Ladder spleenwort (*Asplenium adulterinum* Milde) in Poland – distribution, population state and conservation plan framework

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ABSTRACT: *Asplenium adulterinum* is a rare and endangered European fern species almost strictly related to serpentine rocks. It occurs in SW Poland (Lower Silesia) in 11 sites in the Sudetes Mts. and their foreland. The total Polish quantity of the species is estimated at about 490 individuals. The majority of populations is smaller than 20 individuals, only two reach sizes of about 90 and 200 individuals. Some active protection procedures are proposed to improve the fitness of the populations. The recently developed methods of *in vitro* propagation and *ex situ* preservation should help to create a gene bank of threatened populations. It would be used in case of the necessity to reintroduce any extinct or damaged population in the future.

ABSTRAKT: *Asplenium adulterinum* jest gatunkiem paproci rzadkim i zagrożonym w Europie, w swym występowaniu ograniczonym niemal wyłącznie do skał serpentynitowych. Gatunek w Polsce występuje tylko na Dolnym Śląsku – na 11 stanowiskach w Sudetach i na ich przedpolu. Całkowite polskie zasoby gatunku wynoszą ok. 490 osobników. Większość populacji skupia poniżej 20 roślin, tylko dwie osiągają liczebności ok. 90 i ok. 200 osobników. Proponuje się objęcie zabiegami ochrony czynnej populacji tej paproci dla zwiększenia szans ich przetrwania. Opracowana metoda namnażania zanokcic *in vitro* i ich zabezpieczenia *ex situ* pozwolą stworzyć bank genów zagrożonych populacji. Będzie on mógł być wykorzystany w przyszłości do reintrodukcji w wypadku pogorszenia stanu populacji gatunku.

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KEY WORDS: *Asplenium adulterinum*, Lower Silesia, distribution, endangered species, conservation plan, *in vitro* cultures

Introduction

Ladder spleenwort *Asplenium adulterinum* Milde (Aspleniaceae) is a rare fern, for long believed to be one of the species, which determine the distinctive character of Lower Silesian flora (e.g., Pax 1915; Szafer 1972). *A. adulterinum* was considered to be an European endemic species (Reichstein 1984) but it has also been found on the Vancouver Island at the west coast of Canada (Käsermann 1999; Klinkenberg 2008). Its European sites are concentrated in the Alpine area and in the Middle European mountain ranges of the Hercynian orogeny. The species range also comprises isolated sites scattered around the Fennoscandia as well as those on the Balkan Peninsula (Jalas, Suominen 1972). The species is almost strictly related to serpentine rocks and only exceptionally occurs on the other substrata. Lower Silesia is the only region in Poland with serpentine outcrops and therefore is also the only area of *A. adulterinum* occurrence in the country. The species is regarded as seriously threatened and has the IUCN category ranging from EN – endangered (Żołnierz 2001; Fabiszewski, Kwiatkowski 2002) to CR – critically endangered (Świerkosz, Szczęśniak 2003). The category CR is also attributed to *A. adulterinum* in the Czech Republic and Slovakia (Čeřovský, Klaudisová 1999).

Asplenium adulterinum is an allotetraploid species (2n=144) which originated from a hybrid of the diploid (2n=72) species *A. trichomanes* and *A. viride* (Lovis, Reichstein 1968; Reichstein 1984).

Ladder spleenwort was included in the list of species, which has been proposed by Poland as an extension to the appendix II of the Habitat Directive (92/43/EEC Directive) as Species of Community Interest, whose conservation requires the designation of Special Areas of Conservation. It was also listed in Annex IV among plant species demanding strict protection in all European Union countries. For this reason *Asplenium adulterinum* is listed in Poland as a species which not only needs protection, but also requires establishing a special protection zone around its each locality.

The aim of the investigation was to create an inventory of *A. adulterinum* sites in order to evaluate the condition of the species populations and to identify to what kinds of threats they would be exposed there. The collected data and observations were used to prepare a framework of a conservation plan, which includes some active procedures – these, in our opinion, are crucial for the survival of the *Asplenium adulterinum* in the area.

1. Materials and methods

1.1. Field investigations

Lower Silesian sites of *Asplenium adulterinum* have regularly been monitored for more than twenty years to determine the size and age structure of the populations, namely the number of clumps and share of juvenile individuals, to ascertain their fitness and to evaluate the strength of competition between ferns and other plants. Also, some properties of sites and their vicinities have been monitored, such as the condition of forest communities, kinds and intensity of human influences, etc.

