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EFFECT OF CULTIVATION CONDITIONS ON THE VARIABILITY AND INTERRELATION OF YIELD AND RAW MATERIAL QUALITY IN MILK THISTLE (*Silybum marianum* (L.) GAERTN.)*

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Abstract. The aim of the research was to identify characters which determine the yield and the content of silymarin in the fruits (achenes) of *Silybum marianum* as well as to show the range of their variation. The research was performed based on field experiments conducted at the Mochełek Research Station (53°13' N; 17°51' E) over 2003-2005. Fruit yield and the content of silymarin were affected mainly by weather conditions, the forecrop and the sowing time. The coefficient of variance for fruit yields was 37.3%, and the content of silymarin – 12.7%. Plant height prior to harvest, number of fruits per anthodium on lateral and main shoots, thousand fruit weight, number of anthodiums with pappus and number of all the anthodiums per plant were significantly correlated with fruit yield. The content of silymarin was positively correlated with the number of plants per m^2 prior to harvest, plant height prior to harvest and fruit yield, and negatively correlated with the content of total N in the reserve material of fruits. The content of flavonolignans which make up the silymarin appeared relatively constant and only silydianin changes slightly more than the content of silychristin and isosilybin.

Key words: flavonolignans, milk thistle, *Silybum marianum*, silymarin, yield of achenes

INTRODUCTION

Milk thistle, *Silybum marianum* (L.) Gaertn. (*Asteraceae*), is a species which originated in the Mediterranean region. The pharmaceutical raw material is provided by fruit (achenes) – *Fructus Silybi mariani*. Silymarin is extracted from the dry pericarp; silymarin is a complex of flavonolignans (silybin, silychristin, silydianin, isosilybin) commonly applied in liver treatment [Morazzoni and Bombardelli 1995]. Milk thistle

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fruits and their extracts can be added to animal feed mixtures [Urbańczyk et al. 2002] and can be an ingredient of functional food [Hadolin et al. 2001].

Milk thistle growing was widely launched in the 1980s and only occasionally is research made into factors conditioning the raw material yield and quality. In Poland the plantation area of this crop ranges from 1500 to 2000 ha and is mostly located in the central and northern part of the country (over 53^{o} N in latitude), and thus under climatic conditions much different from the place the species originated from. Research conducted in Poland and its neighboring countries demonstrated that milk thistle accumulates more silymarin in warm years and when exposed to even rainfall distribution in May and June and a high phosphorus and nitrogen fertilization as well as that a delayed harvest date enhances the level of silymarin in fruit [Załęcki and Górna 1983, Kozłowski and Hołyńska 1985, Andrzejewska and Skinder 2006]. It is, therefore, justifiable to search for other relationships conditioning the fruit yield and their content of silymarin. Identifying such relationships could define a direction of both growing guidelines and new cultivar breeding.

Generally, it is believed that the pharmacological activity is conditioned by silybin, which is the main component of silymarin. Dvorak et al. [2003] demonstrated, however, an effective cytoprotective effect of silydianin and silychristin, mostly, and a much less effect of silybin and isosilybin, which suggests a growing demand for raw material with a specific content of respective flavonolignans. Such chances in the future are offered by biotechnological methods [Alikaridis et al. 2000]. However, due to a relatively high and growing demand of the pharmaceutical industry for silymarin, the raw material will still mainly come from field plantations. The share of respective flavonolignans in the composition of silymarin is genetically conditioned. However, it is essential to define to what extent agrotechnical practices can affect the composition of silymarin.

The aims of the paper involved:

– showing the range of variation in the yield and content of silymarin depending upon cultivation conditions,
– determination of correlations as well as regression relationships between components of the yield structure and the chemical composition of milk thistle fruit and the fruit yield and the content of silymarin in fruits,
– evaluation of the range of variation of flavonolignans in the composition of silymarin.

