Edited by **Grażyna Łaska**

Biological diversity

from cell
to ecosystem

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– from cell to ecosystem

Polish Botanical Society
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– from cell to ecosystem

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Preface

The book presents results of studies concerning biological diversity in a wide sense, analysed at different levels of organisation of biological life, from the cell to the ecosystem. The protection of biodiversity and its sustainable use, in the light of the Convention on Biological Diversity, is a complex problem. It involves a comprehensive analysis of all kinds of transformations taking place in the areas of genetics, physiology, biochemistry, flora, demography and phytosociology performed in the aspect of interdisciplinary research as well as the search for mechanisms and determination of directions of measures needed to prevent the loss of biodiversity.

The authors of the papers collected in this monograph have analysed the diversity of fungi, lichens, vascular plants in land and freshwater ecosystems in the aspects of their genetic, population, biocenotic and phytocenotic variations, taking into account the effects of many natural, abiotic and anthropogenic disturbances and mechanisms of plant acclimatisation to variable environmental stresses. The analysis has been made against a background of functioning of biotic and abiotic elements of the natural environment and in the light of their sustainable and rational use. The monograph presents problems related to the diversity of water and soil fungi, ecological-floristic and phytosociological analysis of lichens in the areas subjected to different anthropopressure, genetic and population variation of the taxons of significance to EU member states, mentioned in Enclosure II of the Habitat Directive, and comprehensive analysis of protection of many taxons and valuable habitats mentioned in Enclosure I of the Habitat Directive, taking into account their role for the functioning of Natura 2000 network. Of great interest are also the results concerning the antistress role of plant hormones and different responses of plants aimed at prevention of the effects of oxidation stress, deficiency or excess of mineral components and mechanical damage.

The monograph is a result of integration of many measures undertaken to sustain balance in the natural environment and continuity of basic natural processes at all levels of life organisation. It is intended as a contribution to protection of natural heritage and preservation of biodiversity for future generations.

Grażyna Łaska

Chloroplast movements induced by light: diversity of mechanisms in various taxa

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Abstract

The light-controlled relocation of chloroplasts is widespread among photosynthetic organisms enabling them to optimize energy capture under limiting light conditions and to minimize potential photodamage in excess light. Chloroplasts move passively, driven by forces operating outside the organelles. The photoreceptors involved in light signal perception and transduction are encoded in the nuclear genome and localized at the cell membrane. Different strategies for light-induced chloroplast movements have evolved in various phyla. Some traits are characteristic only of mosses, ferns and water plants. Firstly, the responses of chloroplasts are coupled with cytoplasmic streaming in these organisms, at least at some stages of development. Secondly, apart from blue/UV, red/far red light is also active in controlling the movements. The long wavelengths are absorbed by phytochrome(s) and/or by a hybrid photoreceptor, neochrome. Thirdly, microtubules have been shown to contribute to the motile system in the protonemal cells of *Physcomitrella patens*, apart from the typically involved microfilaments. In contrast, a uniform mechanism seems to operate in higher terrestrial plants. No evidence has been provided for a relationship between cytoplasmic streaming and light-induced chloroplast responses in these plants. Movements are induced only by blue/UV light via the activation of phototropins. While the participation of the actin cytoskeleton in the movement mechanism is unequivocal, the mode of actin involvement remains debatable. According to one hypothesis, the motive force comes from myosin(s) associated with the chloroplast surface interacting with actin filaments. An alternative model puts forward specific short actin filaments as elements entirely responsible for chloroplast movements.

Key words: cytoplasmic streaming, cytoskeleton, blue light, red light, photoreceptor

1. Introduction

Chloroplasts hold a special place among plant organelles as they are the site of photosynthesis – the transformation of light energy into chemical energy. In the vast majority of plants chloroplasts do not occupy a stable position in the cell but are capable of movement alongside its periphery. Light is the major environmental cue that controls these movements and, as a result, the distribution of chloroplasts. According to light direction, colour and intensity, chloroplasts migrate to defined areas of the cell. They accumulate in weakly illuminated regions and avoid regions exposed to strong light. The accumulation response helps to maximize light harvesting under energy-limiting conditions (Zurzycki 1955; Takemiya et al. 2005). On the other hand, the avoidance response protects the photosynthetic apparatus from excess energy in strong light (Park et al. 1996; Kasahara et al. 2002; Sztatelman et al. 2010).

Light-controlled chloroplast movements have been discussed in numerous reviews (Haupt, Scheuerlein 1990; Haupt 1999; Takagi 2003; Wada et al. 2003; Gabryś 2004; Wada, Suetsugu 2004; Gabryś 2012; Banaś et al. 2012).

2. General characterization of motile activity

The motile behavior of chloroplasts is more diversified than that of other organelles. In cells containing multiple chloroplasts it may be roughly divided into three categories shown schematically in Fig. 1. In the large, elongated internodal cells of Characeae, the chloroplasts are stationary, i.e. practically immobilized in the cortical, dense layer of cytoplasm (ectoplasm). The adjacent, inner layer of cytoplasm (endoplasm) steadily streams (Verchot-Lubicz, Goldstein 2009). In contrast, the movement of chloroplasts in several water angiosperms (e.g. *Elodea* and *Vallisneria* sp.) is closely connected with cytoplasmic streaming (Haupt 1982). They swim with the bulk cytoplasm. This is set in motion in response to environmental stimuli, among others – light. Chloroplasts in the mesophyll of terrestrial flowering plants demonstrate a different behavior in that respect – their movement is not linked with cytoplasmic flow. The organelles oscillate around a stationary position in the dark and start to move progressively only in response to light, without any perceptible cytoplasmic movement.

A different type of movement is observed in the filamentous green algae *Mougeotia* and *Mesotaenium* sp. which belong to Zygnemaphyceae (Grölig, Wagner

1988). Each cylindrical *Mougeotia* cell contains a single ribbon-shaped chloroplast which rotates to expose its face to weak light or its edge to strong light. During the rotation the chloroplast edges slide in the thin layer of cytoplasm. The cells of *Selaginella martensii* (Lycophytes) also contain single, giant cup-shaped chloroplasts which perform ameboidal movements (Zurzycki, Zurzycka 1952).

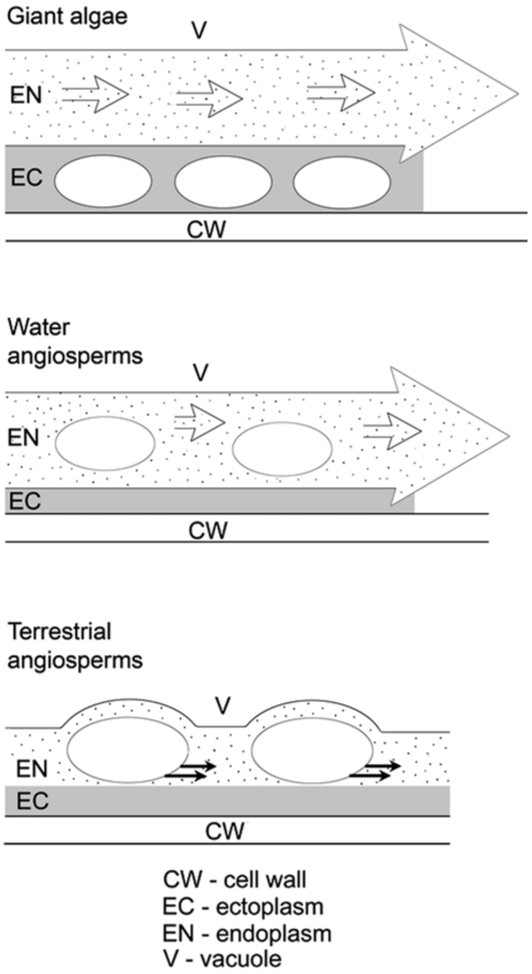

Figure 1. Three basic types of relationships between chloroplast movements and cytoplasmic streaming in various plant groups

Rycina 1. Trzy główne typy relacji pomiędzy ruchami chloroplastów a płynięciem cytoplazmy w różnych grupach roślin

3. Photoreceptors involved

As shown in Fig. 2. chloroplasts respond to blue light in all taxa studied so far. Two photoreceptors associated with the plasma membrane, phototopin 1 and 2, absorb light, which induces their movement responses in the model terrestrial angiosperm, *Arabidopsis thaliana* (Jarillo et al. 2001; Kagawa et al. 2001). It seems logical to assume that phototropins also play this role in other blue-responding species. However, apart from *A. thaliana*, direct evidence is currently available only for the moss *Physcomitrella patens* (Kasahara et al. 2004). Phototropins are light-regulated kinases that also control other movement processes induced by blue light in plants: phototropism, stomatal opening, and leaf positioning and expansion (Sakai et al. 2001; de Carbonnel et al. 2010). In all these processes they act in a redundant way, with phot1 operating in weak light, and phot2 – in strong light. Both phototropins redundantly control chloroplast accumulation, whereas the avoidance response is controlled by phot2 alone (Jarillo et al. 2001; Sakai et al. 2001). It is noteworthy that phot2 alone is also responsible for the blue-light-controlled positioning of nuclei in *A. thaliana* (Iwabuchi et al. 2007). The role of phototropins in chloroplast movements has recently been reviewed by Banaś et al. (2012).

As well as phototropins, red light-absorbing photoreceptors also control chloroplast relocation in several cryptogams (*P. patens, M. scalaris, A. capillus-veneris*). Most responses initiated by red light can be reversed by far-red, indicating the involvement of phytochrome(s). *Mougeotia* has been regarded as a model object for phytochrome-controlled responses of chloroplasts (for a review, see Haupt 1999). The weak light-induced rotation of its chloroplast can be mediated either by a phytochrome (particularly in red light) or by a blue light photoreceptor (a phototropin?). The strong light response requires the cooperation of the two systems (see Haupt, Scheuerlein, 1990; Haupt 1999, and references therein). A time-resolved excitation of both systems using separated blue and red pulses has shown that the interaction takes place downstream of the photoreceptors (Gabryś et al. 1985).

In terrestrial angiosperms, phototropins are indispensable to the activation of chloroplast movements, and phytochromes play only a modulatory role. Arabidopsis mutants lacking phytochrome A or B show a stronger avoidance response. In contrast, the accumulation response is stronger in those plants where phytochromes A or B are overexpressed (DeBlasio et al. 2003). Phytochrome B has been shown to attenuate the avoidance response in cooperation with both phototropins (Luesse et al. 2010).

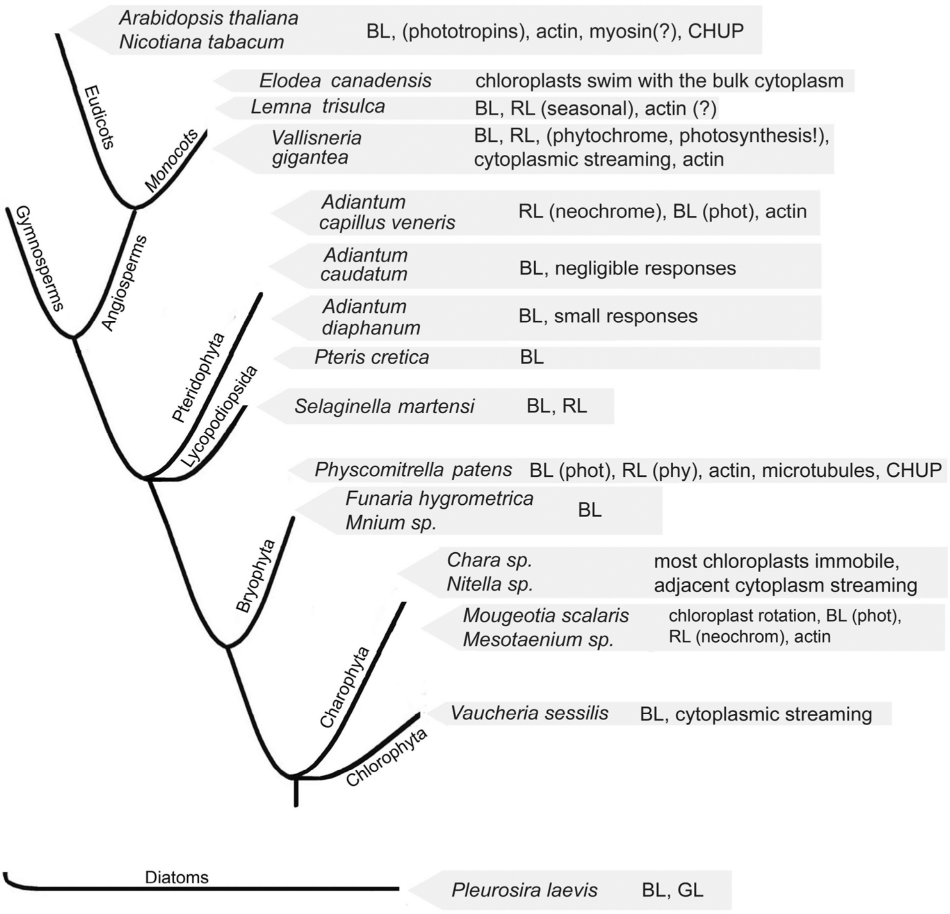

Figure 2. Selected features of light-controlled chloroplast relocations in model plant species. BL, RL, GL: spectral ranges inducing the relocations, blue, red and green light respectively; phot – phototropin, phy – phytochrome. *Pteris* (Augustynowicz, Gabryś 1999), *Mnium* (Zurzycki, Lelątko 1969), *Pleurosira* (Furukawa et al. 1998); other references in the text