1.2. *In vitro* culture and *ex situ* preservation of *Asplenium adulterinum*

Collection and germination of spores

Spores of *A. adulterinum* were collected from ferns growing in the natural habitat on the Kiełczyńskie Hills (KH 4). Single blades of fronds with developed sporangia were collected, packed into paper envelopes and left to dry in room temperature for 7–10 days. After that time, spores pouring out from the sporangium were packed in blotting paper filter bags called further the packets. The spores in the packets were soaked in disinfecting solutions and washed three times with sterile distilled water. A two-step method of disinfection was used: in 70% and in 0.1% NaClO for 3, 5 and 10 min.

Aseptic spores were sown in 100 ml flasks, each containing 35 ml of solid medium. The culture medium for the spore germination was 1/4 MS (Murashige, Skoog 1962) supplemented with 30 $g \cdot l^{-1}$ of sucrose and solidified with 8 $g \cdot l^{-1}$ of agar; the pH value of the medium was adjusted to pH 6.2 before sterilization (1Atm, 121°C, 18 min). Sterile distilled water (2 ml) was added to the flask with sown spore cultures to increase germination. All cultures were incubated at 18–20°C under a cool white fluorescent light of 14.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to provide a 16-h photoperiod. The prothallial colonies were cultured in the following media: full-strength, 1/8, 1/4 and 1/8 dilutions of MS macronutrients (Murashige, Skoog 1962) with 6.8 g l^{-1} of agar, 20 g l^{-1} of sucrose, pH values of 6.8–7.0 and modified MS with changed proportions of macroelements (NH_4NO_3 – 825 mg l^{-1}; KNO_3 – 950 mg l^{-1}; $CaCl_2 \times 2H_2O$ – 110 mg l^{-1}; $MgSO_4 \times 2H_2O$ – 370 mg l^{-1}; KH_2PO_4 – 42.5 mg l^{-1}) and half a portion of glycine.

Gene bank of prothalli

In order to create a "gene bank", prothalli of *Asplenium adulterinum* were stored for a year in low temperature. The single heart-shaped gametophytes were transferred to 50 ml flasks, each containing 20 ml of the medium. Five

explants (gametophytes) were placed in one flask and there were twenty flasks per treatment in every experiment. They were cultured in a 1/2 MS mineral medium with different doses of agar and sucrose. The *in vitro* cultures were stored for a year in low temperature: +2°C, continuous darkness or in +8°C under a cool white fluorescent light of 0.15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in a 16-h photoperiod. After twelve months, colonies of gametophytes were adapted to standard conditions of culture growth for two weeks (18–20°C). After that, prothalli were subcultured on a fresh 1/2 MS medium and the survival rate and size of *Asplenium adulterinum* gametophytes were observed.

Culture of sporophytes

The 2–3 cm high sporophytes formed in tissue culture were separated from gametophytes and subcultured on 1/2 MS medium. Propagated sporophytes were divided and cultured on 1/2 MS containing 1 mg/l of KIN (kinetin) and 0.1 mg/l of NAA.

Acclimation of plants to *ex vitro*

Before planting sporophytes were transferred from agar medium to liquid medium with perlite, where they developed roots, and next, for the last 7 days, plants were placed under light irradiation of 34.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Then, the flasks with ferns were placed in a greenhouse and after 5 days the aluminum foil covers were removed. After next three days, the plants were potted into soil. A few various gardening soils were used, including commercial mixes for ferns of pH 6.5 and pH 7.0. The pots were covered with polyethylene foil, which was then gradually removed. After 4 weeks the number of growing plants was counted. The plants were transferred to a hotbed 5 weeks later.

2. Results and discussion

2.1. Inventory of the sites and population status

Historical sources (reviewed by Schube 1903) listed 13 localities of *Asplenium adulterinum* in Lower Silesia. Currently, this species populations still occupy 11 sites (Fig. 1). Four of them are within the main range of the Sudetes Mts.: the Popiel hill near Janowice Wielkie in the Kaczawskie Mts., Kamionki and Przygórze in the Sowie Mts. and Żmijowiec in the Massif of Śnieżnik. Seven sites are situated in the southern part of the Ślęża Massif in the foreland of the Sudetes Mts. Its western part, the Kiełczyńskie Hills with six sites, is the Polish center of the ladder spleenwort occurrence. In the early 1970s, a known *Asplenium adulterinum* site in Radunia Mt. disappeared (Karpowicz 1963;

