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The pathogens of milk thistle (Silybum marianum [L.] Gaertn.) in the Czech Republic

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ABSTRACT

Milk thistle is grown in the Czech Republic as a medicinal herb; silymarin is isolated from its achenes and used for the production of liver and gallbladder medicine. The quality and content of the active compound is influenced not only by environmental factors, but also by pests and pathogens. The occurrence of pests of milk thistle variety ´Silyb´ was observed in two localities during the years 2011–2013. In the year 2011 the mycoflora of seeds of four milk thistle varieties was determined. Representatives of 15 species were isolated from the seeds, most of them saprophytic. 21 fungal species were isolated and identified from milk thistle plants during the vegetation; *Septoria silybi* among the most important ones. Possibilities of protection of milt thistle against pathogens are discussed.

Keywords: milk thistle; seed pathogen; *Septoria silyb;, Alternaria silybi*.

Academic Discipline And Sub-Disciplines

Agriculture

Subject Classification

Medicinal Plants, pathogens

Type (method/approach)

original research, work, identification of pathogens

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INTRODUCTION

The milk thistle (*Silybum marianum* [L.] Gaertn.), native to Canary Islands, spread gradually to warm sunny areas of many countries. While some states and countries of North and Central America, Africa, Australasia, Middle and Near East consider milk thistle to be a troublesome, invasive weed [6, 10, 15, 26], in the countries of Central and Eastern Europe, Egypt, China and Argentina it is grown commercially as a medicinal herb for seeds from which silymarin is obtained [13, 20, 24]. In Poland, milk thistle is considered to be a weed, medicinal herb, ornamental plant and vegetable at the same time [4]. In the Czech Republic, milk thistle is grown as a medicinal plant and in the years 2011– 2013 it was sown at the average area of 4 500 ha.

A necessity to obtain high quality drug is not only a thorough knowledge of the plant requirements on soil and climatic factors and cultivation technologies, but also the knowledge of pathogens and pests that could damage or devalue the crop. Nowadays medicinal plants – including milk thistle – are not of a peak importance either for growers or phytopathologists and the occurrence of pathogens and pests is paid very little attention.

Gray mold (*Botrytis cinerea*) and powdery mildew (*Golovinomyces orontii*, syn. *Erysiphe cichoracearum*) are among the most frequent causes of the diseases. According to [3] Brandenburger (1985) the leaf blotch is caused by fungi *Ramularia cynarae* and *Septoria silybi,* that is considered an important pathogen also in California [16]. The occurrence of *S. silybi* was recorded also by Zechini [27] *et al*. (1991) in Italy, by Margina and Zheljazkov (1996) in Bulgaria [14] in 1995, by Khan [10] *et al*. (2009) in Pakistan and by Cwalina-Ambroziak [4] *et al.* in Poland (2012). Ostrčilová [17] and Ondřej (2006) consider *Alternaria silybi* to be the most important milk thistle pathogen except a specific fungus *Septoria silybi*. Gannibal (2010) observed [5] only leaf blotches and numerous sporulation of fungus *Cercospora* sp., but he didn't found spores of *Alternaria silybi* in any living tissue. Milk thistle is also a host of rusts *Puccinia cruchetiana*, *P. laschii* var. *laschii*, *P. mariana* and *P. tyrimni* [3]; Berner *et al*. (2002) found [2] *Puccinia punctiformis* on leaves. According to Kováčiková and Kubínek [12] the cause of milk thistle fading are fungi of the *Fusarium* genus; Cwalina-Ambroziak *et al.* [4] identified six *Fusarium* species on the milk thistle stem. The range of pathogens identified in milk thistle was extended also to viruses – *Tomato spotted wilt virus* [7], *Cucumber mosaic virus* [21] and the smut fungus *Microbotryum silybum* [22, 25] were found in milk thistle flower heads in Greece.