Rycina 2. Wybrane cechy kontrolowanych przez światło przemieszczeń chloroplastów w modelowych gatunkach roślin. BL, RL, GL: światło niebieskie, czerwone i zielone, zakresy spektralne wywołujące przemieszczenia chloroplastów; phot – fototropina, phy – fitochrom. *Pteris* (Augustynowicz, Gabryś 1999), *Mnium* (Zurzycki, Lelątko 1969), *Pleurosira* (Furukawa et al. 1998); pozostałe odnośniki w tekście

Both red and blue spectral regions are active in stimulating the accumulation response of chloroplasts in *V. gigantea*. Red light has approximately the triple quantum efficiency of blue light. In contrast, the avoidance response is activated almost exclusively by blue light (Izutani et al. 1990). The effects of red light can be annulled either by far-red light or by DCMU (dichlorophenyl dimethylurea), an inhibitor of the photosynthetic electron transport chain (Dong et al. 1996; Dong et al. 1998). This has been interpreted in terms of a joint regulation of the accumulation response by the P_{FR} form of phytochrome together with photosynthesis. This type of regulation appears to be a rather unique trait of *Vallisneria* as DCMU does not inhibit chloroplast responses to light in other studied species. In particular, it is inactive in *Lemna trisulca* and *Arabidopsis thaliana* (Ślesak, Gabryś 1996).

A chimeric photoreceptor neochrome has been shown to mediate chloroplast movements in the fern *Adiantum capillus-veneris* (Kawai et al. 2003). Neochrome contains a phytochrome chromophore-binding domain attached to the N-terminus of full-length phototropin. Neochrome genes have also been found in *Mougeotia scalaris*, but no evidence has been presented that this chimeric photoreceptor is involved in the light-regulated rotation of its chloroplast. On the other hand, the transient expression of two *MsNEO* genes rescued red-light-induced chloroplast movement in an *A. capillus-veneris* mutant devoid of the neochrome gene, which indicates their functional equivalence (Suetsugu et al. 2005b). Interestingly, no genes encoding neochrome have been found in *P. patens*, whose chloroplasts move under the control of both the red and blue spectral regions (Suetsugu et al. 2005b).

4. Chloroplast responses dependent on cytoplasmic streaming

At least two distinct types of mechanisms seem to operate in chloroplast relocation, one in mosses, ferns and water plants, and another in higher terrestrial plants.

The chloroplast movement concomitant with cytoplasmic streaming has been studied in detail in *Vallisneria sp*. (Izutani et al. 1990, Dong et al. 1996; Dong et al. 1998; Sakurai et al. 2005). Chloroplasts are motionless in the dark. Upon illumination the cytoplasm starts to rotate along the cell. Chloroplasts join the stream of the cytoplasmic matrix after it reaches maximum velocity (Seitz 1979). Under continuous weak light chloroplasts migrate to the periclinal side of the cell, i.e. to the side perpendicular to the light direction. The migration in weak light is slow; the chloroplasts accumulate in the light-exposed areas after several hours. Irradiation with strong light rapidly directs the plastids to the anticlinal sides, parallel to the light direction. This avoidance response is much faster than accumulation and requires several tens of minutes of illumination with strong light to be completed.

A dependence of chloroplast movements on cytoplasmic streaming, similar to that observed in *Vallisneria sp.*, has been described for the coenocytic alga *Vaucheria sessilis* (Blatt, Briggs 1980). In this case, however, chloroplast/cytoplasm movements are activated only by blue light.

A remarkable feature of this type of mechanism is the special rearrangement of the actin cytoskeleton which accompanies the accumulation of chloroplasts at the periclinal wall. Actin bundles are arranged in a network array in dark-adapted cells. Upon irradiation with red light, the actin network is slowly rebuilt into a unique honeycomb structure. The motility of chloroplasts is gradually reduced, and the organelles become trapped inside the cavities of this structure built of light-reorganized actin (Dong et al. 1996; Dong et al. 1998). Strong blue light, absorbed by a blue-specific photoreceptor (phototropin?), initiates a rapid reconstruction of F-actin into linearly arranged bundles and chloroplasts regain mobility. Following a short phase of random small-range activity they start to move progressively along the actin tracks (Sakurai et al. 2005).

Various elements of the mechanism proposed for *Vallisneria* can be found in the light-mediated chloroplast positioning of several species of cryptogams (for review see Wada et al. 2003). For example, characteristic rearrangements of actin filaments associated with single chloroplasts have been demonstrated in the protonemal cells of *A. capillus veneris* during the accumulation response (Kadota, Wada 1992a). However, a video-tracking analysis revealed major discrepancies between trajectories and dynamics of chloroplasts and cytoplasm which suggest that these activities may be independent (Kadota, Wada 1992b).

Unlike most plants studied to date, the cytoskeletal basis of chloroplast movements combines a direct involvement of both microfilament and microtubular systems in the moss *Physcomitrella patens* (Sato et al. 2001). The microtubular cytoskeleton seems to play a secondary role in the mechanism of organelle movements in plants. The motility of organelles is considered to be based on microfilaments while microtubules stabilize the positioning of organelles. However, in the chloroplast movement of *P. patens* a cooperation of microfilament- and microtubule-based systems has been demonstrated by combining blue and/or red microbeam irradiations and treatment with two inhibitors, latrunculin B which disrupts microfilaments, and cremart which disrupts microtubules (Sato et al. 2001). Both systems are differently regulated by two types of photoreceptors. Similar to the system operating in higher plants, actin filaments in *P. patens* are the target of a blue light regulatory pathway. Two more pathways based on microtubules have been proposed. One of them is controlled by phytochrome and the other by a blue light receptor. According to the authors, chloroplast movements in this moss may represent an evolutionary intermediate between a microtubule-dominated movement system operating in algae and a microfilament-based system operating in higher plant cells.

5. Chloroplast responses independent of cytoplasmic streaming

No interdependence has been demonstrated between chloroplast movements in the mesophyll of terrestrial angiosperms and cytoplasmic streaming. In that respect, chloroplasts move in a more autonomous way in terrestrial than in water angiosperms. As with other plant organelles, the F-actin network is the basis of chloroplast motility. Nonetheless, the nature of the involvement of the actin cytoskeleton still needs to be determined. Two contrasting models of this involvement have been proposed recently.

Kadota et al. (2009) proposed a model of blue light-controlled chloroplast redistribution based entirely on rearrangements of short actin filaments (cp-actin) situated on the surface of chloroplasts. The model is based on results obtained with transgenic *Arabidopsis* plants expressing the GFP-mouse talin fusion protein. The motive force for chloroplast movement is deemed to be due to differentiated state of cp-actin filaments at opposite sites of the illuminated organelle. The occurrence of cp-actin filaments depends on an actin binding protein, chloroplast unusual positioning 1 (CHUP1), localized on the chloroplast envelope (Oikawa et al. 2003). CHUP1 has been shown to link chloroplasts to actin filaments (Schmidt von Braun, Schleiff 2008). Short actin filaments are absent in the *chup1* mutant whose chloroplasts are immobile (Kadota et al. 2009). The authors of the model believe that plants have evolved a unique, actin-based mechanism for chloroplast movements, different from those identified to date for other organelles.

Another model assumes that chloroplasts are powered by actin-myosin motors similar to other organelles (Krzeszowiec, Gabryś 2007). Two types of results led to this conclusion. On the one hand, no reconstruction of the actin network was found in the mesophyll of *A. thaliana* or *N. tabacum* in contrast to those depicted for cryptogams and water angiosperms in blue light (Krzeszowiec et al. 2007, Anielska-Mazur et al. 2009). Blue and red light produced similar effects, yet the latter region does not activate chloroplast relocation in terrestrial angiosperms. In addition, blue light does not induce any particular changes in the baskets built of fine F-actin surrounding the chloroplasts, as visualized in the mesophyll of transgenic *N. tabacum* expressing truncated human plastin (an actin-bundling protein) fused with GFP (Anielska-Mazur et al. 2009). On the other hand, investigations using polyclonal antibodies against plant myosin VIII and animal myosins showed differential patterns of myosins on the surface of *Arabidopsis* chloroplasts, which depended on light intensity and colour. Myosins covered the chloroplast envelopes in tissue irradiated with weak blue light. By contrast, in tissue irradiated with strong blue light the chloroplasts were almost myosin-free. No effect occurred either in red light or in the *phot2* mutant which lacks phototropin 2 and, in consequence, any avoidance response of chloroplasts. On the contrary, the redistribution of myosins was similar to wild type at the surface of chloroplasts in a *phot1* mutant. Both accumulation and avoidance responses are normal in this mutant, and only slightly shifted towards higher light intensities as a result of the impaired expression of phototropin 1. Thus, actin-myosin interaction has been suggested to power chloroplast movement, similar to movements of other plant organelles.

Attempts to identify the motor proteins active in chloroplast movements have so far been unsuccessful. Myosin has been shown to participate in movements of plant organelles (Avisar et al. 2008). Suppression of the myosin XI-K function and/or RNA interference dramatically reduced the movement of peroxisomes, mitochondria and Golgi stacks in *N. benthamiana* leaf cells. However, none of the tested myosins appeared to be involved in light-induced movements of chloroplasts. These data point to a principal role for myosin XI-K in the trafficking of peroxisomes, mitochondria and Golgi stacks but not in chloroplast movement.

Differences in the mechanisms operating in various plant groups extend also to the regulation of chloroplast movements. For example, their regulation by Ca^{2+} differs fundamentally in water plants and terrestrial angiosperms. Whereas extracellular Ca^{2+} at millimolar concentrations has an inhibitory effect on the chloroplasts of *V. gigantea* (Takagi, Nagai 1986) and *E. densa* (Forde, Steer 1976), it does not affect chloroplast redistribution in *N. tabacum* (Anielska-Mazur et al. 2009) and *A. thaliana* (unpublished results). Ca^{2+} ions have been reported to inhibit cytoplasmic streaming in pollen tubes and the hair cells of higher plants (Kohno, Shimmen 1988). These facts additionally support the view that chloroplast relocation in water angiosperms is linked with cytoplasmic movements while it is more autonomous in terrestrial angiosperms.

6. Long-term environmental and developmental impacts

Light conditions of growth have a significant influence on chloroplast distribution in cells and on the movement control system. The re-positioning of chloroplasts in darkness following irradiation is the least investigated chloroplast response mainly because it is very slow. While responses to light require about 1,5 h of constant illumination for chloroplasts to attain a stationary position in higher land plants, the return to dark arrangement lasts several hours. Conventionally, chloroplasts are assumed to adopt a random distribution under all cell walls. This is not always true. For example, the chloroplasts of *Funaria hygrometrica* gather at anticlinal walls in the dark, thus their dark distribution is indistinguishable from that obtained during the avoidance response (Zurzycki 1967). Studies on *A. thaliana* grown under different intensities of white light (close to the compensation point of photosynthesis and in excess, non damaging light) have shown striking differences in the dark arrangement of chloroplasts (Trojan, Gabryś 1996). While the percentage of chloroplasts on anticlinal walls was 36,5 in weak-light cultivated plants, it increased to 71% in strong-light cultivated plants. Thus, chloroplasts are closer to the position characteristic of the accumulation/avoidance response in leaves grown in weak/strong light respectively. Less striking but still obvious differences can be observed in both types of chloroplast response to light. It has to be kept in mind that most experiments are usually performed on plants cultivated under artificial laboratory conditions, often in light enriched in some spectral (e.g. red) region and thus the responses may differ, at least quantitatively, from those occurring in nature. The results of measurements taken in the field support this observation. Chloroplast movement in response to bright light was rapid allowing responses to brief sunflecks. Movements measured in four species were qualitatively similar, with differing kinetics and magnitudes. It should be noted that chloroplasts were in motion most of the time, rarely achieving extreme anticlinal or periclinal positions.