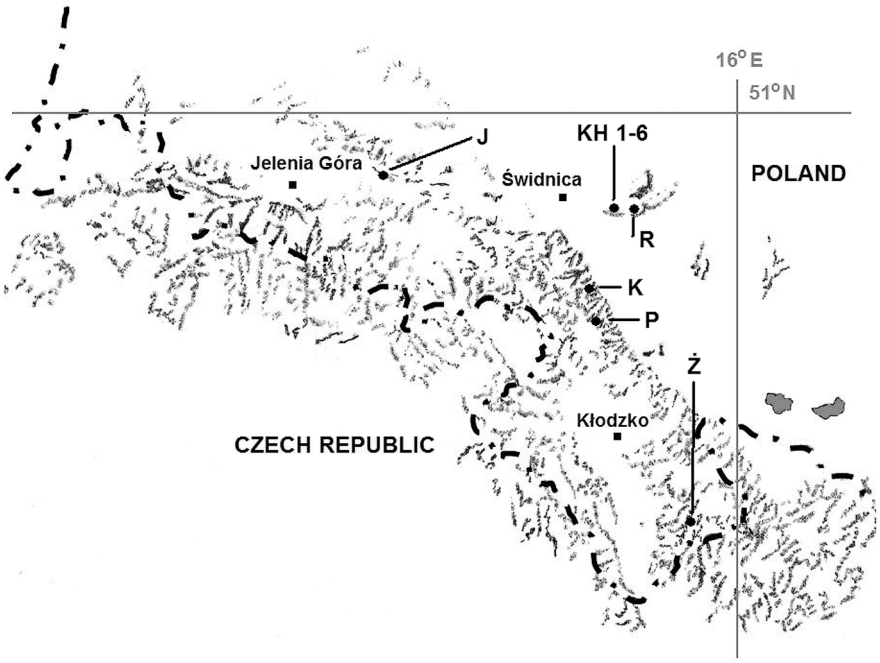

Fig. 1. Distribution of *Asplenium adulterinum* in Lower Silesia. For the explanation of population symbols see Tab. 1

Rys. 1. Rozmieszczenie *Asplenium adulterinum* na Dolnym Śląsku. Wyjaśnienie symboli populacji – por. tab. 1

J. Fabiszewski – personal communication). In 2005 Żołnierz (unpbl. data) found another small population of *A. adulterinum* growing in this mountain together with *A. cuneifolium*. It was not possible to confirm any populations of *A. adulterinum* specified by Schube (1903) in the Grochowa Massif, in two anthropogenic sites on walls in the city of Świdnica, and the Witoszów village. In the both latter sites *A. trichomanes* occurs, thus an incorrect species identification should be taken into account. Lower Silesian sites of *Asplenium adulterinum* are situated within the range of altitudes of about 300 m a.s.l. (Kiełczyńskie Hills) to 1550 m a.s.l. (Żmijowiec).

Investigated populations differ in size (Tab. 1); six of them do not exceed 20 individuals and only two from the Kiełczyńskie Hills with about 90 and 200 individuals may be recognized as relatively abundant. The total species quantity in Lower Silesia is as high as *c*. 490 individuals, but 76% of them are concentrated in the small area of the Kiełczyńskie Hills. None of the populations has shown a decreasing tendency in size for the last 8–20 years (3 years the Radunia population). The tendency to increase the size may be observed in

populations of Janowice Wielkie (Fig. 2) and Przygórze. In the first half of the 1990s both populations were close to extinction with about 10 individuals each (Świerkosz 1992). Populations also differ in the contribution of the juvenile individuals. They usually do not exceed 5% of all plants in population, but their share may be sometimes as high as 15–20% (Janowice Wielkie, Kiełczyńskie Hills – KH2).

Tab. 1. Sizes of Lower Silesian *Asplenium adulterinum* populations. Population size tendency concerns the period of the last 8–20 years (3 years in case of the Radunia population)

Tab. 1. Wielkość dolnośląskich populacji *Asplenium adulterinum*. Określenie tendencji dynamicznych populacji obejmuje okres ostatnich 8–20 lat (3 lat w przypadku populacji z Raduni)

No. (Nr)	Localization (Stanowisko)	Population symbol (Symbol populacji)	Population size (Wielkość populacji)	Last observation year (Ostatnia obserwacja)	Population size tendency (Tendencja wielkości populacji)
1	Janowice Wlk.	J	32	2008	↗
2	Kiełczyńskie Hills	KH1	ca 200	2008	→
3	Kiełczyńskie Hills	KH2	54	2008	→
4	Kiełczyńskie Hills	KH3	2	2007	→
5	Kiełczyńskie Hills	KH4	11	2005	→
6	Kiełczyńskie Hills	KH5	14	2005	→
7	Kiełczyńskie Hills	KH6	ca 90	2008	→
8	Radunia	R	15	2008	→
9	Kamionki	K	50	2008	→
10	Przygórze	P	16	2008	↗
11	Żmijowiec	Ż	10	2005	→
	Total:		ca 490		