MATERIAL AND METHODS

The research material was obtained from two field experiments (as a monoculture and in crop rotation) carried out over 2003 and 2005 at the Mochełek Experiment Station of the University of Technology and Life Sciences in Bydgoszcz, located 53^{o}13' N latitude and 17^{o}51' E longitude, 98.5 m above sea level. The milk thistle of the Polish cultivar 'Silma' was grown on a typical lessive soil, produced from heavy loamy sand of pH 5.9, with high phosphorus content, average content of potassium and average or low content of magnesium. The field experiments were made following the growing practices in Poland; sowing rate of 18 kg·ha^{-1}, nitrogen fertilization of 50 kg·ha^{-1}, weed control with herbicide Stomp. The harvest with the use of combine harvester was made in the last decade of July or at the beginning of August, when 40-50% of inflorescences had pappus. The following conditions varied:

– the weather over the growing periods; the first year of study was warmer than the others and mean precipitations were, generally, similar; however, the distributions May through July were different (Table 1);
– growing in typical agricultural crop rotation after cereal forecrop (barley) and in monoculture (2004 was the second, and 2005 the third year of milk thistle growing in monoculture);
– two sowing dates: April 1 and 22;
– three levels of fertilization with potassium: no fertilization, 58 and 116 kg·ha^{-1} K;
– two kinds of raw-spacing: 25 and 50 cm.

Table 1. Meteorological conditions during the growing season of milk thistle against many-years data at the Mochełek Research Station
Tabela 1. Warunki meteorologiczne w latach uprawy ostropestu plamistego w Stacji Badawczej Mochełek

Year Rok	Mean twenty four hours air temperature, °C Średnia dobowa temperatura powietrza					Precipitation – Opady, mm				
	April kwiecień	May maj	June czerwiec	July lipiec	mean średnia	April kwiecień	May maj	June czerwiec	July lipiec	sum suma
2003	6.4	14.4	17.7	19.2	14.4	18.5	18.1	30.4	106.2	173.2
2004	7.5	11.3	14.7	16.4	12.5	32.1	54.4	39.6	53.5	179.6
2005	7.4	12.1	14.9	19.4	13.5	34.8	82.6	30.5	33.6	181.5
1949-2003	7.2	12.8	16.2	17.8	13.5	27.0	40.4	54.4	72.7	194.5

Correlation and regression analyses were made for the years 2004 and 2005, when milk thistle was cultivated both in monoculture and in crop rotation. The total of 48 treatments were analyzed; each treatment included 4 plots 24 m^2 each. The results of the silymarin composition come from 16 collective samples.

The measurements of the number of anthodiums, fruits per anthodium per main stem, fruits per anthodium of lateral shoots and the plant height prior to harvest included 10 plants per plot. The fruit yield was dried at the temperature of 40°C to the moisture of 8%. The dry matter of the pericarp was separated as follows: 100 fruits were weighed, placed into bolting-cloth bags in the steam bath over boiling water for one hour, and rinsed in cold water. Then the fruits were crashed and the pericarp was separated from the seed. Having been dried at 40°C, the air-dried pericarp was weighed.

The reserve material of achenes (obtained as a result of fruit grinding and pericarp separation) was used to determine the content of crude fat with the Soxhlet method and the content of total nitrogen with the Kiejdahl method. The content of silymarin in fruits was evaluated with the spectrophotometric methods reading the absorbance of the methanolic extract at 490 nm after derivatization with 2.4 dinitrophenylhydrazine. The silymarin components were determined with the RPHPLC-DAD method at the Research Institute of Medical Plants in Poznań.

Coefficients of variation (C.V.) and of correlation between the characters evaluated were calculated. For the characters significantly correlated with the yield and content of silymarin, linear and quadratic regressions were calculated, coefficients of determinance (D) were given and the values of dependent variables for which the highest yields of fruits or the highest content of silymarin were achieved. If the coefficient of determinance for quadratic regression was at least 5% higher than the coefficient for linear regression, then maximum values of the dependent variable only for quadratic regression were given. The calculations were made with Statistica™ PL software.

RESULTS AND DISCUSSION

Weather conditions over the research years and the cultivation method (crop rotation, monoculture) mostly diversified the milk thistle yields (Table 2). A decrease in the yield of milk thistle grown in monoculture was a result of a high intensity of insect feeding [Andrzejewska et al. 2006]. A three-week delay in the sowing date made ripening occur later in summer, when it was warmer, which enhanced the accumulation of silymarin. Varied fertilization with potassium and row spacing did not affect the fruit yield and their content of silymarin.