Plants adapted to dissimilar environmental conditions have diverse patterns and mechanisms of chloroplasts to light. This has been revealed among others in a methodical study of the sporophytes of three fern species belonging to the genus *Adiantum: A. caudatum, A. capillus-veneris* and *A. diaphanum* (Augustynowicz, Gabryś 1999). *A. caudatum* tolerates dry conditions and can be found on earth banks and rock faces, in full sun. *A. capillus-veneris* is the most flexible plant naturalized in many places, and it tolerates a broad range of light conditions. The third one is a shade and wet loving species. Two spectral regions, red and blue, cooperate in the control of chloroplast distribution in the *A. capillus-veneris* sporophyte, similar to its protonema. Red light activates an accumulation response irrespective of intensity. Chloroplast responses are much less dynamic in the two species which exhibit a low degree of environmental flexibility. The limited (or zero) responsiveness of chloroplasts to light seems to be a general feature of obligate sun-loving plants, e.g. *Pisum sativum* (Park et al. 1996), *Petunia hybrida* and several species of grasses (unpublished results). Apparently, these plants have developed and/or intensified other mechanisms to protect the photosynthetic apparatus from excess light.

An interesting developmental effect has been observed in the duckweed *Lemna trisulca*. This water angiosperm differs from the ones described above in that the relocation of its chloroplasts is activated only to blue light and does not involve any noticeable cytoplasmic movement (Zurzycki 1962). Sporadically, in periods of intense development, orientation movements of *L. trisulca* chloroplasts also become controlled by red light (unpublished results). As the exact conditions necessary to activate these red-light-induced responses have not been determined and the directional effect of red light lasts only a few weeks, this episodic phenomenon awaits investigation.

7. Concluding remark

In the last decade, the cellular and molecular background of chloroplast relocation in *Arabidopsis thaliana* has been the focus of most laboratories involved in the studies of mechanisms of chloroplast movements. Among the most interesting results (so far limited to *Arabidopsis* and *Physcomitrella*) are discoveries of new regulatory proteins, apart from CHUP1 also WEB1, Thrumin, KAC1 and KAC2, PMI2 and Jac1 (DeBlasio et al. 2005, Suetsugu et al. 2005a, Kodama et al. 2010, Suetsugu et al. 2010, also see Banaś et al. 2012). The role of these proteins in the response mechanism needs to be clarified. This short review shows how many other issues need to be investigated before reliable models of this mechanism can be proposed for various taxa.

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Indukowane światłem ruchy chloroplastów: rozmaitość mechanizmów u różnych taksonów

Streszczenie

Kontrolowane światłem przemieszczenia chloroplastów występują powszechnie wśród organizmów fotosyntetycznych, z jednej strony umożliwiając im optymalne wykorzystanie energii w warunkach słabego światła, zaś z drugiej minimalizując możliwość uszkodzenia przez silne światło. Siły poruszające chloroplasty wytwarzane są poza tymi organellami. Fotoreceptory zaangażowane w odbiorze sygnału świetlnego są kodowane w genomie jądrowym i zlokalizowane przy błonie komórkowej. W procesie ewolucji, w różnych taksonach rozwinęły się różnorodne strategie indukowanych światłem przemieszczeń chloroplastów. Niektóre cechy tych przemieszczeń są charakterystyczne tylko dla mchów, paproci i roślin wodnych. Po pierwsze, w organizmach tych odpowiedzi chloroplastów są ściślej powiązane z ruchami cytoplazmy,

przynajmniej w niektórych stadiach rozwojowych. Po drugie, poza światłem niebieskim i ultrafioletowym ruchy chloroplastów są kontrolowane również przez światło czerwone i daleką czerwień. Światło długofalowe jest absorbowane przez fitochrom(y) i/lub przez chimeryczny fotoreceptor neochrom. Po trzecie, w komórkach przedrośli Physcomitrella patens, poza mikrofilamentami stwierdzono zaangażowanie w układ ruchowy chloroplastów mikrotubul. Natomiast mechanizm odpowiedzi chloroplastów w wyższych roślinach lądowych wydaje się być jednolity. W roślinach tych nie wykazano dotychczas związku pomiędzy płynięciem cytoplazmy a indukowanymi światłem ruchami chloroplastów. Ruchy te indukowane są tylko światłem niebieskim i bliskim ultrafioletem za pośrednictwem fototropin. Podczas gdy udział cytoszkieletu aktynowego w mechanizmie ruchowym nie budzi najmniejszych wątpliwości, dyskusyjny pozostaje sposób zaangażowania aktyny. Zgodnie z jedną hipotezą siła motoryczna jest wytwarzana przez miozyny związane z powierzchnią chloroplastów, które oddziałują z filamentami aktynowymi. Pod tym względem mechanizm ruchów chloroplastów nie odbiegałby od mechanizmów ruchu innych organelli. Według odmiennego modelu elementami wyłącznie odpowiedzialnymi za przemieszczenia chloroplastów są specyficzne, krótkie filamenty aktynowe występujące jedynie na powierzchnie chloroplastów.

Morphogenesis of plants *in vitro* under stress conditions

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Abstract

The plants ability to regenerate a new organism from fragments of stem, leaf, flower, tissue, and even single somatic cell under *in vitro* culture conditions has been used in both research and commercial applications. Many factors influence the plants *in vitro* culture growth and its final effect. They include the genetical, biochemical and physiological properties of explants, the plant growth regulator sources (mainly auxins and cytokinins and also gibberellins, abscisic acid or ethylene), mineral nutrient compositions, and physical culture environment. To improve the efficiency of regeneration, apart from plant growth regulators, many treatments have been applied. One of them is incubation of cultures for a certain time in the conditions of physical (low and high temperature, desiccation) and chemical (pH, salinity, deficit of selected nutrients) stress. These types of stress have been found to have a positive effect on regeneration in some crop plants, like flax and oat. Stress conditions caused enhanced production of reactive oxygen species (ROS), whose destructive effect is connected with damage of plant cell membranes, organelles, also nucleic acids and proteins. It is followed by destruction of some organs or a whole plant, inhibition of growth, which can finally lead to the plant death. On the other hand, ROS as signalling molecules may be involved in signal transduction pathways that change gene expression, protein synthesis, and some metabolic processes. Several described modifications or factors may improve the efficiency of organogenesis in plant *in vitro* culture.

Key Words: abiotic stress, antioxidants, *in vitro* culture, reactive oxygen species

1. Introduction

Cells of higher plants often retain their usual potentialities when cultivated *in vitro*. The totipotency of these cells is demonstrated by the ability of *in vitro* cultured plant organs (e.g. fragments of stem, leaf, flower) tissues, cells and protoplasts to develop meristematic centers of cells that can develop into roots, shoots, flowers or whole plants. Currently, the tissue culture is used in both research and commercial applications. Tissue culture not only provides a method of mass propagation, but it also permits the production of disease-free plants, mutants, and secondary plant products. A new and important area of their use is the genetic engineering of plants. Thus, various culture treatments can be applied to optimize the morphogenic processes in plant *in vitro*. Some stress conditions leading to overproduction of reactive oxygen species (ROS) have been reported to be beneficial for embryogenesis and plant regeneration *in vitro* (Haq et al. 2009). On the other hand, ROS can also be toxic to plant cells. Thus, the main purpose of this paper is to review the possible role of stress conditions, and especially ROS, in the regulation of some morphogenic processes in plants *in vitro*, focusing attention on the dual function of these compounds.

2. Fundamental aspects of *in vitro* morphogenesis

Morphogenesis is the sum of processes that give form to an organism, it includes organization of cells into tissues, tissues into organs, and organs into the entire organism. In plants, morphogenesis is a continual process. Two centers of division, the root and shoot meristems, are established in the embryo, and the whole plant is formed from these meristematic centers (Mohnen et al. 1990). Useful model systems for studying morphological, biochemical and molecular processes connected with early stages of plants development are tissue cultures *in vitro*. Such cultures allow: 1) regulation of aquatic and trophic conditions of vegetation, 2) elimination of the metabolism of microorganisms, 3) research into the physiological processes in the different phases of vegetation, 4) comparison of the process at the level of cells, tissues, and a whole plant, 5) production of genetically the same plant material, 6) limitation of the impact of aging process (Mitrović et al. 2012).

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions in a nutrient culture medium of known composition.

The two primary morphogenic pathways leading to the whole plant regeneration involve either somatic embryogenesis, or shoot organogenesis followed by root organogenesis (Fig. 1). Both developmental pathways can occur either directly without the callus intermediate stage, termed adventitious; or indirectly following the unorganized callus stage, termed *de novo* (Philips 2004). Regeneration by both, organogenic and somatic embryogenic pathways is possible only for a few plant species. Many plant species can regenerate by one or the other of these pathways (Philips 2004). Another pathway through which whole plants are regenerated is the method in which explants that include a meristem are grown in appropriate media supplemented with plant growth regulators to induce proliferation of multiple shoots, followed by rooting of the excised shoots to regenerate whole plants (Fig. 1).

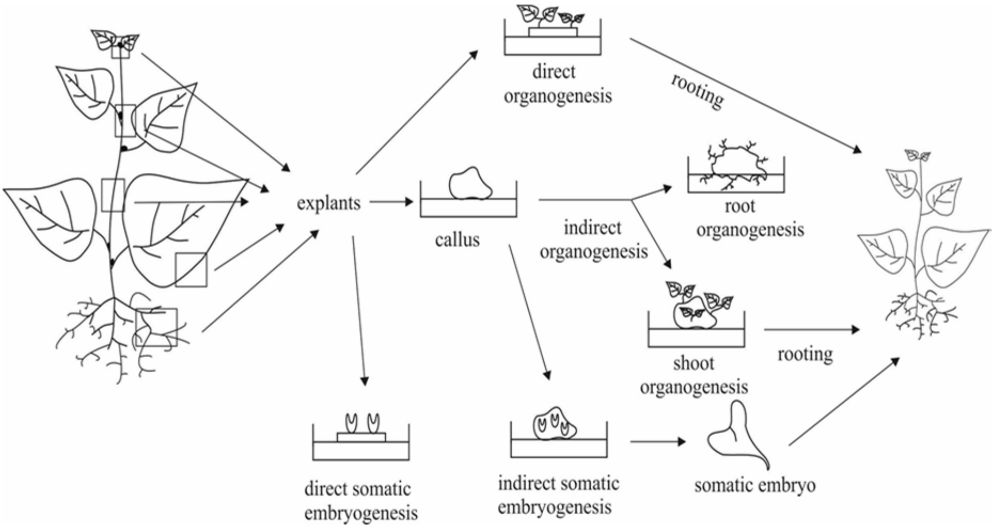

Figure 1. Routes of plant regeneration in tissue cultures *in vitro* from different explants. *Adapted from Malepszy (2009), with modifications*

Rycina 1. Drogi regeneracji roślin w kulturach tkankowych *in vitro* z różnych eksplantatów. *Zmodyfikowano wg Malepszy (2009)*

Many culture factors have been applied to induce and optimize organogenesis. Typical treatments include introduction of plant growth regulator sources and variation in their concentrations, choice of explants, nutrient medium composition (especially inorganic and organic nitrogen sources, carbohydrate sources and variation in their concentrations) culture environment (e.g. humidity, temperature, quality of illumination, gas environment) and osmotic potential (Philips 2004).

Organogenesis *in vitro* depends not only on the application of exogenous phytohormones, in particular auxin and cytokinin, but also on the ability of the tissue to respond to changes in concentration of the phytohormones during culture growth (Sugiyama 1999). Other plant hormones: gibberellins, ethylene or abscisic acid are rather rarely used in plant *in vitro* culture. Plant hormones are the critical media components determining the developmental pathway of plant cells. Hormone balance is apparently more important than the absolute concentration of any individual hormone. Usually a high cytokinin to auxin ratio (or high cytokinin with no auxin) is required to induce shoot organogenesis. Root initiation typically requires a moderate to high auxin signal. A high auxin signal (e.g. 2,4-dichlorophenoxyacetic acid) is also important to induce somatic embryogenesis (Philips 2004). Generally, organogenesis *in vitro* is composed of three distinct phases of different dependence on exogenous phytohormones: 1) cells are dedifferentiated to acquire organogenic competence; 2) dedifferentiated cells are determined for specific organ formation in response to exogenous phytohormones; 3) organs morphogenesis proceeds independently of exogenous phytohormones (Christianson, Warnick 1985).