↗ – increasing tendency, → – constant size

↗ – tendencja wzrostowa, → – stan stały

Żołnierz (in prep.) examined biometrical features of *Asplenium* ferns (*A. adulterinum, A. cuneifolium* and *A. adiantum-nigrum*) growing in Lower Silesian serpentine outcrops. Some results regarding *A. adulterinum* are shown in Figures 3 and 4. The biggest population from the Kiełczyńskie Hills (KH1) also include the most vigorous plants. Their clumps possess the highest number of leaves, nearly 30 on the average. Those leaves are significantly longer than in other populations; the mean length of five longest leaves in the most vigorous ferns reaches almost 24 cm. Other populations do not differ significantly between each other in the number of leaves in clumps nor in the mean length of the five longest leaves.

Fig. 2. *Asplenium adulterinum* in the site in Janowice Wielkie (J; phot. L. Żołnierz)

Rys. 2. *Asplenium adulterinum* na stanowisku w Janowicach Wielkich (J; fot. L. Żołnierz)

Four of eleven sites (Janowice Wielkie and the Kiełczyńskie Hills: KH1, KH4, KH5) have an anthropogenic character; they are abandoned serpentine quarries. Other sites are of a natural origin being the small outcrops with the exception of Żmijowiec, where a group of large rocks with many crevices forms a lot of microhabitats, which can be occupied by ferns.

The kind and the strength of threats to the *Asplenium adulterinum* sites are specified in Table 2. The most important threats noticed in the sites of *A. adulterinum* are as follows: human penetration, disturbances in plant communities in the vicinity of the fern habitats, pressure from the competing species and lack of free microsites (crevices), which could be occupied by spleenworts.

Lower Silesian sites of *A. adulterinum* occur in the areas which are visited by large number of tourists. Some of them are situated close to villages and this is why, in all cases, the human impact on the ferns and their habitats have to be seriously taken into account. This kind of threat is mostly visible in Janowice Wielkie. The quarry with *Asplenium* ferns (*A. adulterinum* and also endangered *A. adiantum-nigrum*) is a recreational place frequently visited by inhabitants of the village and therefore ferns, which are gathered in a very small area of only few short crevices, may become extinct at any moment. Sites KH4 and KH5 in the Kiełczyńskie Hills are also abandoned quarries. Small fern populations grow there in places which are hard to reach but, un-

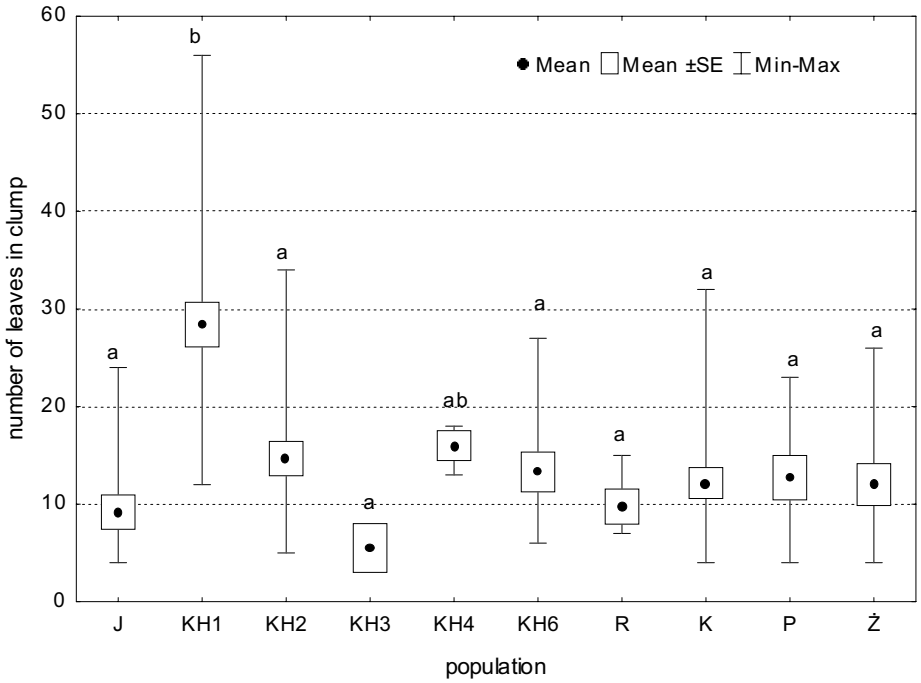

Fig. 3. Mean number of leaves in clumps of *Asplenium adulterinum* (from L. Żołnierz, unpbl.). Populations sharing the same letter indexes do not differ at p <0.05. (HSD Tukey's test after one-way ANOWA). For the explanation of population symbols see Tab. 1