Table 2. Response of milk thistle to the cultivation conditions
Tabela 2. Reakcja ostropestu plamistego na warunki uprawy

Treatment – Czynnik		Fruit yield Plon owoców $t \cdot ha^{-1}$	Content of silymarin, % d.m. of fruits Zawartość sylimaryny, % s.m. owoców
Research years Rok uprawy	2003	0.94 ± 0.13*	2.60 ± 0.295
	2004	1.58 ± 0.25	2.75 ± 0.301
	2005	1.00 ± 0.48	2.62 ± 0.339
Cultivation method Metoda uprawy	crop rotation – zmianowanie	1.61 ± 0.28	2.78 ± 0.172
	monoculture – monokultura	0.96 ± 0.44	2.60 ± 0.420
Sowing date Termin siewu	April 1	1.30 ± 0.48	2.50 ± 0.286
	April 22	1.27 ± 0.50	2.89 ± 0.210
Potassium fertilization Nawożenie potasem	no fertilization – bez nawożenia	1.34 ± 0.45	2.65 ± 0.359
	58 $kg \cdot ha^{-1}$ K	1.24 ± 0.48	2.72 ± 0.307
	116 $kg \cdot ha^{-1}$ K	1.29 ± 0.49	2.73 ± 0.333
Row spacing Rozstawa rzędów	20 cm	1.27 ± 0.49	2.69 ± 0.329
	50 cm	1.30 ± 0.48	2.69 ± 0.335

* standard deviation – odchylenie standardowe

Milk thistle grown in the field demonstrated a great variation in the plant density, number of inflorescences and the number of fruits per anthodium (Table 3). As a result, the variation coefficient of yield was as much as 37.3%. Haban et al. [2007] also reports high yield variability in experiments conducted in Slovakia. The characters which were relatively constant were the content of total nitrogen, thousand fruit weight, pericarp weight and the content of fat. The content of silymarin ranged from 2.02 to 3.38% of the dry weight of fruits, while the coefficient of variation was 12.7%. Ram et al. [2005], for most characters, report on higher values of coefficients of variation, but they compared the stocks which originated in different parts of the world.

There were found numerous correlation relationships between yield structure components and the chemical composition of fruits and the fruit yields (Table 3). Plant height prior to harvest, number of fruits per anthodium on lateral shoots, thousand fruit weight, number of all the anthodiums per plant, number of fruits per anthodium on the main stem, and number of anthodiums with pappus per plant were significantly correlated with the fruit yield. The content of silymarin was positively correlated with the number of plants per m^2 prior to harvest, plant height prior to harvest and fruit yield, and negatively with the content of total N in the reserve material of fruits.

Table 3. Value and variation of yield structure components and the chemical composition of milk thistle fruits and their correlation relationships
Tabela 3. Wartość i zmienność elementów struktury plonu oraz składu chemicznego owoców ostropestu plamistego i ich zależności korelacyjne

Parameter Parametr	A	B	C	D	E	F	G	H	I	J	K	L
Max – Maks.	59.0	3.40	6	160.6	125.2	32.9	140	1.91	16.27	35.2	4.2	3.38
Min – Min.	14.0	1.00	1.1	43.2	22.9	24.3	36	0.35	11.89	25.6	3.5	2.02
Mean Średnia	39.8	2.28	3.36	92.0	75.2	28.6	96.3	1.29	14.07	30.9	3.89	2.68
C.V., %	29.3	32.7	34.0	34.9	35.4	10.5	27.6	37.3	8.1	6.9	4.6	12.7
Correlation coefficients – Współczynniki korelacji												
A	1.00	-0.1011	-0.274	-0.179	-0.057	-0.145	-0.134	0.035	-0.301	**0.438**	-0.241	**0.584**
B		1.00	**0.811**	**0.386**	**0.484**	0.093	**0.522**	**0.533**	0.163	-0.186	-0.149	0.119
C			1.00	0.257	**0.405**	**0.469**	**0.686**	**0.649**	**0.500**	**-0.376**	**-0.371**	0.167
D				1.00	**0.859**	0.109	**0.486**	**0.575**	0.016	0.042	-0.216	-0.053
E					1.00	0.183	**0.622**	**0.731**	**0.076**	0.107	**-0.337**	0.141
F						1.00	**0.639**	**0.610**	**0.922**	**-0.482**	**-0.597**	0.246
G							1.00	**0.873**	**0.565**	-0.181	**-0.634**	**0.451**
H								1.00	**0.490**	-0.141	**-0.594**	**0.458**
I									1.00	**-0.495**	**-0.481**	0.148
J										1.00	-0.130	0.191
K											1.00	**-0.591**
L												1.00