Cultures are generally initiated from sterile pieces of a whole plant. These pieces are termed explants, and may consist of pieces of organs, such as leaves or roots (Fig.1), or may be specific cell types, such as pollen or endosperm. Many features of the explant are known to affect the efficiency of culture initiation. Generally, the younger, more rapidly growing tissue (or a tissue at an early stage of development) is more effective (Christianson, Warnick 1985).

The introduction and proliferation of plants (or their parts) *in vitro* may alter the oxidative metabolism and predispose tissues to the damaging effects of reactive oxygen species (ROS). In parallel, oxidative processes and ROS may positively affect the morphogenic responses of cells grown *in vitro* (Haq et. al 2009). Selected stress conditions that generate ROS have been applied to find methods for improvement of plant regeneration *in vitro*.

3. ROS: nature, origin and scavenging

The level of ROS in plants under normal growth conditions is low. Environmental stresses such as drought, salinity, chilling, metal toxicity, and UV-B radiation as well as pathogen attack lead to enhanced generation of ROS in plants as a result of disruption in cellular homeostasis. ROS are group of free radicals, reactive molecules, and ions derived from O_2 (Kreslavski et al. 2012).

The most common ROS include: singlet oxygen ($^1O_2$), superoxide radical ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$). Both $^{\bullet}O_2^-$ and H_2O_2 are only moderately reactive. H_2O_2, the most long-living ROS, is uncharged and a rather stable molecule (half-life of 1 ms). The most active oxygen species is a short-living (the lifetime is only 10^{-9}s) extremely reactive $^{\bullet}OH$. Therefore, ROS include the radical derivatives of oxygen ($^{\bullet}O_2^-$, $^{\bullet}OH$, but also the peroxyl, alkoxyl or hydroperoxyl radicals), which are called free radicals, i.e. molecule species containing one or more unpaired electrons, but they also include non-radical derivatives of oxygen such as H_2O_2, ozone and singlet oxygen (Sharma et al. 2012).

Plants have a complex antioxidative defence system comprising nonenzymatic and enzymatic components to scavenge ROS, which are found in different organelles such as chloroplasts, mitochondria and peroxisomes. The important low-molecular antioxidants (nonenzymatic) that reduce the level of ROS include: tocopherols, carotenoids, numerous phenolic compounds, such as flavonoids and anthocyanins, and also proline and glycine betaine. They interact with numerous cellular component, but osmolytes can function also as stabilizers of protein structures (Sharma et al. 2012). The enzymatic components of antioxidative defence system includes enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), enzymes of ascorbate-glutathione (AsA-GSH) cycle: ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) (Batková et al. 2008).

4. Abiotic stress conditions, ROS and morphogenesis *in vitro*

Unfavourable environmental factors shift the balance between oxidants and antioxidants toward oxidants, which stimulates development of intracellular oxidative stress (Konieczny et al. 2008). The enhanced production of ROS during environmental stress causes peroxidation of lipids, oxidation of protein, damage to nucleic acids, enzyme inhibition, activation of programmed cell death (PCD) pathway and can lead to the death of cells (Miller 2002). Oxidative stress also affects the tissues cultured *in vitro*. Visible symptoms of the stress include a reduction of water content, culture growth and regeneration ability, culture ageing and finally cell necrosis (Benson 2000; Abbasi 2011). On the other hand, several stress conditions increase the plant regeneration ability (Puijalon et al. 2008). Thus, to improve some organogenic processes in plants *in vitro* the effect of exposition of tissue cultures to action of short-term stress conditions was tested. For example, water stress has been reported to stimulate regeneration of rice (Jain et al. 1996) and wheat (Khanna, Daggard 2001) cultures. Heat-, salt- and mineral stress (mineral nutrients deprivation), positively affect the shoot-buds induction on hypocotyl of flax (*Linum usitatissimum*) seedlings *in vitro* (Mundhara, Rashid 2001). Some other data indicate that the induction of meristems in the epidermis of the hypocotyl of flax plantlets was positively influenced by calcium (Ca^{2+}) deprivation and some physical stimuli, like drought and wind (Verdus et al. 1997). The results of our study indicate that chemical desiccation of hypocotyl-derived explants of flax may improve the efficiency of rhizogenesis (Fig. 2). However, the rate of shoot organogenesis, was similar in control and stressed cultures (Fig. 3) (Adamczuk et al., unpublished data).

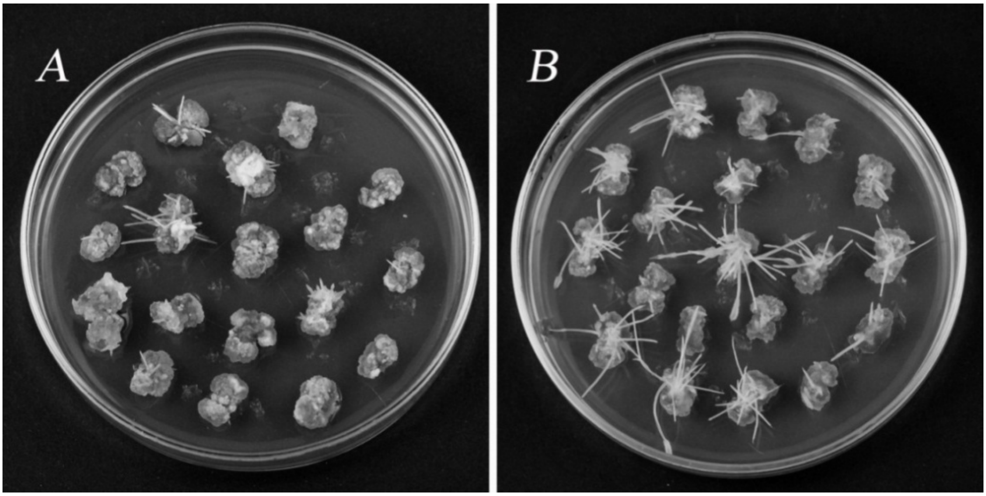

Figure 2. Roots regeneration in 21-day old cultures of flax (*Linum usitatissimum* L.) on Murashige and Skoog (MS) medium supplemented with α-naphtaleneacetic acid (NAA, 1 mg l^{-1}) and benzyladenine (BA, 0.05 mg l^{-1}) under control (A) and osmotic stress conditions (B) (Adamczuk et al., unpublished data)

Rycina 2. Regeneracja korzeni w kulturach *in vitro* lnu (*Linum usitatissimum* L.) po 21 dniach wzrostu na pożywce Murashige and Skoog (MS) wzbogaconej o kwas naftylo-1-octowy (NAA, 1 mg l^{-1}) i benzyloadeninę (BA, 0.05 mg l^{-1}) w warunkach kontrolnych (A) oraz w warunkach stresu osmotycznego (B) (Adamczuk i in., dane nieopublikowane)

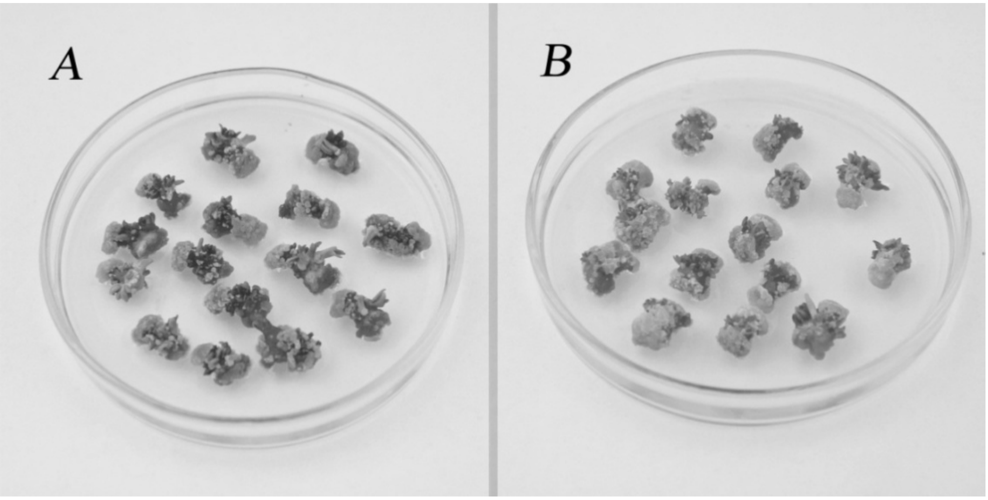

Figure 3. Shoots regeneration in 21-day old cultures of flax (*Linum usitatissimum* L.) on Murashige and Skoog (MS) medium supplemented with benzyladenine (BA, 1 mg l^{-1}) and 2,4-dichlorophenoxyacetic acid (2,4-D, 0.05 mg l^{-1}) under control (A) and osmotic stress conditions (B) (Adamczuk et al., unpublished data)

Rycina 3. Regeneracja pędów w kulturach *in vitro* lnu (*Linum usitatissimum* L.) po 21 dniach wzrostu na pożywce Murashige and Skoog (MS) wzbogaconej o benzyloadeninę (BA, 1 mg l^{-1}) i kwas 2,4-dichlorofenoksyoctowy (2,4-D, 0.05 mg l^{-1}) w warunkach kontrolnych (A) oraz w warunkach stresu osmotycznego (B) (Adamczuk i in., dane nieopublikowane)

Positive effect of stress action on plant morphogenesis is also connected with ROS, especially with H_2O_2 (Kreslavski et al. 2012). H_2O_2 has been known not only as stress signal molecule, but also a signal in plant growth and development (Ślesak et al. 2007).

H_2O_2 has been involved in developmental processes during plant morphogenesis *in vitro* in several species (Papadakis et al. 2001; Tian et al. 2003; Mitrović et al. 2012). The results obtained by exogenous application of H_2O_2 indicate that this compound at a low concentration might be involved in regulation of shoot organogenesis in gladiolus (*Gladiolus hybridus* Hort.) (Gupta, Datta 2003). H_2O_2 is also correlated with the morphogenetic process in strawberry callus, and may actually serve as a messenger in the process of bud primodium formation (Tian et al. 2003). A high concentration of H_2O_2 in *Mesembryanthemum crystallinum* L. was found in callus, which showed high regeneration potential (Libik et al. 2005). A dual role of H_2O_2 has been shown in plant protoplast division and regeneration. Thus, in grapevine, H_2O_2 reduces regeneration potential, but contrastingly it is required for protoplast division (de Marco, Roubelakis-Angelakis 1996; Papadakis et al. 2001). Elevated level of H_2O_2 detected in flax *in vitro* cultures subjected to osmotic stress

(Fig. 4B) leads to improved regeneration of roots (82% – control, 97% – stress). On the other hand, the efficiency of shoot organogenesis is the same (around 100%; Adamczuk et al., unpublished data), although stressed cultures showed higher H_2O_2 content than control (Fig. 4A).