Rys. 3. Średnia liczba liści w kępach *Asplenium adulterinum* (L. Żołnierz, npbl.). Populacje oznaczone tymi samymi indeksami literowymi nie różnią się przy p <0.05. (test HSD Tukey'a po jednoczynnikowej analizie wariancji). Wyjaśnienie symboli populacji – por. tab. 1

fortunately, both sites are visited by rock climbers, so the risk of human impact cannot also be excluded. This kind of threat is also present in the Kamionki (K) site, which is distant from the village no more than 100 m.

Disturbances in the neighbourhood of the fern sites may significantly modify environmental factors, especially those concerning microclimate such as insolation, air humidity, wind speed, evaporation rate, etc. Disturbances of the highest degree occur in the vicinity of the Żmijowiec (Ż) site. Rocks, which were previously surrounded and partially covered by spruce wood of the upper mountain belt, for about thirty years now are in the middle of the large deforested area. This forest decline appeared in that part of the Sudetes Mts. in the first half of the1970s. Probably, this is why the small population of *A. adulterinum* is currently restricted only to few shaded places on rocks, even though there are a lot of crevices, which potentially could be occupied by ferns, if the microclimate conditions are more suitable. Severe disturbances in the

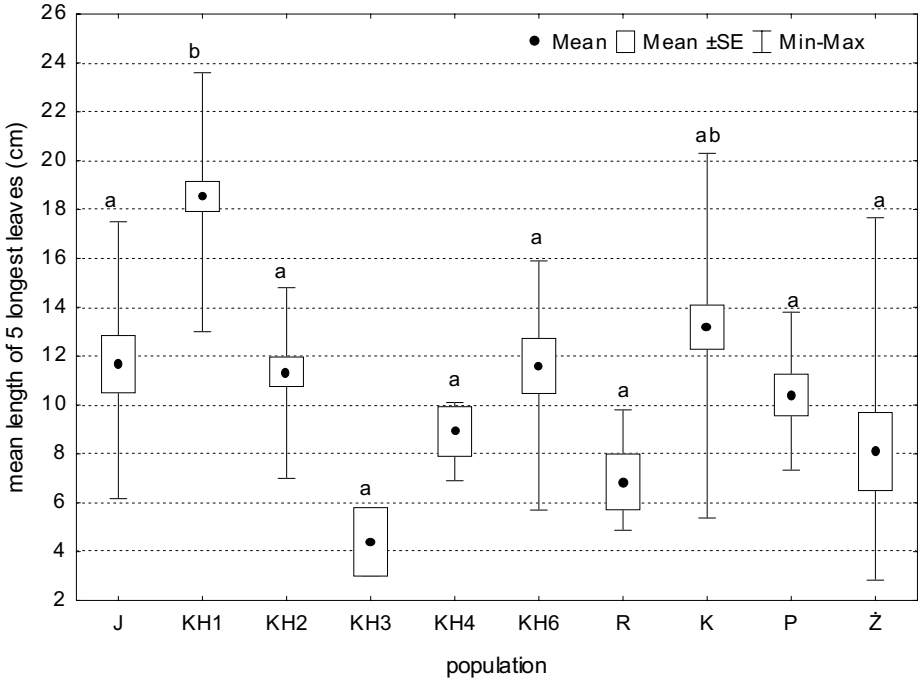

Fig. 4. Mean length of five longest leaves in clumps of *Asplenium adulterinum* (from L. Żołnierz, unpbl.). Populations sharing the same letter indexes do not differ at p <0.05. (HSD Tukey's test after one-way ANOWA). For the explanation of population symbols see Tab. 1

Rys. 4. Średnia długość pięciu najdłuższych liści w kępach *Asplenium adulterinum* (z L. Żołnierz, npbl.). Populacje oznaczone tymi samymi indeksami literowymi nie różnią się przy p <0.05. (test HSD Tukey'a po jednoczynnikowej analizie wariancji). Wyjaśnienie symboli populacji – por. tab. 1

surrounding of *A. adulterinum* sites also occur in the Kiełczyńskie Hills (KH6), where deforestation is a result of strong wind, and in Przygórze, where timber is harvested in the immediate vicinity of rocks with ferns.