In Egypt [1] observed serious damages due to depreciation of seeds-head by weevil larvae *Larinus latus* Herbst (Coleoptera: Curculionidae). In Greece Kavallieratos [22] *et al*. (2007) described the plant damage caused by aphids *Dysaphis lappae cynarae* and in Iran [18] by *Aphis fabae* sp. *cirsiiacanthoidis* Scop. Khan [10] *et al*. (2009) recorded the leaf harm caused by caterpillars of *Spodoptera* sp. at the end of flowering. Snails are frequent pests during wet weather.

The aim of this work was to determine the range of pathogens that could affect negatively the health state of plants and the seed quality of milk thistle in the conditions of the Czech Republic.

MATERIALS AND METHODS

The occurrence of pests and pathogens of the milk thistle variety ´Silyb´ was studied in two south-Moravian localities in the years 2011–2013.

The locality Javůrek (district Brno-country) is in a mildly warm region (MT5), average year temperatures vary around 7.5 °C. Summer is normal or short here, mildly cold and mildly dry. Transitional periods are usually mildly warm, normal or long. Winter is of normal length with short snow cover period. The soils are deep, loamy brown, and are subject of ilimerisation. Average altitude is 400 m above the sea level [23].

The locality Citonice in the district Znojmo is in a warm, mildly dry climatic region in the altitude 360 m above the sea level. The terrain is largely planar. The soil is deep to moderately deep, mostly without soil skeleton [11].

No fungicide treatment of plants against pathogens was performed.

Determination of the seed microflora

In the year 2011 the seed microflora was determined in 50 milk thistle seed samples from three international and two local breeding locations (Bulgaria – regional variety, Czech Republic – Žabčice and Citonice – the variety ´Silyb´, Slovak Republic – Piešťany, Serbia – Beograd – regional variety). Seeds with unsterilized surface (10 pcs, 5 repetitions) were put into petri dishes (PD) on a moist filter paper and left at the temperature 21/16 °C with alternating periods of light and darkness (12/12 h.). The presence of fungi was evaluated on the 5th day using stereomicroscope (Olympus SX12) and individual species were determined microscopically (Olympus SX41) according to their morphological characteristics.

Identification of micro fungi in milk thistle plants

Symptomatic milk thistle plants were collected during the vegetation period. In the cases when reproduction organs of microfungi were found the identification was performed directly from the plant material. Otherwise, tissue segments were taken from leaves, stems, anthodia and roots (0.25 cm^2), the surface was disinfected with 1 % solution of sodium hypochloride, rinsed with distilled water and dried. The segments were placed into petri dishes with potato-sucrose agar and cultivated for 5 days (at the temperature 22/18 °C at the alternation of light and darkness 14/10 h). Growing fungi hyphes were transferred on PDA and the pathogens were further cultivated for their subsequent identification. The determination of pathogens was performed by classic microscope methods.

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The size of fruiting bodies and spores (100) was measured in preparatives stored in 10% lactic acid under 40×magnification. Identification of the pathogens was performed according to their morphological characteristics.

RESULTS AND DISCUSSION

Limiting factors for the milk thistle growth and development are the altitude (up to 500 m above the sea level) and especially the weather course during the sprouting and maturation of the anthodia. The occurrence of pathogens in the seeds depends not only on the factors affecting it during the vegetation but also on the storage method and length.

Microflora of milk thistle seeds in the year 2011

Fifteen fungi species (Table 1) were isolated from the seeds, mostly saprophytic.

Table 1: Microflora of milk thistle seeds in the year 2011

Pathogen	variets/provenience				
	BG 3	´Silyb´ 1	´Silyb´ 2	SVK 2	4
Alternaria alternata (Fr.) Keissl. (1912)	+	+	+		+
Alternaria tenuissima (Kunze) Wiltshire (1933)			+		
Botrytis cinerea Pers. (1794)			+	+	
Cladosporium cladosporioides (Fresen.) G.A. de Vries,(1952)		+	+	+	
Curvularia lunata (Wakker) Boedijn (1933)	+				
Epicoccum purpurascens Ehrenb.: Schlecht.	+		+		
Gonatobotrys sp. Corda			+		
Humicola sp.			+		
Rhizopus stolonifer (Ehrenb.) Vuill.					+
Mucor hiemalis Wehmer (1903)		+			
Penicillium spp.	+	+	+	+	+
Pirella circinans Bainer 1882					
Septoria silybi Pass.			+		
Stemphylium botryosum Wallr. (1833)			+		
Streptomyces sp.					+
Trichothecium roseum (Pers.) Link (1809)	+			+	
non-sporulating cultures	2	1	1	3	1