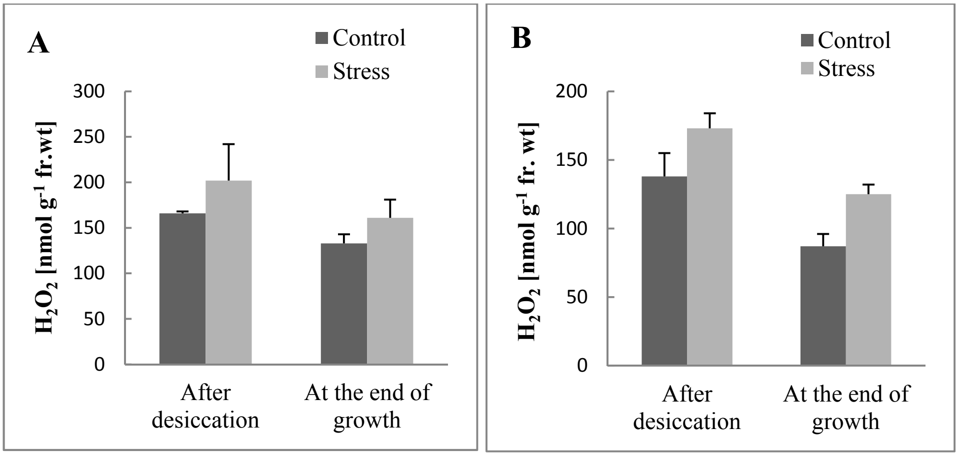

Figure 4. The concentration of H_2O_2 in cultures of flax *Linum usitatissimum* L. during regeneration of shoots (A) and roots (B) on Murashige and Skoog (MS) medium supplemented with: (A) benzyladenine (BA, 1 mg l^{-1}) and 2,4-dichlorophenoxyacetic acid (2,4-D, 0.05 mg l^{-1}); and (B) α-naphtaleneacetic acid (NAA, 1 mg l^{-1}) and BA (0.05 mg l^{-1}). Stress – osmotic stress (Adamczuk et al., unpublished data)

Rycina 4. Stężenie H_2O_2 w kulturach pędowych (A) i korzeniowych (B) lnu (*Linum usitatissimum* L.) rosnących na pożywce Murashige and Skoog (MS) z dodatkiem: (A) benzyloadenina (BA, 1 mg l^{-1}) i kwas 2,4-dichlorofenoksyoctowy (2,4-D, 0.05 mg l^{-1}); (B) – kwas naftylo-1-octowy (NAA, 1 mg l^{-1}) i BA (0.05 mg l^{-1}). Stres – stres osmotyczny (Adamczuk i in., dane nieopublikowane)

Nitric oxide (NO) has also emerged as an important signalling molecule that participates in many plant physiological and developmental processes and in plant responses to stress stimuli (Kopyra, Gwóźdź 2004). The role of NO during some morphogenic processes *in vitro* in several plant species has also been reported (Petřivalský et al. 2012). Supplementation of NO donor – sodium nitroprusside (SNP) alleviates browning of tuber explants by reducing H_2O_2 accumulation and promotes callus induction frequency and shoots differentiation of *Dioscorea opposita* Thunb. (Xu et al. 2009). Scavenging of ROS and RNS (Reactive Nitrogen Species) induced the formation of cucumber microcalli (*Cucumis sativus* cv. Marketer), thus suggesting a differential role of NO in the maintenance of cell viability and in the control of cell division. The crucial role of controlled ROS and RNS production in both protoplasts regeneration and cellular growth and differentiation was shown during regeneration of cucumber (*Cucumis sativus*) cells from isolated protoplast (Petřivalský et al. 2012). NO donors (SNP, 2µM S-nitroso-N-acetylpenicillamine (SNAP), 2µM 3-morpholinsydnonimine (SIN–1)) significantly promoted shoot differentiation from the hypocotyl explants of flax. SNP also augmented the rhizogenic response of the microshoots in terms of percentage of responding explants, number of roots per responding explant and average root length (Kalra, Babbar 2010). Experiments on *Cimbidium* suggest that the process of dedifferentiation and redifferentiation leading to rhizome formation under the condition of Mg^{2+} deficiency is NO mediated (Guha, Rao 2012).

Some data indicate that the antioxidative enzymatic system is involved in the regulation of plant morphogenesis (Chen, Ziv 2001). Several examples described below indicate that the activity of different antioxidant enzymes may be changed during different stages of regeneration. During *in vitro* morphogenesis of gladiolus the somatic embryogenesis is increased when the activity of SOD is also increased, but CAT and peroxidase (POX) activities are decreased. In contrast, increase in CAT and POX activity and concomitant decrease in SOD activity have been noted during shoot organogenesis (Gupta, Datta 2003). These authors concluded, that somatic embryogenesis prefer more stressful environment than shoot organogenesis (Gupta, Datta 2003). High SOD and no CAT and POX activity in primary explants, and a decrease in SOD and an increase in CAT and POX activity during *Tacitus bellus* L. shoot organogenesis, suggest higher H_2O_2 content in intact plant than in the tissue subjected to organogenesis (Mitrović et al. 2012). Changes in H_2O_2 – producing (SOD) and consuming enzymes (CAT and POD) suggest the importance of maintenance of a low H_2O_2 content during *Tacitus bellus* shoot organogenesis (Mitrović et al. 2012). During *in vitro* regeneration of sunflower (*Helianthus annuus* L.) the differences in endogenous H_2O_2 level, and in some antioxidant enzymes activities (SOD, CAT, POX) between the explants showing embryogenic and organogenic proliferation, have been observed (Konieczny et al. 2008).

It is worth noting, that plant tissue cultures grown under apparent optimal conditions may be exposed to oxidative stress and ROS action, which may positively affect morphogenesis. Explant preparation involves wounding of the tissues which is known to cause oxidative stress. Elicitors of oxidative stress include also hypochlorite and mercuric salts, used to sterilize the surface of primary explants (Cassels, Curry 2001). Oxidative processes and lipid peroxidation take place also at the early stages of cells dedifferentiation, as it has been detected in *Vitis vinifera* L. *in vitro* (Benson, Roubelakis-Angelakis 1992). Indirect evidence indicates that ($^{\bullet}OH$) and ($^{\bullet}O_2^-$), both highly toxic oxygen species, are produced by dedifferentiated plant cultures (Benson, Withers 1987). As follows from the above examples, the oxidative stress caused by overproduction of ROS may positively affect the morphogenic response of plant tissue *in vitro*. Interesting results have been obtained during regeneration process of wheat, which suggests that callus regeneration is associated with ROS production induced by plant hormones added to culture medium (Szechyńska-Hebda et al. 2007). These authors concluded that ROS being under control of antioxidant enzymes can mediate signalling pathways between exogenously applied hormones and the induction of a direction of morphogenesis (Szechyńska-Hebda et al. 2007).

5. Signaling role of ROS in plant morphogenesis *in vitro*

It is now obvious that H_2O_2 and some other ROS, especially ($^{\bullet}O_2^-$), could function in the cell as "double agents". They directly initiate strong oxidative stress that can lead to injuries and death of the organisms or they function as signalling molecules including some molecular, biochemical, and physiological responses, which help to develop the adaptive mechanisms and improve the organism tolerance (Kreslavski et al. 2012). The questions arise: i) if these adaptive mechanisms may also lead to enhanced regeneration efficiency in tissue cultures *in vitro*; ii) if some elements of signal transduction initiated by H_2O_2 involved in signalling cascade can result in regeneration improvement.

ROS/H_2O_2 are the second messengers in several plant hormone responses, including stomatal closure, root gravitropism, seed germination, lignin biosynthesis, programmed cell death, hypersensitive responses, and acquisition of tolerance to both biotic and abiotic stresses (Sharma et al. 2012). Lately, some information on the components of cellular signalling pathways leading to stress tolerance acquisition has been provided (Kreslavski et al. 2012; Ślesak et al. 2007; Sharma et al. 2012). In brief, plants can sense, transduce and translate ROS signal into appropriate cellular responses *via* some redox-sensitive proteins, calcium mobilization, G-protein, protein phosphorylation, and adequate gene expression (Batková et al. 2008). ROS can also modulate the activities of many components in signalling, such as protein phosphatases, protein kinases and transcription factors (Xiong et al. 2002).

The perception and transduction of ROS signals and molecular mechanisms by which ROS affect some morphogenic processes in plant tissue cultures *in vitro* are generally not well known (Abbasi et al. 2011; Verdus et al. 1997). On the basis of indirect data, Verdus et al. (1997) have suggested that the positive effect of mechanical stimuli on some organogenic processes in flax tissues may be connected with fast activation of mitogen-activated protein kinase (MAPK), a component of the universal kinase cascade, and calcium-dependent protein kinase (CDPK). Obert et al. (2005) have reported that cell differentiation and development is regulated by differential expression of genes, therefore the metabolism of ROS may play a decisive role in cell differentiation and development. Physiological experiments and phenotypic examination of organogenesis-defective mutants allowed concluding that the dedifferentiation processes may be associated with active expression of a different subset of cell cycle genes, whereas in root and shoot organogenesis, the genes of auxin and cytokinin signal transduction may be involved (Sugiyama 1999). The expression of some of them could be under control of ROS.

Some experimental data, described in the previous chapter indicate that the antioxidant enzymatic system is involved in the regulation of plant morphogenesis. It remains an open question whether activation of genes encoding antioxidant proteins depends on ROS generated under stress conditions in tissue culture *in vitro*. If yes, it may lead to high efficiency organogenesis. It is worth noting that plant treatment with H_2O_2 induces the expression of genes related to plant protective responses to stress: ascorbate peroxidase, glutathione reductase, catalase, MAPK, and phosphatase (Kreslavski et al. 2012). It has been suggested that exogenous H_2O_2 content may mimic the signalling induced by endogenously produced H_2O_2 (Forman 2007), thus we can expect that future experiments at the molecular level may give new significant data, concerning the mechanisms of ROS action, especially during some morphogenic processes *in vitro*. Understanding of these mechanisms may be useful for development of methods enhancing the regeneration ability of some plant species of considerable economic importance.

Hypothetical routes of ROS action (signalling and toxic), which may be involved in the regulation of morphogenesis, also in tissues *in vitro* culture, are proposed in figure 5.

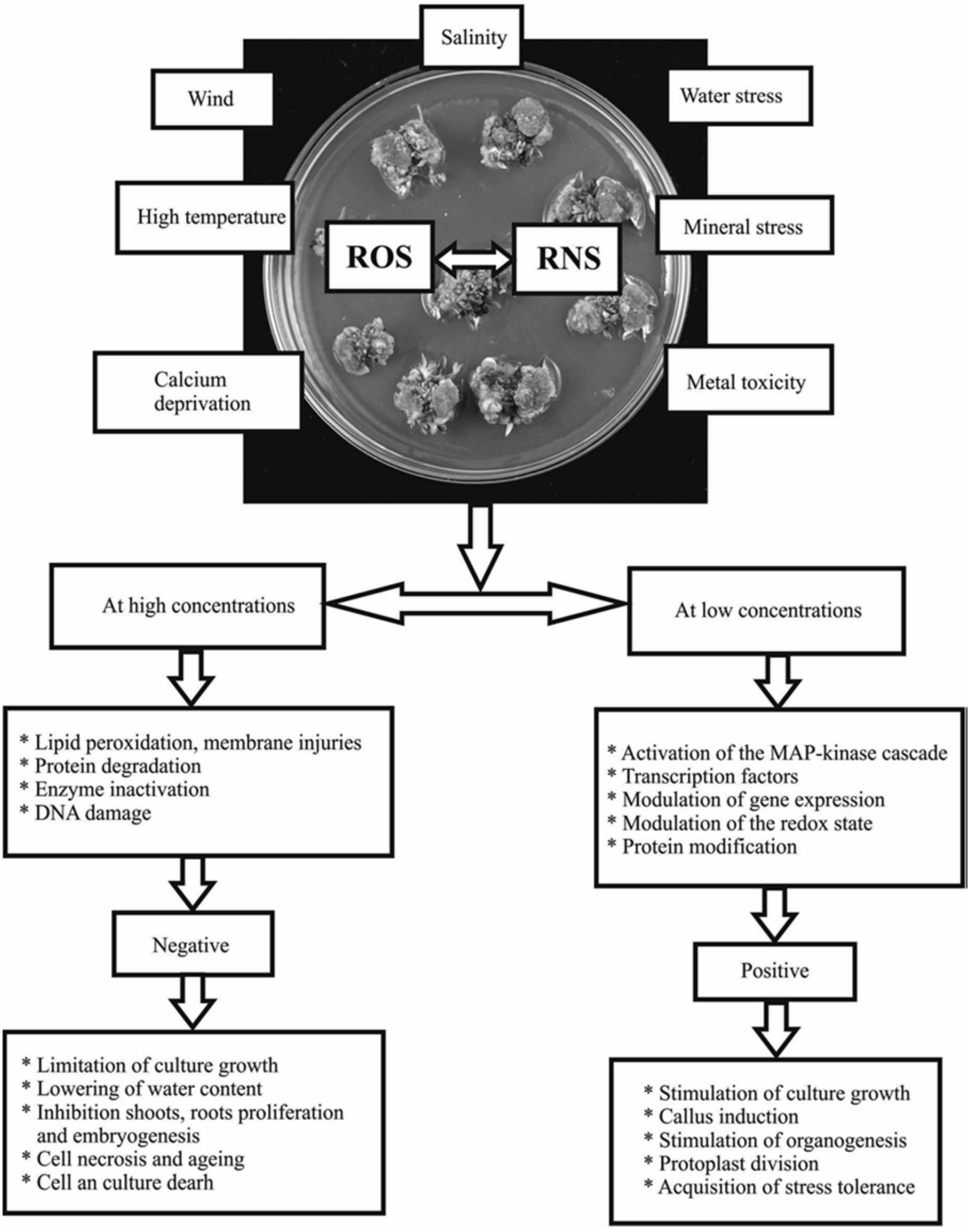

Figure 5. Dual action of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the regulation of plant morphogenesis *in vitro. Adapted from Sharma et al. (2012), with modifications*

Rycina 5. Podwójna rola reaktywnych form tlenu (ROS) oraz reaktywnych form azotu (RNS) w regulacji morfogenezy roślin w kulturach *in vitro. Zmodyfikowano wg Sharma i in. (2012)*