The competition between *Asplenium adulterinum* and other vascular plants, which expand into fern microsites on serpentine rocks, is a very common phenomenon. Some grass species, above all *Calamagrostis epigeios* and *C. arundinacea*, and such nitrophilous shrubs as *Rubus idaeus* and *R. plicatus,* are particularly expansive and strong competitors. The expansion of competitor-species is stimulated by disturbances mentioned above leading to the decrease of free crevices and other microsites suitable for ferns. Additionally, the risk of *Asplenium adulterinum* extinction may result from their propable low genetic diversity. Because of the sites' isolation and the small population

Tab. 2. Kind and intensity of threats to *Asplenium adulterinum* populations in the investigated sites

Tab. 2. Rodzaj i wielkość zagrożenia populacji *Asplenium adulterinum* na badanych stanowiskach

No. (Nr)	Localization (Stanowisko)	Population symbol (Symbol populacji)	Human preassure intensity (Natężenie antropopresji)	Disturbances in the surrounding area (Zaburzenia w otaczającym terenie)	Pressure from the competitor species (Wpływ konkurencji)	Lack of free microsites (Brak dostępnych mikrosiedlisk)
1	Janowice Wielkie	J	***	***	**	**
2	Kiełczyńskie Hills	KH1	*	–	*	*
3	Kiełczyńskie Hills	KH2	*	–	**	*
4	Kiełczyńskie Hills	KH3	*	–	***	**
5	Kiełczyńskie Hills	KH4	**	*	**	**
6	Kiełczyńskie Hills	KH5	**	*	–	**
7	Kiełczyńskie Hills	KH6	*	**	***	**
8	Radunia	R	*	*	*	*
9	Kamionki	K	**	*	**	*
10	Przygórze	P	**	***	–	*
11	Żmijowiec	Ż	*	***	*	*

– – no threat, * – low threat; ** – moderate threat; *** – high threat
– – brak zagrożenia, * – niskie zagrożenie; ** – umiarkowane zagrożenie; *** – wysokie zagrożenie

size the probability of successful gene flow between them seems to be extremely low, even within the same massif, suggesting that all populations are highly inbred.

2.2. Conservation action framework

2.2.1. Existing law status of protection

Since 2004 *Asplenium adulterinum* has been under law protection in Poland (Minister of Environment 2004). The regulations contain the requirements of active protection procedures and demand an establishment of the 30 m radius protective zone around the species site (Minister of Environment 2004). *A. adulterinum* as a species "particularly important for the Community" is included in the appendix II of the European Union Habitat Directive (92/43/EEC) and protected in the territory of EU.

All Lower Silesian sites of *Asplenium adulterinum* except Kamionki are situated within the boundaries of landscape parks (Rudawski, Ślężański, Gór Sowich and Śnieżnicki) as well as Natura 2000 network areas. Three out of six sites on the Kiełczyńskie Hills (KH1, KH4 and KH5) are recognized as ecological areas ("użytek ekologiczny" – the form of nature protection in Poland of lower status in comparison to a nature reserve; Governor of the Lower Silesia Province 2003).

2.2.2. Concept of further procedures

Proposal of the law status improvement

There is an urgent need to correct the law status of the Kamionki site. It has to be included in the enlarged Natura 2000 habitat area (PLH020005) and the landscape park (Gór Sowich). On the other hand, Kamionki is the only site where all rare *Asplenium* "serpentine ferns", i.e. *A. adulterinum*, *A. cuneifolium* and *A. adiantum-nigrum*, which in Poland are limited only to serpentine habitats, occur together. In our opinion that is a sufficient reason to establish a floristic reserve with an area of about 1.2 ha.

The network of the ecological protection areas was planned (Żołnierz 1997) and established (Governor of the Lower Silesia Province 2003) for protection of rare *Asplenium* species in the area of the Ślęża Massif in the landscape park Ślężański. It seems that this network should be expanded to include all the known *Asplenium* sites in Poland.

Proposal of the active protection and monitoring procedures

We formulate the main aims of the *Asplenium adulterinum* protection plan as follows:

- To sustain the current species area in Poland (*c*. 60 km^2).
- To maintain all existing (11) sites of species.
- To maintain current species resources (*c*. 500 individuals).
- To keep *ex situ* protected collections of plants representing natural populations, at least those, whose size is smaller than 50 individuals. The *ex situ* maintained collection of fern individuals would be used to reintroduce declining or becoming extinct populations.