bold – significant at 5%
A – number of plants per m^2 prior to harvest – liczba roślin na m^2 przed zbiorem
B – number of anthodiums with pappus per plant – liczba kwiatostanów z puchem na roślinie
C – number of all the anthodiums per plant – liczba wszystkich kwiatostanów na roślinie
D – number of fruits per anthodium on the main stem – liczba owoców w kwiatostanie na pędzie głównym
E – number of fruits per anthodium on lateral shoots – liczba owoców w kwiatostanie na pędach bocznych
F – thousand fruit weight – masa tysiąca owoców, g
G – plant height prior to harvest – wysokość roślin przed zbiorem, cm
H – fruit yield – plon owoców, $t \cdot ha^{-1}$
I – dry weight of thousand fruit pericarp – sucha masa perykarpu z tysiąca owoców, g
J – content of fat in the reserve material of fruits – zawartość tłuszczu w materiale zapasowym owoców, %
K – content of total N in the reserve material of fruits – zawartość N ogólnego w materiale zapasowym owoców, %
L – content of silymarin in fruits – zawartość sylimaryny w owocach, %

The coefficient of determination, which accounts for the degree of dependence of the parameters studied, was much higher for quadratic than for the linear regression when the dependent variable was made up of thousand fruit weight and the content of total nitrogen in the reserve material of achenes, as well as the number of ripe anthodiums per plant (Table 4). The quadratic regression equations show that the maximum yields were recorded when the number of all the anthodiums per plant ranged from 4.5 to 5, thousand fruit weight – from 28 to 32 g, the content of total nitrogen – from 3.6 to 3.9%, and the content of silymarin in fruits – from 2.7 to 3.2%. The relationship between the fruit yield and the number of ripe anthodiums, milk thistle plant height, the number of fruits per anthodiums and the dry weight of pericarp were linear for that measurement range, which means that the highest yields were recorded when the number of anthodiums with pappus per plant was 3.4, the number of fruits per main stem – 160, fruits per anthodium on lateral shoots – 125, the plant height – 140 cm, and the dry matter of the pericarp – 16 g.

Table 4. Regression relationships of milk thistle yields
Tabela 4. Zależności regresyjne plonów ostropestu plamistego

Independent variable – Zmienna niezależna	Regression equation Równanie regresji	D, %	Max* Maks.
Number of anthodiums with the pappus per plant Liczba kwiatostanów z puchem kwiatostanowym na roślinie	$y = 5.074 + 3.456x$	28.4	3.4
Number of all anthodiums per plant Liczba wszystkich kwiatostanów na roślinie	$y = -3.832 – 0.803x^2 + 7.994x$	48.2	4.5-5.0
Number of fruits per anthodium on the main stem Liczba owoców w kwiatostanie na pędzie głównym	$y = 4.985 + 0.865x$	33.0	160
Number of fruits per anthodium on lateral shoots Liczba owoców w kwiatostanie na pędach bocznych	$y = 2.964 + 0.133x$	53.4	120
Thousand fruit weight, g Masa 1000 owoców	$y = -218.27 – 0.2489x^2 + 15.279x$	47.7	28-33
Plant height prior to harvest, cm Wysokość roślin przed zbiorem	$y = -2.349 + 0.159x$	76.2	140
Thousand fruit pericarp weight, g Masa perykarpu z 1000 owoców	$y = -16.084 + 2.062x$	24.0	16.0
Silymarin content, % of d.m. of fruits Zawartość sylimaryny, % s.m. owoców	$y = -67.946 – 9.535x^2 + 56.135x$	25.9	2.7-3.2