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Morfogeneza roślin w kulturach *in vitro* w warunkach stresowych

Streszczenie

Zdolność roślin do regeneracji, czyli odtwarzania całej rośliny z fragmentu pędu, liścia, kwiatu, a nawet z jednej komórki somatycznej wykorzystano w praktyce do wegetatywnego rozmnażania roślin za pośrednictwem izolowanych protoplastów, komórek, tkanek i organów hodowanych in vitro, jak i w szeroko stosowanych metodach tradycyjnych. Prowadzenie kultury in vitro i końcowy jej efekt zależy od właściwości biochemiczno-fizjologicznych i genetycznych użytego materiału roślinnego, właściwości fizycznych podłoża oraz od substancji odżywczych i regulatorów wzrostu w nim zawartych, głównie auksyn i cytokinin, ale także giberelin, kwasu abscysynowego i etylenu. Oprócz roślinnych regulatorów wzrostu, w kulturach in vitro stosuje się szereg zabiegów mających usprawnić procesy regeneracji roślin. Jednym z nich jest inkubacja kultur przez odpowiednio dobrany czas w warunkach stresu fizycznego (niska i wysoka temperatura, desykacja) i chemicznego (pH, zasolenie, deficyt wybranych składników pożywki). Pozytywne efekty wybranych stresów na organogenezę stwierdzono m.in. u kilku gatunków roślin użytkowych, takich jak len, czy owies. Pod wpływem czynników stresowych dochodzi w komórkach roślinnych do wzmożonego wytwarzania reaktywnych form tlenu (RFT), których destrukcyjne działanie polega na uszkodzeniach błon komórkowych i innych organelli, jak też organicznych składników komórki, takich jak kwasy nukleinowe czy białka. Zewnętrznym przejawem tych zmian są uszkodzenia poszczególnych organów i całych roślin, ograniczenie wzrostu a nawet ich śmierci. Z drugiej strony RFT, jako cząstki sygnalne mogą uczestniczyć w przekazywaniu sygnałów, co prowadzi do zmian ekspresji wielu genów, biosyntezy białek czy przebiegu ważnych procesów metabolicznych, prowadzących m.in. do efektywniejszej organogenezy.

The role of antioxidants in plant response to oxidative stress

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Abstract

Plants can resort to a complex antioxidant defense machinery to protect themselves against oxidative stress damages. They are endowed with very efficient enzymatic antioxidant defense systems based on the use of superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione peroxidase, phospholipid hydroperoxide glutathione peroxidase) and non-enzymatic (ascorbic acid, glutathione, phenolic compounds, flavonoids, proline, carotenoids, tocopherols. This antioxidant machinery works in concert to control the cascades of uncontrolled oxidation and protects plant cells from oxidative damage by scavenging reactive oxygen species. In this chapter, the role of antioxidants in plant response to oxidative stress induced by abiotic factors is discussed.

Key words: abiotic stress, non-enzymatic and enzymatic antioxidants, reactive oxygen species

1. Introduction

Stress is defined as any environmental variable, which can induce a potentially injurious strain in plants. A number of abiotic stresses, such as extreme temperatures, high light intensity, osmotic stresses, salinity, drought, flooding, heavy metals, herbicides and toxins lead to overproduction of reactive oxygen species (ROS) causing extensive cellular damage and inhibition of photosynthesis (Foyer, Shigeoka 2011). The most common ROS are hydrogen peroxide (H_2O_2), superoxide ($^{\bullet}O_2^{-}$), hydroxyl radical ($^{\bullet}OH$) and singlet oxygen ($^1O_2$). ROS can inactivate enzymes and damage important cellular components. They are responsible for protein, lipid and nucleic acids modification and are assumed to play a major role in ageing and cell death (Gill, Tuteja 2010). To prevent or repair these damages, plant cells use a complex defense system, involving a number of antioxidant molecules that, in turn, induce changes in the biochemical plant machinery. Antioxidants are found in almost all cellular compartments, which points to the importance of ROS detoxification for cellular survival. Two main classes of plant defenses have been described and referred to as non-enzymatic and enzymatic antioxidant systems (Ahmad et al. 2010).

2. Non-enzymatic antioxidants

2.1. Ascorbate

Ascorbate (AA), commonly known as vitamin C, is one of the most studied and powerful antioxidant. It has been detected in the majority of plant cell types, organelles and in the apoplast. Under physiological conditions AA exists mostly in the reduced form in leaves and chloroplasts. Its intracellular concentration can build up to millimolar range, e.g. 20 mM in the cytosol and 20–300 mM in the chloroplast stroma (Pastori et al. 2003).

It is the best known water soluble molecule for detoxifying H_2O_2, especially as a substrate of ascorbate peroxidase (APX), an essential enzyme involved in ROS detoxification. AA can also react with $^1O_2$, $^{\bullet}O_2^{-}$, $^{\bullet}OH$, lipid hydroperoxidases and regenerate α-tocopherol from tocopheroxyl radicals, thus providing membrane protection. AA is co-factor of violaxanthin de-epoxidase, thus sustaining dissipation of excess excitation energy (Müller-Moulé et al. 2002). AA, while scavenging ROS (Huang et al. 2005), is oxidized to monodehydroascorbate (MDHA) and then to dehydroascorbate (DHA). DHA is very unstable and only ascorbate possesses antioxidant and free radical scavenger properties. DHA must be reduced back to AA; otherwise under physiological conditions it is lost within minutes (Chen, Gallie 2008). Oxidized AA can be recycled at the expense of glutathione or NADPH by the enzymes of the ascorbate-glutathione cycle which is often called the Halliwell-Asada cycle (Asada 1999).

2.2. Glutathione

Tripeptide (γ-Glu-Cys-Gly) glutathione (GSH) is one of the crucial metabolites in plants which is considered as most important intracellular defense against ROS induced oxidative damage. It occurs abundantly in the reduced form (GSH) in plant tissues and is localized in all cell compartments like cytosol, endoplasmic reticulum, vacuole, mitochondria, chloroplasts, peroxisomes as well as in apoplast and plays a central role in regulation of sulphate transport, signal transduction, conjugation of metabolites, detoxification of xenobiotics and the expression of stress-responsive genes. The reduced form of GSH is necessary to maintain the normal reduced state of cells so as to offset all the injurious effects of oxidative stress. It can potentially scavenge $^{\bullet}O_2^-$, $^{\bullet}OH$ and H_2O_2 (Rennenberg 1980; De Kok et al. 1986).

In addition GSH plays a key role in the antioxidative defense system by regenerating another water soluble antioxidant, AA, *via* the ascorbate-glutathione cycle (Asada 1999). In combination with its oxidized form (GSSG), GSH maintains redox equilibrium in the cellular compartments. It also plays an indirect role in protecting membranes by maintaining α-tocopherol and zeaxanthin in the reduced state (Gill, Tuteja 2010). It has been reported that when the intensity of stress increases, glutathione concentrations usually decline and redox state becomes more oxidized, leading to deterioration of the system (Piotrowska et al. 2010).

2.3. Proline

Proline (Pro) is an α-amino acid, one of the twenty DNA-encoded amino acids. It is unique among the 20 protein-forming amino acids because its amine nitrogen is bonded to not one but two alkyl groups, thus making it a secondary amine. The more common L form has *S* stereochemistry. In plants Pro is an antioxidant needed to mitigate the adverse effects of ROS. Pro can protect plants from UV light, salt, drought, heat and H_2O_2 stress and can prevent cell death. Enhanced synthesis of Pro under abiotic stress has been implicated as a mechanism to alleviate cytoplasmic acidosis and maintain $NADP^+$:NADPH at values compatible with metabolism. The important role in Pro synthesis plays the enhancement of pentose-phosphate pathway activity. This pathway is an important component of antioxidative defense mechanisms, which need NADPH to maintain GSH and AA in the reduced state (Hare et al. 1998).

2.4. Tocopherols

Tocopherols (vitamin E) are lipid soluble antioxidants found in all plant parts. Out of four isomers of tocopherols (α-, β-, γ-, δ-) found in plants, α-tocopherol has the highest antioxidative activity due to the presence of three methyl groups in its molecular structure. One molecule of α-tocopherol scavenges up to 120 $^{\bullet}O_2^-$ molecules by resonance energy transfer. Tocopherols prevent the chain propagation step in lipid autooxidation by reducing lipid peroxyl radicals to their corresponding hydroperoxides and this makes it an effective free radical trap (Havaux et al. 2005).

The α-tocopherol located in the membranes can link with polyunsaturated fatty acid (PUFA) to form complexes. When PUFA is oxidized by $^{\bullet}OH$ and superoxide into lipid peroxyl radical, α-tocopherol can convert the lipid peroxyl radical into lipid hydroperoxide and it is itself converted into tocopheroxyl radical. α-tocopherol can also protect hydrosulfide groups of proteins from oxidization and the protection can be achieved in two main ways. Directly, α-tocopherol can react with protein sulfur-radical and convert the radical back into hydrosulfide and it is itself converted to tocopheroxyl radical (Havaux et al. 2005; Maeda et al. 2006). Indirectly, it can enhance the biosynthesis and intracellular level of other antioxidants, such as GSH, which can reduce the sulfur-radical. All of the reactions end with generating tocopheroxyl radical, if the radical is not reduced, it will react further on to yield tocopherylquinone. However, plants have mechanisms to convert the tocopheroxyl radicals back in time with the help of Halliwell-Asada cycle (Asada 1999; Caretto et al. 2002).

2.5. Carotenoids

Carotenoids are lipid soluble antioxidants which play a multitude of functions in plant metabolism including enhancement of their abiotic stress tolerance. These pigments are most likely involved in the scavenging of two ROS, i.e. $^1O_2$ and peroxyl radicals. The efficacy of carotenoids for physical ROS quenching is related to the number of conjugated double bonds present in the molecule which determines their lowest triplet energy level (Ramel et al. 2012).

The interaction of carotenoids with $^1O_2$ depends largely on physical quenching which involves direct energy transfer between both molecules. The energy of $^1O_2$ is transferred to the carotenoid molecule to yield ground state oxygen and a triplet excited carotene. Instead of further chemical reactions, the carotenoid returns to ground state dissipating its energy by interaction with the surrounding solvent. In contrast to physical quenching, chemical reactions between the excited oxygen and carotenoids is of minor importance, contributing less than 0.05% to the total quenching rate. Since the carotenoids remain intact during physical quenching of $^1O_2$ or excited sensitizers, they can be reused several times in such quenching cycles. Among various carotenoids, xanthophylls (zeaxanthin, cryptoxanthin) as well as carotenes (α- and β-carotene) proved to be efficient quenchers of $^1O_2$ interacting with reaction rates that approach those of a diffusion control reaction (Gill, Tuteja 2010).

2.6. Flavonoids

These plant pigments occur widely in the plant kingdom, and are commonly found in leaves, floral parts and pollens. They show high antioxidant activity against a variety of oxidizable compounds (Amalesh et al. 2011). Many flavonoid biosynthetic genes are induced under stress conditions, such as wounding, drought, cold, metal toxicity, UV-B and nutrient deprivation (Keilig, Ludwig-Müller 2009).

Flavonoids are ideal scavengers of H_2O_2 due to alkyl peroxyl radicals and thus, in principle, they are effective inhibitors of lipid peroxidation. Several flavonoids have been shown to be potent inhibitors of lipoxygenase and prostaglandin synthetase, which convert polyunsaturated fatty acids to oxygen-containing derivatives. Flavonoids, with strong absorption in the 300–400 UV regions, also act as internal light filters protecting chloroplasts and other organelles from UV damage (Hernández et al. 2008).

2.7. Phenolic acids

Plant phenolics have often been referred to as secondary metabolites and many of these compounds play an essential role in the regulation of plant growth development, interaction with other organisms and antioxidant response. Chlorogenic acid has been found to be the most abundant phenolic acid in the plant extract and also the most active antioxidant inhibiting peroxide formation in a linoleic acid test system (Michalak 2006).

3. Enzymatic antioxidants

3.1. Superoxide dismutase (SOD)

Superoxide dismutase (EC 1.15.1.1) provides the first line of defense against the toxic effects of elevated levels of ROS in plants (Wang et al. 2009). The SODs remove $^{\bullet}O_2^-$ by catalyzing its dismutation, one molecule of $^{\bullet}O_2^-$ being reduced to H_2O_2 and another oxidized to O_2:

$$2^{\bullet}O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

The compartmentalization of different forms of SOD throughout the plant makes them counteract stress very effectively. For example, Fe SODs are most abundantly localized inside plant chloroplasts. Mn SODs are present in mitochondria and peroxisomes. Cu/Zn SODs are concentrated in the chloroplast, cytosol and in some cases the extracellular space (Miszalski et al. 1998).