BG3 - Institute of Plant Genetic Resources K. Malkovo Bulgaria

´Silyb´1 - Žabčice Czech Republic

´Silyb´2 - Citonice Czech Republic

SVK 2 - Gene Bank Crop Research Institute Piešťany Slovak Republic

4 - Institut za proučavanje lekovitog bilja „Dr Josif Pančić“ Beograd Serbia

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Kováčiková [12] and Kubínek (1986) describe only the genera *Alternaria*, *Rhizopus*, *Cladosporium*, *Mucor* and *Botrytis.* Pycnidia of the parasitical fungi *Septoria silybi* were found only rarely, namely in the seeds of the ´Silyb´ variety from Citonice, where 1.6 % of the total stored seeds were infected. The fungi of *Septoria* genus are contagious through the seeds and the infection causes rapid decrease of germination ability and emergence of the plants, optionally also falling of seedlings. Dead plants are a source of infection for subsequent leaf blotch.

Pathogens isolated from milk thistle plants during the vegetation in the years 2011– 2013

The occurrence of milk thistle leaf blotches was generally weak in both localities during the whole time of the investigation (see Table 2).

Table 2: The list of pathogens isolated from milk thistle plants during the vegetation in the years 2011– 2013

Pathogen	year		
	2011	2012	2013
Acremonium roseum (G. Sm.) W. Gams	5	1	3
Acremonium strictum W. Gams	1	3	1
Alternaria alternata (Fr.) Kiesel	57	41	46
Alternaria silybi Gannibal	-	-	2
Botrytis cinerea Pers.	22	16	32
Cladosporium cladosporioides (Fres.) de Vries	5	3	10
Epicoccum spp.	3	7	2
Fusarium avenaceum (Fr.) Sacc.	2	5	1
Fusarium culmorum W.G.Sm. Sacc.	4	6	2
Fusarium oxysporum Schlecht.	2	-	3
Fusarium poae (Peck.) Wollenweber	1	1	2
Golovinomyces orontii	25	38	31
Humicolla spp.		1	
Mucor circinelloides von Tiegh.	-	2	1
Penicillium spp.	5	4	7
Phoma exiqua Desm.	-	1	-
Puccinia punctiformis	8	11	9
Rhizoctonia solani J.G.Kühn	1	1	-
Rhizopus stolonifer (Ehrenb.) Vuill.	-	1	3
Septoria silybi	10	13	8
Trichoderma harzianum Rifai	1		1
yeast	4	5	2
non-sporulating cultures	4	2	1

Round to slightly elongated shiny grey-brown spots with light grey centres occurred on basal leaves of milk thistle during the rainy summer periods in all investigated years. Concentric rings were visible on gradually increasing spots, occasionally the stains pooled into irregular shapes. The size of spots with already visible pycnidia varied in the range 3.5–12×4.0–14 mm (6.7×7.3 mm in average). Black spherical pycnidia formed on both sides of spots that released light yellow strands of colourless, filamentous, straight or slightly curved unicellular pycnospores after moistening (Table 3).

Table 3: Comparison of the *Septoria silybi* pycnides and pycnospores sizes found during experiments and given in literature

Pathogen	Pycnospores (µm)	Pyknidia (µm)	Source
Septoria silybi	65–70		Brandenburger (1984)
	28.2–70.7×2.35	70.5–75.2×79.9–84.6	Margina, Zheljazkov (1996)
	27–89×2.9–4.0	54.6–79.2×82.6–110.4	Šafránková (2011)

The pathogen was identified as *Septoria silybi* Pass. based on the morphological characteristics.