* the value of the independent variable at which the yield of achenes was the highest – wartość zmiennej niezależnej, przy której wartość plonu niełupek była najwyższa

Despite a far geographical distance and different climatic conditions, the results of the present studies much coincide with the results reported by Ram et al. [2005]. The greatest difference concerns the number of inflorescences per plant, however it comes from a considerable differentiation in the plant density. A dense sowing limits the formation of inflorescences on lateral shoots (a negative correlation with the number of anthodium per plant), which also enhances the leveling of flowering and ripening, which, in turn, affects the content of silymarin. There was noted a negative correlation between the content of total nitrogen and the content of silymarin, for which it is difficult to provide a final explanation, which would still require a confirmation in successive years. Similarly, the reports by other authors offer no information on the relationship between the content of silymarin and the chemical composition of the reserve material of the milk thistle achenes. The share of the pericarp in the weight of the achenes ranged from 48.5 to 55.8%. However, no correlation was found between the weight of dry pericarp and the content of silymarin. Although the flavonolignans are compounds soluble in fat, there was no significant correlation between the content of fat and the content of silymarin.

The highest content of silymarin occurred when prior to harvest the number of plants ranged from 40 to 55 per 1 m^2 (Table 5). The content of silymarin was also positively correlated with the plant height and the fruit yield. The pattern of the relationship between the plant height and the content of silymarin was linear, while the highest content of silymarin was found when the fruit yields ranged from 1.4 to 1.8 kg·ha^{-1}.

Table 5. Regression relationships of the content of silymarin in milk thistle fruits
Tabela 5. Zależności regresyjne zawartości sylimaryny w owocach ostropestu plamistego

Independent variable – Zmienna niezależna	Regression equation Równanie regresji	D, %	Max* Maks.
Number of plants per 1 m^2 Liczba roślin na 1 m^2	$y = 0.73 – 0.001x^2 + 0.87x$	51.3	40-55
Plant height prior to harvest, cm Wysokość roślin przed zbiorem	$y = 2.13 + 0.006x$	20.6	140
Total nitrogen content in the reserve material of achenes, % Zawartość azotu ogólnego w materiale zapasowym niełupek	$y = -36.20 – 2.85x^2 + 21.11x$	45.9	3.6-3.8
Fruit yield, t·ha^{-1} Plony owoców	$y = 1.86 – 0.003x^2 + 0.114x$	25.9	1.4-1.8

* the value of the independent variable at which the content of silymarin was the highest – wartość zmiennej niezależnej, przy której zawartość sylimaryny była najwyższa

Silybin and silychristin were dominant components of silymarin. However, the silybin and isosilybin (called silybinin) shares made up 49-51% of the all flavonolignans (Fig. 1). The content of flavonolignans appeared relatively constant and only silydianin showed a tendency to change. Hammonda et al. [1993] showed that due to a change in moisture conditions and nitrogen fertilization, the range of changes in the content of silydianin was greater than in the other flavonolignans. In the raw material obtained from Israel, no silydianin was recorded [Chiavari et al. 1991]. The lability of this flavonolignan, therefore, shows that to some extent it is possible to modify the composition of silymarin due to milk thistle growing conditions.