SOD genes have been shown to be sensitive to environmental stresses, presumably as a consequence of increased ROS formation. This has been shown in an experiment with *Zea mays* where a 7-day flooding treatment resulted in a significant increase in lipid peroxidation, membrane permeability and the production of superoxide anionradical and hydrogen peroxide in the leaves (Hegedüs et al. 2001). Significant increase in SOD activity under salt stress has been observed in various higher plants, e.g. *Cicer arietinum* and *Lycopersicon esculentum* (Gill, Tuteja 2010).

Analysis of transgenic plants that overexpress these protective enzymes should provide interesting insights into their relative contributions to abiotic stress tolerance. Transgenic tobacco plants that overexpressed chloroplast-localized pea Cu/Zn SOD had greater resistance to photooxidative damage and to methyl viologen-mediated oxidative stress (Gupta et al. 1993). Bowler et al. (1992) have demonstrated increased resistance to methyl viologen in transgenic tobacco plants that overexpressed mitochondrial Mn SOD. Transgenic rice plants overexpressing SOD demonstrated enhanced drought tolerance. Overexpression of Mn SOD in transgenic *Arabidopsis thaliana* plants also showed increased salt tolerance (Wang et al. 2007).

3.2. Catalases (CAT)

Catalases (EC 1.11.1.6) were the first antioxidant enzymes to be discovered and characterized. CAT are highly expressed enzymes, particularly in certain plant cell types and are thus an integral part of the plant antioxidative system. CAT are present in the peroxisomes and mitochondria of nearly all aerobic cells (Mhamdi et al. 2010).

The typical CAT reaction is dismutation of two molecules of H_2O_2 to water and O_2. CAT has one of the highest turnover rates off all antioxidant enzymes: one molecule of CAT can convert 6 million molecules of H_2O_2 to H_2O and O_2 per minute (Yang et al. 2008). The variable response of CAT activity has been observed under abiotic stress. Its activity declined in *Glycine max*, *Phragmites australis*, *Capsicum annuum* and *Arabidopsis thaliana*, whereas its activity increased in *Oryza sativa*, *Brassica juncea*, *Triticum aestivum*, *Cicer arietinum* and *Vigna mungo* (Gill, Tuteja 2010) under heavy metal stress. However, the stimulation of CAT activity in response to heavy metal stress was noted in aquatic plant *Wolffia arrhiza* and green microalga *Chlorella vulgaris* (Bajguz 2010; Piotrowska et al. 2010). Hsu and Kao (2007) reported that pretreatment of rice seedlings with H_2O_2 under non-heat shock conditions resulted in an increase in CAT activity and protected rice seedlings from subsequent heavy metal stress. Increase in CAT activity in *Cicer arietinum* roots following salinity stress was noted by Kukreja et al. (2005). In another study, Sharma and Dubey (2005) reported a decrease in CAT activity in rice seedlings following drought stress. It has also been reported that high insolation increased the CAT activity in *Picea asperata* under drought stress (Yang et al. 2008).

3.3. Ascorbate peroxidases (APX)

Plants are rich in peroxidases, the enzymes that remove H_2O_2 by using it to oxidize a cosubstrate (De Gara 2004). Ascorbate peroxidases (EC 1.11.1.11) present in plant chloroplast and cytosol can remove H_2O_2 by using AA as a cosubstrate, oxidizing it to a (poorly reactive) ascorbyl free radical in the Halliwell-Asada cycle (Asada 1999). The reaction they catalyze is the transfer of electrons from AA to peroxide, producing dehydroascorbate and water as products:

$$\text{ascorbate} + H_2O_2 \rightarrow \text{dehydroascorbate} + 2H_2O$$

APX has higher affinity to H_2O_2 (µM range) than CAT and POD (mM range) and it may have a more crucial role in the management of ROS during stress (Shigeoka et al. 2002). Enhanced expression of APX in plants has been demonstrated during different stress conditions (Davletova et al. 2005). APX activity increased during exposure of plants to ozone, sulphur dioxide chilling and UV-B stress. Increased APX activity under Cd and Pb stress has been reported in *Wolffia arrhiza* and *Chlorella vulgaris* (Bajguz 2010; Piotrowska et al. 2010). Hsu and Kao (2007) reported that pretreatment of *Oryza sativa* seedlings with H_2O_2 under non-heat shock conditions resulted in an increase in APX activity and protected rice seedlings from subsequent Cd stress. Enhanced activity of APX was also found in salt stressed *Anabena doliolum* (Srivastava et al. 2005). Significant increase in APX activity was noted under water stress in three cultivars of *Proteus vulgaris* and *Proteus asperata* (Gill, Tuteja 2010). Sharma and Dubey (2005) have found that mild drought stressed plants had higher chloroplastic APX activity than control grown plants but the activity declined at a higher level of drought stress.

3.4. Glutathione reductase (GR)

Glutathione reductase (1.8.1.7) reduces glutathione disulfide (GSSG) in NADPH dependent reaction to the sulfhydryl form GSH, which is an important cellular antioxidant. It is localized predominantly in chloroplasts, but small amount of this enzyme has also been found in mitochondria and cytosol. Therefore this enzyme is important for maintenance of the GSH pool. The glutathione/GR system is involved in H_2O_2 metabolism by reducing dehydroascorbate generated following the (per)oxidation of AA in the Halliwell-Asada cycle (Asada 1999; Yannarelli et al. 2010).

GR activity has been found to be increased in the presence of Cd in *Cicer annuum*, *Arabidopsis thaliana*, *Vigna mungo*, *Triticum aestivum* and *Brassica juncea* (Gill, Tuteja 2010; Yannarelli et al. 2010). Kukreja et al. (2005) noted increased GR activity in *Cicer arietinum* roots in response to salt stress. Srivastava et al. (2005) reported a decline in GR activity in *Anabena doliolum* under Cu stress but its increase under salt stress. Sharma and Dubey (2005) noted a significant increase in GR activity in drought stressed *Oryza sativa* seedlings. Under high insolation drought increased the GR activity in *Proteus asperata* seedlings but no prominently drought-induced differences in GR activities were observed in low light seedlings (Gill, Tuteja 2010).

3.5. Monodehydroascorbate reductase (MDHAR)

In plants, monodehydroascorbate reductase (EC 1.6.5.4) is an enzymatic component of the Halliwell-Asada cycle that is one of the major antioxidant systems of plant cells developed for the protection against the damages produced by ROS (Asada 1999). The MDHAR activity has been described in several cell compartments, such as chloroplasts, cytosol, mitochondria, glyoxysomes, and leaf peroxisomes (Wang et al. 2009). This enzyme catalyzes the chemical reaction:

$$\text{NADH} + \text{H}^+ + 2\ \text{monodehydroascorbate} \rightarrow \text{NAD}^+ + 2\ \text{ascorbate}$$

Schutzendübel et al. (2001) have noted enhanced MDHAR activity in Cd-exposed *Pinus sylvestris* and declined MDHAR activity in Cd exposed poplar hybrids. Sharma and Dubey (2005) reported that the activities of enzymes involved in regeneration of AA, i.e. MDHAR, DHAR and GR were higher in drought stressed rice seedlings. It has also been reported that the increase in MDAR activity contributes towards chilling tolerance in tomato fruit (Stevens et al. 2008).

3.6. Dehydroascorbate reductase (DHAR)

Dehydroascorbate reductase (EC 1.8.5.1) is an enzyme that is critical for maintenance of an appropriate level of AA in plant cells. DHAR is responsible for regenerating AA from an oxidized state in the reaction:

$$2GSH + \text{dehydroascorbate} \rightarrow GSSG + \text{ascorbate}$$

This enzyme also regulates the cellular AA redox state, which in turn affects cell responsiveness and tolerance to environmental ROS (Chen, Gallie 2008). Thus, DHAR is a physiologically important reducing enzyme in Halliwell-Asada recycling reaction in higher plants. DHAR also plays important roles in plant adaptation to environmental stresses. Enhanced tolerance to ozone and drought stress was observed in transgenic tobacco overexpressing DHAR in the cytosol. Furthermore, transgenic seedlings showed enhanced tolerance to low temperature and high concentration of NaCl (Ushimaru et al. 2006).

3.7. Glutathione peroxidase (GPX)

Glutathione peroxidase (EC 1.11.1.9) is a general name of the enzyme family showing peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water (Gill, Tuteja 2010). An exemplary reaction catalysed by glutathione peroxidase is:

$$2\ GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

where GSH represents reduced monomeric glutathione, and GSSG represents glutathione disulfide. Glutathione reductase then reduces the oxidized glutathione to complete the cycle:

$$GSSG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$$

The major function of GPXs in plants appears to be the scavenging of phospholipid hydroperoxides and thereby the protection of cell membranes from peroxidative damage. GPXs are also involved in redox transduction under stressful conditions. Consistent with these two functions, the expression of many GPXs is enhanced in response to abiotic and biotic stresses, including salinity, heavy metal toxicity and infection with bacterial or viral pathogens (Miao et al. 2006).

3.8. Phospholipid hydroperoxide glutathione peroxidase (PHGPX)

Phospholipid hydroperoxide glutathione peroxidase (EC 1.11. 1.12), a member of the glutathione peroxidase (GPx) family, is a unique antioxidant enzyme that can directly reduced phospholipid hydroperoxides and complex hydroperoxy lipids which comprise the biomembrane lipid layers, in addition to H_2O_2 and other organic hydroperoxides which are substrates of the rest of the GPx family. Accordingly, PHGPx is considered crucial for protecting membranes from oxidative stress. In addition to using the glutathione system for its regeneration, PHGPX can also utilize a large variety of other reducing compounds, including cysteine (Cys) and Cys-containing proteins. In plants, the thioredoxin-regenerating system has been shown to be much more efficient than the glutathione system, and therefore the plant PHGPX is in fact a thioredoxin peroxidase as well (Chen et al. 2004).

4. Cooperation between different antioxidant systems

It is very important for plant survival under stress conditions that antioxidants can work in co-operation, thus providing better defense and regeneration of the active reduced forms. The most studied example of the antioxidant network is the ascorbate-glutathione (Halliwell-Asada) pathway in the chloroplasts, where it provides photoprotection by removing H_2O_2 (Fig. 1).

Generally speaking, superoxide dismutase (SOD) converts hydrogen superoxide into hydrogen peroxide. Hydrogen peroxide is converted into water by the Halliwell-Asada cycle. The first reaction is catalyzed by ascorbate peroxidase (APX) which converts hydrogen peroxide into water with the AA being oxidized into monodehydroascorbate (MDHA). Monodehydroascorbate reductase (MDHAR) reduces MDHA into AA with the help of NAD(P)H. Dehydroascorbate (DHA) is spontaneously produced from MDHA and can be reduced to AA by dehydroascorbate reductase (DHAR) with the help of glutathione (GSH) which becomes oxidized (GSSG). The cycle closes with glutathione reductase (GR) converting GSSG back into GSH with the reducing agent NAD(P)H (Asada 1999).

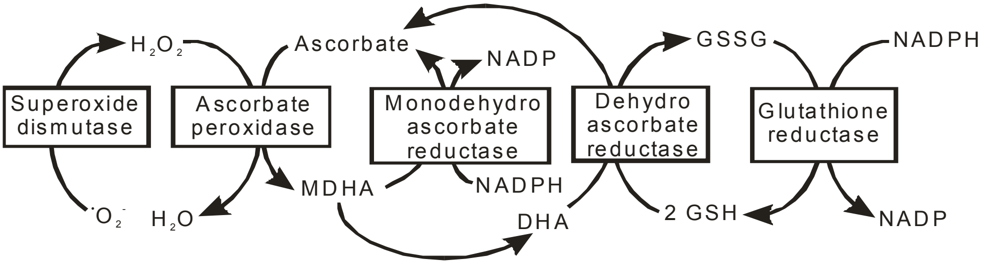

Figure 1. The Halliwell-Asada pathway (Asada 1999, modified)

Rycina 1. Szlak Halliwella-Asady (Asada 1999, zmienione)

AA works in co-operation not only with GSH, but also takes part in the regeneration of α-tocopherol, providing synergetic protection of the membranes. Tocopherol has been reported to be in direct interaction also with reduced GSH and reduced coenzyme Q. When they are present together in a membrane, they show a combined antioxidant activity which is markedly synergetic. Recently, redox coupling of plant phenolics with AA in the H_2O_2-peroxidase system has been shown. It takes place in the vacuole, where H_2O_2 diffuses and can be reduced by peroxidases using phenolics as primary electron donors. Both AA and the MDHA radical can reduce phenoxy radicals generated by this oxidation. If regeneration of AA is performed in the cytosol and AA is supplied back to the vacuole, a peroxidase/phenolics/ascorbate system could function in vacuoles and scavenge H_2O_2 (Gill, Tuneja 2010).

