1.583    |              |

Explanations as in Table 2 – objaśnienia jak w tabeli 2

The growing season of 2010 was least conducive to the development (in particular to the emergence) of milk thistle. The fruit yield of milk thistle is largely determined by weather conditions [Habán et al. 2009, Sadowska et al. 2011] as well as by sowing date [Andrzejewska et al. 2011]), forecrop and cultivation regime [Andrzejewska and Sadowska 2008]. The most probable reason for the reduced yield was the absence of N fertilization combined with cold and wet weather during seedling emergence. In that season, N fertilization had the most significant effect on achene yield, which increased by more than 70% with increasing N rates. Achene yield was significantly higher in 2011 than in 2009 and 2010. The highest achene weight per hectare (1.6 – 1.8 t) was achieved in treatments with N fertilization at 80 and 120 kg · ha<sup>-1</sup> – a 27.8% increase in comparison with the unfertilized treatment (tab. 3). In 2009, a similar achene yield was noted in the corresponding treatments, although the yield decrease in the unfertilized treatment was higher (31.3% relative to the treatment fertilized with 120 kg N · ha<sup>-1</sup>).



Mean achene yield over three years ( $1.449 \text{ t} \cdot \text{ha}^{-1}$ ) was significantly higher in treatments with N fertilization at 80 and  $120 \text{ kg ha}^{-1}$ , compared to the remaining treatments. Kozera and Nowak [2004] noted a positive response of milk thistle to increasing rates of mineral fertilizers, and reported the highest achene yield for fertilization at  $261 \text{ NPK} \cdot \text{ha}^{-1}$ . The cited authors observed also a significant yield increase after foliar application of Basfoliar 36 Ex. In a study by Andrzejewska and Sadowska [2008], increasing potassium rates had no effect on the aboveground and achene yield of milk thistle, and silymarin content. Omer et al. [1993] reported that the fruit yield of milk thistle increased proportionally to nitrogen rates, while Geneva et al. [2008] found that foliar application of NPK and microelements contributed to a more than 70% increase in milk thistle yield, in comparison with the unfertilized control treatment.

## CONCLUSIONS

1. The composition of fungal communities colonizing the stems of milk thistle was affected by weather conditions during the growing season, while mineral fertilizers exerted variable effects.

2. Potential pathogens were isolated from the stems of milk thistle in high numbers. The predominant species was *Alternaria alternata*. Others pathogens fungi were less frequent (*Fusarium* spp.) or were encountered only sporadically (*Rhizoctonia* spp., *B. cinerea* and *Phoma* spp.).

3. The *A. alternata* fungus, in contrast to *Fusarium* species, was more abundant in treatments with supplemental Mg and Mg+B fertilization than in those with NPK fertilization.

4. The yield of milk thistle achenes increased in response to increasing rates of nitrogen fertilization.

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## NAWOŻENIE MINERALNE A GRZYBY ZASIEDLAJĄCE ŁODYGI OSTROPESTU PŁAMISTEGO *Silybum marianum* (L.) Gaertner ORAZ JEGO PŁONOWANIE

**Streszczenie:** Niełupki ostropestu płamistego ze względu na zawartość cennych związków tłuszczowych, białkowych oraz substancji biologicznie czynnych znalazły się w kręgu zainteresowań producentów farmaceutyków, kosmetyków, żywności funkcjonalnej, a także specjalistów od pasz i żywienia zwierząt. O plonie niełupek tej rośliny obok czynników agrotechnicznych (terminu siewu, rodzaju uprawy, nawożenia) decyduje też stan fitosanitarny roślin. Badania przeprowadzono w latach 2009–2011 na poletkach doświadczalnych zlokalizowanych w Tomaszkanie (północno-wschodnia Polska). Ostropest płamisty uprawiano na następujących obiektach nawozowych: 1.  $N_0PK$ , 2.  $N_0PKMg$ , 3.  $N_0PKMg$  + mikroelement B, 4.  $N_1PK$ , 5.  $N_1PKMg$ , 6.  $N_1PKMg$  + B, 7.  $N_2PK$ , 8.  $N_2PKMg$ , 9.  $N_2PKMg$  + B, 10.  $N_3PK$ , 11.  $N_3PKMg$ , 12.  $N_3PKMg$  + B (gdzie:  $N_0$  – bez nawożenia azotem,  $N_1$  –  $40 \text{ kg} \cdot \text{ha}^{-1}$  /azotan amonowy/,  $N_2$  –  $80 \text{ kg} \cdot \text{ha}^{-1}$ ,  $N_3$  –  $120 \text{ kg} \cdot \text{ha}^{-1}$ , P –  $40 \text{ kg} \cdot \text{ha}^{-1}$  /superfosfat potrójny/, K –  $117 \text{ kg} \cdot \text{ha}^{-1}$  /60% sól potasowa/, Mg –  $20 \text{ kg} \cdot \text{ha}^{-1}$  /kizeryt/ przedsięwzięcie, B – dolistnie /Bormax/). W laboratorium analizowano strukturę zbiorowiska grzybów zasiedlających łodygi ostropestu. Po zbiorze określono plon niełupek. Warunki pogodowe oraz nawożenie makroelementami i borem wpływały na strukturę zbiorowiska grzybów zasiedlających łodygi ostropestu. Potencjalne patogeny miały 50–80% udział w zbiorowisku grzybów. Wśród nich dominował gatunek *Alternaria alternata*, rzadziej identyfikowano grzyby rodzaju *Fusarium* (6 gatunków) i *Rhizoctonia*, a sporadycznie *Botrytis cinerea* i *Phoma* spp. Mniejszą liczebność *A. alternata* otrzymano z łodyg roślin z obiektów bez nawożenia N i z nawożeniem N w dawce  $40 \text{ kg P} \cdot \text{ha}^{-1}$ . Grzyby rodzaju *Fusarium*, odmiennie niż *A. alternata*, mniej licznie występowały w zbiorowisku grzybów izolowanych z łodyg z obiektów z nawożeniem magnezem oraz Mg i borem łącznie. Wraz ze zwiększającymi się dawkami N notowano wzrost plonu niełupek ostropestu.

**Słowa kluczowe:** ostropest płamisty, nawożenie mineralne, grzyby, plon niełupek

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