The survival of small populations of *Asplenium adulterinum* in sites lacking free microsites and where ferns have to compete with expansive grasses, herbs and shrubs requires active protection procedures, which are postulated by many authors (Vogel, Breckle 1992; Fabiszewski 1993; Żołnierz 1993, 2001, 2004; Čeřovský, Klaudisová 1999; Kromer *et al.* 2006; Szczęśniak 2006).

Our idea for active protection procedures primarily concerns the shaping of the sites and their protective zones on purpose to obtain optimal microclimate conditions. It could be achieved by the following activities:

- Controlling of the density of tree stands.
- Planting trees in deforested surroundings of the sites in Przygórze and Żmijowiec.
- Removing *Robinia pseudoacacia* trees from sites in the Kiełczyńskie Hills to stop the nitrogen input.
- Removing the competitor species from the fern microsites. This operation must be performed very carefully to avoid the damage of ferns and the destruction of the soil filling the crevices. In addition, it is planned to carry out an experiment in a similar site to check the effectiveness of the herbicide treatment targeted on shrubs.
- Preparation of the microsites to make them available for ferns. The removal of plants from the crevices, especially below those already occupied by ferns and shortly before the spore release, may significantly increase the chance for the juvenile individuals to appear.

All sites and populations should be monitored regularly and frequently enough to make it possible to quickly react if any adverse effects of fern fitness emerge. The usage of *ex situ* maintained individuals for reintroduction is taken into account only if any population is driven into extinction or declines significantly.

2.3. Development of the *in vitro* culture method and *ex situ* preservation of *Asplenium adulterinum*

As a result of biotope degradation, the protection *in situ* of ecosystems and biocoenoses is frequently impossible and therefore the protection of perishing species in *ex situ* conditions, meaning beyond their natural biotopes, is more often mentioned (Łukasiewicz 1985).

The *ex situ* methods rely, first of all, upon cultivation and conservation, which is common in botanical gardens, and then on the reintroduction to the natural environment. One of the *ex situ* methods of preserving the threatened plant species is the technique of the *in vitro* cultures, which is based on the growth of isolated tissues or cells in suitable conditions. *In vitro* cultures are a very good way of propagating many plant species, especially the recalcitrant species, i.e. species difficult in generative reproduction. Moreover, cultures help us to recognize the nutritional requirements and to get known details of the plant-life cycle. Cousens *et al*. (1989) mentioned that the majority of fern gametophytes perish in natural conditions. Gametophytes frequently do not survive the most critical developmental phases such as germination of spore, prothallial growth, fertilization and development of the young sporophyte. Therefore, *in vitro* cultures ensure the undisturbed growth and development of ferns (Zenkteler 1992).

In the Botanical Garden of the University of Wrocław in the Plant Tissue Culture Laboratory we carried out research on a serpentine fern species, *Asplenium adulterinum.* Results of studies on propagation of *Asplenium adulterinum* under *in vitro* conditions are satisfactory. We could observe the growth of prothalli from sown spores, development of sex organs, egg fertilization and formation of zygote that begins the sporophyte generation. Gametophytes growing in the culture were able to propagate forming the secondary prothalli, similarly like sporophytes regenerating new shoots, fronds and rhizomes. This means that the whole reproductive life cycle of the fern was completed *in vitro* showing the exceptional usefulness of this technique in generative as well as vegetative propagation of *A. adulterinum*.

The *in vitro* culture is also an effective method of preserving genetic diversity of the *A. adulterinum* fern in the conservation program. Heart-shaped gametophytes of this plant species well tolerated storage in +8°C and light intensity of 0.15 $\mu mol \cdot m^{-2} \cdot s^{-1}$, on the medium of 1/2MS, without growth regulators. The influence of full-strength, 1/2, 1/4 and 1/8 of MS basal medium was tested in experiments. The growth and development of gametophytes were affected by the medium composition. Media of 1/2 MS gave the best results in terms of the fresh weight increase. Gametophytes growing on this medium had the correct shape, were the most numerous and formed many secondary gametophytes. On both media, rich (full-strength MS medium) and poor (1/8 MS) in nutrients, the fresh weight and the number of primary gametophytes were low. Some gametophytes became necrotic on the full-strength MS medium. The effect of medium concentration proved that this species requirements connected with the level of nutrition are not so low. They can be estimated as moderate, because the MS medium formula belongs to the richest the used in tissue cultures.

The influence of various soils on the survival of sporophytes transferred from *in vitro* culture to the greenhouse was evaluated. The best soil for *ex vitro* cultivation was Kronen commercial mix for ferns at pH 6.5; soil taken from the natural environment of pH 7.2 was equally good. It can be concluded that for the *A. adulterinum* growth the soil from the natural environment is not absolutely required and can be substituted. Cultivation of *A. adulterinum* proceeded satisfactorily; plants acclimated well outside in a garden frame if they were initially shaded. *A. adulterinum* reached the sporophytic phase in the second year of cultivation (Fig. 5 and 6).