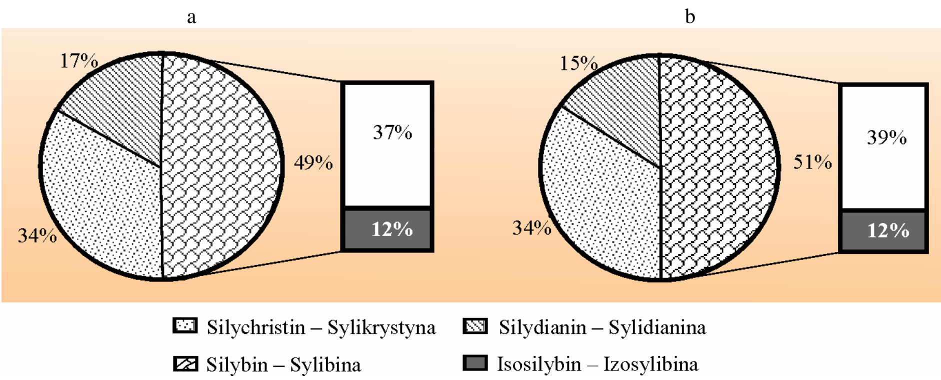

Fig. 1. Share of flavonolignans in silymarin (a – the highest content of silymarin, b – the lowest content of silymarin)
Rys. 1. Udział flawonolignanów w sylimarynie (a – najwyższa zawartość sylimaryny, b – najniższa zawartość sylimaryny)

The results of the present research demonstrate that milk thistle grown in the moderate climate of Poland shows a high variation in the characters determining yield, although the experiment involved 'Silma' cultivar, where such characters as the length of the vegetation period, plant height, thousand fruit weight and uniformity of ripening were clearly improved as compared with the initial populations [Kaźmierczak and Seidler-Łożykowska 1997]. The variation in the content of silymarin is clearly lower than the variation in yield. The level of silymarin is comparable with the data reported by other authors who made investigations in much warmer climatic conditions [Hammouda et al. 1993, Ram et al. 2005]. The content of silymarin of about 3.0% is possible when the harvest is made when about 50% of inflorescences produce fruits with pappus [Załęcki and Górna 1983], the sowing date is delayed until the third decade of April, the plant density ranges from 40 to 55 per 1 m^2 , and the height of plants prior to harvest reaches 140 cm. A high content of silymarin is obtained from plants producing high fruit yields, which also coincides with other reports [Hetz et al. 1995]. One of the main conditions of high yields is keeping the plantation pest-free [Andrzejewska et al. 2006]. Of the flavonolignans which make up the silymarin, the content of silydianin changes more than the content of silychristin and isosilybin.

CONCLUSIONS

1. A high variability of milk thistle fruit yield results from moisture and thermal conditions and pest infestation in monoculture. The variability range of the content of silymarin was considerably smaller and resulted mainly from the difference in sowing time.
2. Fruit yields of milk thistle are correlated with all yield structure components, while the content of silymarin is correlated with canopy architecture components.
3. Proportions of flavonolignans in silymarin are determined genetically, and only sylidianin indicated a tendency to vary.

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WPŁYW WARUNKÓW UPRAWY NA ZMIENNOŚĆ ORAZ WSPÓŁZALEŻNOŚĆ PLONU I JAKOŚCI SUROWCA OSTROPESTU PLAMISTEGO (*Silybum marianum* (L.) GAERTN.)

Streszczenie. Celem badań było wykazanie zakresu zmienności plonów ostropestu plamistego (*Silybum marianum*) i zawartości oraz składu sylimaryny w zależności od warunków uprawy. Wskazano cechy skorelowane z plonem i zawartością sylimaryny. Podstawę badań stanowiły doświadczenia polowe prowadzone w Stacji Badawczej Mochełek (53°13’ N; 17°51’ E) w latach 2003-2005. Plon owoców zależał głównie od warunków pogodowych i przedplonu, a zawartość sylimaryny od terminu siewu. Współczynnik zmienności plonów owoców wynosił 37,3%, a zawartości sylimaryny – 12,7%. Z plonem owoców istotnie skorelowane były: wysokość roślin przed zbiorem, liczba owoców w kwiatostanach na pędach bocznych i pędzie głównym, masa tysiąca owoców, liczba wszystkich koszyczków na roślinie i liczba koszyczków z puchem kwiatostanowym. Zawartość sylimaryny była skorelowana dodatnio z liczbą roślin na m^2 przed zbiorem, wysokością roślin przed zbiorem i plonem owoców, a ujemnie z zawartością azotu ogólnego w materiale zapasowym niełupek. Udział poszczególnych flawonolignanów w sylimarynie ulegał tylko minimalnym zmianom i jedynie sylidianina wykazywała pewną labilność.

Słowa kluczowe: flawonolignany, ostropest plamisty, *Silybum marianum*, sylimaryna, plon niełupek

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