S. sylibi is considered to be an important pathogen mostly during humid, rainy summers [14, 16], when its pycnospores are spreading quickly and the spots are formed not only on older leaves of the rosette, but also on young leaves on the stem. Moscow [14] and Lindow (1989) state that the spots are present on older leaves up to 2.5-times more often than on young leaves. The infections occur primarily during days with high humidity, because the pathogen penetrates the leaves through opened ducts [19]. As for our observed plants, the spots with pycnides were found only on the eldest leaves, stems and stem leaves were not infected. Neither the biocycle nor the infection cycle of the fungi has been studied in detail yet. Although the *Septoria* genus is contagious via the seeds, the pycnids were found only in one seed sample (Citonice), probably thanks to the absence of this pathogen in given locality or because the seeds were obtained from a recognized growth. The pathogen hibernates on infected post-harvest residua, on overwintering plants or on the seeds as pycnides. With respect to the minimal presence of pycnidia on seed, contested crop residues may be considered the main source of infection in the Czech Republic.

Uredo- and teliosori of biotrophic brachyform rust *Puccinia punctiformis* (F. Strauss) Röhl. (1813) that often occur on *Cirsium arvense* (L.) Scop. were found in milk thistle leaves. Unique bearings were found on the reverse side of the leaves, occasionally also on the face side.

Description of the isolated pathogen: the uredospores are unicellular, spherical to ellipsoidal, with fine nipples on the surface, 19–25×31–35 µm. Teliospores are pedunculated, elliptical, ovate to pear shape, bicellular, usually slightly strangled in the middle, with fine nipples on the surface, the length is 27–31×24–28 µm. The biocycle of the rust isn't fully understood yet, especially the emergence of systemic infection. However, thanks to the method of cultivation it can be assumed that there is no risk of systemic infection at our conditions. Nowadays the occurrence of the rust in milk thistle is a marginal issue and the need for chemical protection is not considered.

Odstrčilová [17] and Ondřej (2006) state *Alternaria sylibi* and *Septoria silybi* to be important milk thistle pathogens, but only saprophytic species of the *Alternaria* genus were found in the investigated samples. *Alternaria silybi*, described by [5] Gannibal (2010) in Russia as a new species pathogenic for milk thistle, was found only in peripheral lesions on several leaves originating from the site Citonice (2013).

Description of the isolated pathogen *Alternaria silybi*: the conidiophores were individual, unicellular, with ellipsoidal or ovoid conidia of the size 49–81×7–12 µm, with 5–8 transversal septa and one longitudinal septum in 1–4 cells. The rostrum was fibrous, 55–164×2.5–3 µm with 2–4 transversal septa.

At the end of the vegetation period the occurrence of mildew *Golovinomyces orontii* was observed in all aboveground parts of plants including milk thistle anthodia and seeds (in the years 2011, 2012 and 2013) and also the gray mold (*Botrytis cinerea*) occurred during rainy weather. Vast infestation of leaves, stems and anthodia influenced especially the seed ripening. The occurrence and intensity of milk thistle infestation by both pathogens depends mostly on the source of infection and on the weather. Both *G. orontii* and *B. cinerea* are important pathogens that can influence the drug yield and quality negatively at favourable conditions, but there aren't any fungicides registered for the milk thistle protection in the Czech Republic.

In addition to the pathogens found on the aboveground parts of plants, the pathogens of *Fusarium* and *Rhizoctonia* sp. were found in milk thistle roots in 2012. The roots and root collars of several plants were damaged by the *Rhizoglyphus* sp. pests.

As for animal pests, the most leaf damage was done by slugs. In 2013 the occurrence of bean aphid (*Aphis fabae*) was observed in the time of terminal anthodia flowering. Due to the fact that there is no product registered for the milk thistle protection against aphids the plant treatment wasn't performed.

Plant protection

Odstrčilová [17] and Ondřej (2006) tested the fungicides containing metconazol as the active compound (Caramba) for the protection against *S. silybi*, but the compound isn't currently registered for medicinal plants protection in the Czech Republic. Because of the field area sown with milk thistle in the Czech Republic the proliferation of *S. silybi* can be expected and thus also the necessity to solve this problem.