Results of the studies proved that *in vitro* culture is an excellent method of generative and vegetative propagation of *A. adulterinum* and it is a perfect tool of gaining knowledge on the biology of this species.

Fig. 5. Propagation of *A. adulterinum* sporophytes growing *in vitro* (phot. D. Poturała)

Rys. 5. Namnażanie sporofitów *A. adulterinum in vitro* (fot. D. Poturała)

Fig. 6. Mature sporophytes originated from the *in vitro* culture and cultivated *ex situ* in the Botanical Garden of the University of Wrocław (phot. D. Poturała)

Rys. 6. Dojrzałe sporofity pochodzące z kultur *in vitro*, utrzymywane *ex situ* w Ogrodzie Botanicznym Uniwersytetu Wrocławskiego (fot. D. Poturała)

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Zanokcica serpentynowa (*Asplenium adulterinum* Milde) w Polsce – rozmieszczenie, stan zachowania populacji i założenia programu ochrony

Zanokcica serpentynowa *Asplenium adulterinum* Milde to paproć ściśle związana z siedliskami serpentynitowymi, dlatego na terenie Polski spotykana jest tylko na Dolnym Śląsku, jako jedynym rejonie występowania tych skał. W początkach XX w. gatunek podawany był z 13 lokalizacji. Do naszych czasów dotrwało 11 niewielkich populacji. Większość z nich osiąga liczebność poniżej 20 osobników, tylko na Wzgórzach Kiełczyńskich znajdują się dwa stanowiska z ok. 90 i 200 kępami zanokcicy serpentynowej. Gatunek ten – choć w zróżnicowanym stopniu – zagrożony jest na wszystkich swoich stanowiskach. Poza niską liczebnością populacji oraz ich wsobnością wynikającą z izolacji

stanowisk, zagrożenia związane są przede wszystkim z: niewielką powierzchnią większości stanowisk, ograniczoną dostępnością odpowiednich mikrosiedlisk w obrębie odsłonięć skalnych oraz silną presją konkurencyjną ze strony współwystępujących gatunków. Ponadto na właściwości siedlisk i stan populacji niekorzystnie wpływają zaburzenia w otaczających zbiorowiskach roślinnych. Stanowiska gatunku w większości znajdują się na terenach silnie penetrowanych przez ludzi.

Od 2004 r. zanokcica serpentynowa objęta jest ochroną gatunkową. W zapisach dotyczących statusu prawnego gatunku uwzględniono konieczność prowadzenia zabiegów ochrony czynnej, a także zaznaczono wymóg tworzenia wokół stanowisk stref ochronnych o promieniu 30 m. Zanokcica serpentynowa znalazła się na liście gatunków zgłoszonych przez Polskę do poszerzonego załącznika II Dyrektywy Siedliskowej UE, leżącej u podstaw europejskiej sieci obszarów chronionych Natura 2000. Stan prawny gatunku wydaje się zadowalający, proponuje się jednak dodatkowe rozwiązania, takie jak: poszerzenie sieci użytków ekologicznych na stanowiskach nieobjętych dotąd ochroną prawną oraz korektę granic obszaru Natura 2000 w rejonie Kamionek w Górach Sowich. Na stanowisku tym postuluje się utworzenie rezerwatu florystycznego z uwagi na to, że jest to jedyne w Polsce miejsce jednoczesnego występowania trzech rzadkich i zagrożonych gatunków „serpentynitowych" zanokcic: *Asplenium adulterinum, A. cuneifolium* i *A. adiantum-nigrum*.

Warunkiem przetrwania gatunku jest objęcie jego populacji i siedlisk ochroną czynną. Przewidziano takie przedsięwzięcia jak: kontrola zwarcia drzew i krzewów na stanowiskach paproci, usuwanie gatunków konkurencyjnych, przygotowywanie mikrosiedlisk w celu wspomagania ich zasiedlania przez zanokcice. Dla populacji o liczebnościach poniżej 50 osobników przewidziano tworzenie *ex situ* banków genów, przy czym wykorzystane zostaną techniki hodowli gatunku *in vitro*, rozwinięte w Pracowni Kultur Tkankowych Ogrodu Botanicznego Uniwersytetu Wrocławskiego. Efekty wdrożonego programu będą regularnie kontrolowane w toku wieloletniego monitoringu stanu populacji i ich siedlisk.