With the increasing area sown with medicinal plants in the Czech Republic it is necessary to solve the problem of their protection against both known and newly identified pathogens and pests. Nowadays the protection against *Septoria silybi*

is based on preventive measures, i.e. healthy seeds, 2–3-year break of crop rotation and the destruction of crop residues by deep ploughing. The occurrence of milk thistle pathogens is influenced also by amounts of nutrients, especially N, P, K, Mg and B. Cwalina-Ambroziak [4] *et al.* (2012) observed lower occurrence of *Fusarium* fungi after the application of fertilizers containing Mg and Mg+B. Low doses of N or growing without fertilization with N led to the decrease of *Alternaria alternata* occurrence, but higher doses of N influenced the yield of achenes positively. The occurrence of milk thistle pathogens is influenced mostly by the weather course during the growing season while the mineral fertilization shown only variable effects. Agrotechnical measures can affect the occurrence of pathogens to the certain extent, but when conditions are favourable for the pathogen development these measures are insufficient and some chemical protection is necessary.

CONCLUSION

The aim of the work was to determine the pathogens of Milk thistle [*Silybum marianum* (L.) Gaertn.] which would affect its growing and consequently the quality of final drug. In the years 2011–2013, the occurrence of the pathogens of Milk thistle, variety ´Silyb´, on two localities was monitored. 21 fungi species were isolated and identified from the Milk thistle plants during the vegetation period. The most important was *Septoria silybi*. The isolates of Milk thistle pathogens were described, e.g. *Septoria silybi, Alternaria silybi* and *Puccinia punctiformis*. Because the transfer of *Septoria silybi* by the seeds is possible, the microflora of the seeds of four Milk thistle varieties was studied. 15 species of fungi were determined on that seeds. There were 15 genera isolated from the seeds, from which the majority belong to the saprophytes. The occurrence of the pycnides of the pathogen *S. silybi* on the seeds was recorded in 1.6 % cases only. The possibilities of the precautions and pesticide control of the Milk thistle pathogens are discussed.

REFERENCES

[1] Abdel-Moniem A. S. H. 2002. The seed-head weevil, *Larinus latus* Herbst (Coleoptera: Curculionidae) as a new record in Egypt on the milk thistle, *Silybum marianum* (L.) (Asteraceae: Compositae). *Arch. Phytopathol. Plant Prot*. 35: 157–160.

[2] Berner D. K., Paxson L. K., Bruckart W. L., Luster D. G., McMahon M., Michael J. L. 2002. First report of *Silybum marianum* as a host of *Puccinia punctiformis*. *Plant Disease* 86(11): 1271.

[3] Brandenburger W. 1985. Parazitische Pilze an Gefässpflanzen in Europe. Fischer Verlag, Stuttgart, p. 672.

[4] Cwalina-Ambroziak B., Wierzbowska J., Damszel M., Bowszys T. 2012. The effect of mineral fertilization on achenes yield and fungal communities isolated from the stems of milk thistle *Silybum marianum* (L.) *Gaertner. Acta Sci. Pol., Hortorum Cultus* 11(4): 157– 168.

[5] Gannibal P.B. 2010. Taxonomic studies of *Alternaria* from Russia: new species on Asteraceae. *Mycotaxon* 114(10–11): 109–114.

[6] Holm L.G., Doll J., Holm, E., Pancho, J., Herberger J. 1997. World Weeds. *Natural Histories and Distribution*. Wiley, NewYork.

[7] Chatzivasiliou E.K., Bourakas I., Drossos E., Eleftherohorinos I., Jenser G., Peter D., Katis 2001. Weeds in greenhouses and tobacco fields are differentiallyinfected by tomato spotted wilt virus and infested by its vector species. *Plant Dis.* 85: 40–46.

[8] Karkanis, A., Bilalis, D., Efthimiadou, A. 2011. Cultivation of milk thistle (*Silybum marianum* L. Gaertn.), a medicinal weed. Industrial Crops and Products 34: 825– 830.

[9] Kavallieratos N.G., Tomanovic Z., Sarlis G.P., Vayias B.J., Zekic V., Emmanouel N.E. 2007. Aphids (Hemiptera: Aphidoidea) on cultivated and self-sown plants in Greece. Biology 62: 335–344.

[10] Khan M. A., Blackshaw R. E., Marwat K. B. 2009. Biology of milk thistle (*Silybum marianum*) and the management options for growers in north-western Pakistan. Weed Biology and Management 9: 99–105.

[11] Koláčková P. 2011. Qualitative parameters of healthy and infected MAP plants. Diploma hesis, Mendel University in Brno.

[12] Kováčiková E., Kubínek J. 1986. Fading of the milk thistle (*Silybum marianum* L. Gaertn.). Proceedings of the Institute of Scientific and Technical Information for Agriculture – Plant Protection, 22(3): 217–222.

[13] Kroll D.J., Shaw H.S., Oberlies N.H., (2007): Milk thistle nomenclature: why it matters in cancer research and pharmacokinetic studies. *Integr. Cancer Therapy* 6(2): 110–119.

[14] Margina A., Zheljazkov V. D. 1996. Leaf spot on milk thistle (*Silybum marianum* L. Gaerth.) in Bulgaria. *Beitrage zur Züchtungsforschung* 2(1): 254–256.

[15] Montemurro P., Fracchiolla M., Lonigro A. 2007. Effect of some environmental factors on seed germination and

spreading potential of *Silybum marianum*. *Gaertner. Ital. J. Agron* 3: 315–320.

[16] Moscow D., Lindow S.W. 1989. Infection of milk thistle (*Silybum marianum* L. Gaertn.) leaves by *Septoria silybi*. Phytopathology, 79(10): 1085–1090.

[17] Odstrčilová L., Ondřej M. 2006. Diseases of special crops. p. 48–53. In: Proceedings, XII. conference on the Actual questions of growing, processing and usage of medicinal, aromatic plants and spice. Prague, December 7th 2006, p. 129.

[18] Rezwani A. 2008. A new species, and a new record of a subspecies belonging to the genus *Dysaphis* (Homoptera: Aphididae) from Iran. *J. Entomol. Soc. Iran* 27: 23–26.

[19] Roche C. 1991. Milk Thistle (*Silybum marianum* (L.) Gaertn.). Pacific Northwest extension publication no. PNW382.Washington State University/Oregon State University/University of Idaho, Pullman,WA/Corvallis, OR/Moscow, ID.

[20] Růžičková G., Fojtová J., Součková M. 2011. The yield and quality of milk thistle ((*Silybum marianum* L. Gaertn.) seed oil from the perspective of environment and genotype - a pilot study. *Acta Fytotech Zootech* 14 (1): 9–12.

[21] Sacristan S., Fraile A., Garcia-Arenal F. 2004. Population dynamics of cucumbermosaic virus in melon crops and in weeds in central Spain. *Phytopathology* 94: 992–998.

[22] Souissi T., Berner D.K., Smallwood, E.L., (2005): First report of smut caused by *Microbotryum silybum* on inovy thistle. *Plant Dis*. 89: 1242.

[23] Suchá M. 2007. Complex geographic characteristics and risk factors in the Rosice-Oslavany region. Bachelor thesis. Masaryk University in Brno.

[24] Szilágy I., Tététnzi P., Antus S., Selingmann O., Charim V.M., Seitz M., Wagnerm H. 1981. Structure of silimarin and silymonin. 2. New flavonolignans from a white blooming *Silybum marianum* variety. *Planta med*. 43: 121–127.

[25] Vánky K., Berner D. 2003. *Microbotryum silybum* sp. nov. (Microbotryales). *Mycotaxon*, 85(1– 3): 307–311.

[26] Veres S., Tyr T. 2012. Milk thistle (*Silybum marianum* (L.) Gaern.) as a weed in sustainable crop rotation. *Res. J. Agric. Sci.* 44: 118–122.

[27] Zechini, A., Zambonelli A., Morara M. 1991. Ulteriori indagini sulle malatine crittogamiche di piante officinale in *Emilia Romagna*. *Informatore Fitopatologico*, 41(12): 45–52.