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Rola antyoksydantów w odpowiedzi roślin na stres oksydacyjny

Streszczenie

Stres oksydacyjny jest odpowiedzią roślin na różne czynniki stresowe takie, jak: deficyt wody, stres osmotyczny, stres solny, deficyt niektórych soli mineralnych (np. fosforu), zbyt niska lub zbyt wysoka temperatura, działanie patogenów, ucisk mechaniczny, zranienie, promieniowanie UV, nadmiar promieniowania fotosyntetycznie czynnego, duża dostępność tlenu po okresie niedotlenienia, działanie zanieczyszczeń atmosferycznych (SO_2, NO, NO_2, O_3), metali ciężkich czy herbicydów. Stres oksydacyjny występuje wówczas, gdy wzrasta w komórkach poziom reaktywnych form tlenu (ROS) takich, jak: wolnorodnikowy anion ponadtlenkowy, nadtlenek wodoru i rodnik hydroksylowy. Formy aktywnego tlenu powstają również w komórkach w warunkach normalnych jako uboczny produkt wielu reakcji oksydoredukcyjnych. Nadmiar ROS powoduje uszkodzenie wielu makrocząsteczek takich, jak: białka, tłuszcze oraz kwasy nukleinowe, powodując modyfikację struktury, utratę aktywności i właściwości biologicznych tych cząsteczek. Ponadto reakcje ROS biorą udział w przyspieszeniu procesu starzenia się. Rośliny przed uszkodzeniami spowodowanymi przez ROS są chronione przez antyoksydanty. W zależności od właściwości fizycznych komórek rozróżniamy antyoksydanty hydrofilowe, chroniące środowisko wodne komórek i antyoksydanty hydrofobowe, chroniące wnętrze błon komórkowych. Organizmy zawierają specjalne enzymy katalizujące rozkład anionorodnika ponadtlenkowego i nadtlenku wodoru takie, jak: dysmutaza ponadtlenkowa, katalaza, peroksydazy. Odrębną grupę stanowią antyoksydanty nieenzymatyczne, np. witaminy (askorbinian, tokoferole), glutation, barwniki roślinne (karotenoidy, antocyjaniny). Skutecznymi antyoksydantami są również flawonoidy, np. epikatechina, kwercetyna. Antyoksydanty są wspomagane w ochronie roślin przed stresem oksydacyjnym przez egzogenne stosowane hormony roślinne (auksyny, cytokininy i brassinosteroidy), które zwiększają ich poziom.

Mechanisms of heavy metals detoxification in plants

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Abstract

Heavy metal pollution is one of the most important environmental problems today. Most of the metals are easily absorbed by the plants and accumulate in different organs. Heavy metals hamper the growth of plants by disturbing many biochemical, physiological and metabolic processes. They trigger changes in the rate of transcript level of numerous genes coding for proteins to have protective changes against damage caused by stress. An important mechanism of heavy metal toxicity is their ability to bind strongly to oxygen, nitrogen and sulphur atoms. Plants have a range of potential mechanisms at the cellular level that might be involved in the detoxification and thus are responsible for tolerance to heavy metals stress. Once metal ions enter the cell, they are bound by chelators and chaperones. Chelators contribute to metal detoxification by buffering cytosolic metal concentrations; while chaperones specifically deliver metal ions to organelles and metal-requiring proteins. There are several known metal-chelators in plants. These include phytochelatins, metallothioneins, organic acids, and amino acids. Among heavy metal-binding ligands in plant cells, phytochelatins and metallothioneins are the best characterized. Heat shock proteins have been also found in plants treated with heavy metals. However, the specific functions or structures of heat shock proteins remain unidentified.

Key words: detoxification, heat shock proteins, heavy metals, metallothioneins, phytochelatins

1. Introduction

Continuously increasing environmental contamination by chemical compounds is one of the most important and unsolved problems. Nevertheless, members of the plant kingdom (microorganisms, lower and higher plants) can assimilate environmental contaminants, and be successfully directed to remove toxic compounds from the environment, providing long-term protection against their environmental dispersal in ever increasing doses. Plants respond to different factors in the environment, including heavy metals, wounding, drought, high salt concentration and changes in temperature and light, pathogen and pest attacks. Stress leads to a morphological, physiological, biochemical and molecular changes. As a consequence, these diverse environmental stresses often activate similar cell signalling pathways and cellular responses, such as the production of stress proteins, up-regulation of antioxidants and accumulation of compatible solutes. Biochemical adaptation in plants involves various changes in the biochemistry of the cell. These changes include development of new metabolic pathways, accumulation of low molecular weight metabolites, synthesis of special proteins, detoxification mechanisms and changes in phytohormone levels (Khan et al. 2000; Prasad 2004; Sharma and Dietz 2009).

In the remainder of this chapter, biochemical mechanisms of heavy metals detoxification in plants will be discussed in more detail.

2. Toxicity of heavy metals

Heavy metal pollution is one of the most important environmental problems today. Wastes containing different heavy metals originate mostly from: mining, metalliferous mining and smelting, surface finishing industry, energy and fuel production, fertilisers and pesticides production and use, metallurgy, electroplating, electrolysis, electroosmosis, leatherworking, photography, electric appliance manufacturing, metal surface treating, aerospace and atomic energy installation, etc. The most common heavy metals that cause toxicity in plants and animals are arsenic, lead, mercury, cadmium, nickel, iron and aluminium. Most of the metals are easily absorbed by plants and bioaccumulate in different organs. Visible symptoms of plant exposure to heavy metals are preceded by changes induced at the structural and ultrastructural levels. These changes at the cell, tissue and organ level in turn are either a result of direct interaction of the toxic metals with structural components at these sites or direct consequence of changes in signal transduction and/or metabolism (Clemens 2006). The non specific symptoms of heavy metal toxicity in plants are rapid inhibition of root growth, stunted growth of the plant and chlorosis. Heavy metal phytotoxicity leads to inhibition of enzyme activities, disturbed mineral nutrition, water imbalance, changes in hormonal status and alternation in membrane permeability. These disorders upset normal physiological activities of the plant. At high concentrations heavy metals may eventually induce cell death (Sanità di Toppi, Gabbrielli 1999; Sharma, Dubey 2005).

3. Mechanisms of heavy metals detoxification

Plants have a range of potential mechanisms at the cellular level that might be involved in the detoxification and thus improve tolerance to heavy metal stress. Tolerance to heavy metals in plants may be defined as the ability to survive in a soil that is toxic to other plants, and is manifested by an interaction between a genotype and its environment (Macnair et al. 2000), although the term is frequently used in literature to include changes that may occur experimentally in the sensitive response to heavy metals. Tolerance could result from a less specific mechanism that confers a broad resistance to several different metals (co-tolerance) or may involve a series of independent metal-specific mechanisms (multiple tolerance) (Schat et al. 2000). Chelation of metals in the cytosol by high-affinity ligands is one of the mechanism of detoxification. Ligands include some amino acids and organic acids, and three classes of peptides, i.e. glutathione (GSH), phytochelatins (PCs) and metallothioneins (MTs). In the plants growing under non-optimal temperature, there is high expression of heat shock proteins (HSPs), which normally act as molecular chaperones in protein folding, but may also function in the protection and repair of protein under metal-stress (Fig. 1) (Zenk 1996; Khan et al. 2000; Cobbett, Goldsbrough 2002; Hall 2002).

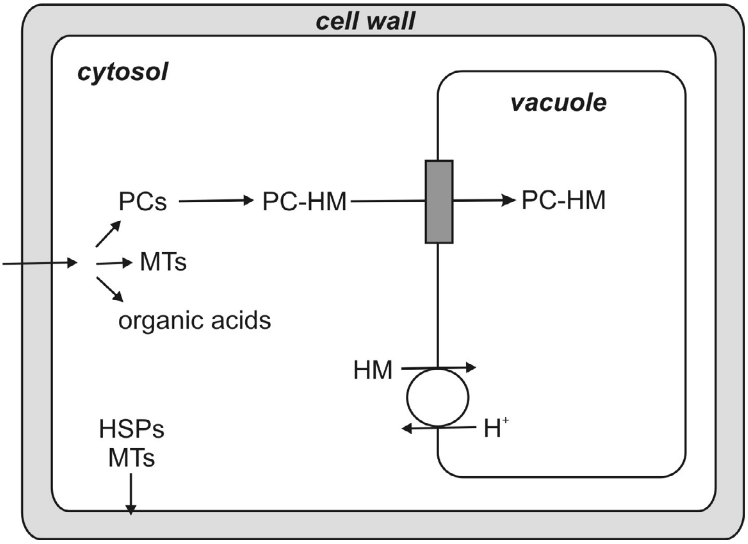

Figure 1. Potential cellular mechanisms for heavy metal detoxification: chelation in cytosol by various ligands; transport of PC-HM complex into the vacuole; transport and accumulation of heavy metals in vacuole (Hall 2002). Abbreviations: HM, heavy metals; PC, phytochelatin; MT, metallothioneins; HSP, heat shock proteins

Rycina 1. Potencjalne mechanizmy komórkowych detoksyfikacji metali ciężkich: chelacja w cytozolu przez różne ligandy; transport kompleksu PC-HM do wakuoli; transport i akumulacja metali ciężkich w wakuoli (Hall 2000). Skróty: HM, metale ciężkie; PC, fitochelatyny; MT, metalotioneiny; HSP, białka szoku termicznego

3.1. Peptide ligands

Three classes of peptides, glutathione (GSH), metallothioneins (MTs), and phytochelatins (PCs), have been implicated in heavy-metal homeostasis in plants. Thiol peptide, GSH (γ-Glu-Cys-Gly), and in some species its variant homoglutathione (h-GSH, γ-Glu-Cys-β-Ala), is considered to influence the form and toxicity of heavy metals such as As, Cd, Cu, Hg, and Zn, in several ways. These include direct metal binding, promotion of the transfer of metals to other ligands, such as MTs and PCs, provision of reducing equivalents for the generation of metal oxidation states more amenable to binding by MTs and possibly PCs, removal of the active oxygen species formed as a result of exposure of cells to heavy metals, and/or the formation of transport-active metal complexes. MTs are small gene-encoded, cysteine-rich polypeptides. In contrast, PCs are enzymatically synthesized cysteine-rich peptides (Cobbett, Goldsbrough 2002; Szalai et al. 2009).

3.1.1. Glutathione

Glutathione (GSH), the main non-protein thiol found in cells, is synthesized exclusively in cytosol in two steps that require ATP. The first step is the unusual coupling of γ-carboxylic acid of glutamic acid to cysteine by enzyme γ-glutamylcysteine synthetase (γ-GCS), followed by the formation of GSH by GSH synthetase (GS), which uses ATP and γ-glutamylcysteine and glycine as substrates. The formation of γ-glutamylcysteine is the rate-limiting reaction in GSH synthesis and is feedback inhibited by GSH itself, a mechanism responsible for the regulation of cellular GSH concentration. After synthesis, GSH is widely distributed in many intracellular organelles, including the endoplasmic reticulum, nucleus, and mitochondria. The compartmentalization of GSH includes redox pools that are separate from the cytoplasmic pool. The balance between GSH and GSSG forms, their redox potential, and their control of cellular activities is different in each location. GSH is found predominantly in its reduced form, except in the endoplasmic reticulum, where it is found mainly as oxidized dimeric glutathione (GSSG). GSSG constitutes the main source of oxidizing equivalents to provide the adequate environment necessary for favouring disulfide bond formation and the proper folding of nascent proteins. GSSG is reduced to GSH using NADPH via glutathione reductase (Szalai et al. 2009).

GSH is a critical source of reducing power and it is involved in a number of diverse functions that include apoptosis, disulfide bond formation, detoxification, antioxidant defense, maintenance of thiol status, and modulation of cell proliferation. The central cysteine group in the backbone of GSH is essential in the regulation of disulfide bonds of proteins and in the disposal of electrophiles and oxidants. This antioxidant function of GSH is realised through the redox-active thiol group, that becomes oxidized when GSH reduces target molecules. The exact role of GSH depletion in apoptosis is still not clear but the decrease in cellular reducing power is a clear indicator of the initiation of cell death (Navrot et al. 2006; Szalai et al. 2009).

3.1.2. Phytochelatins

Phytochelatins (PCs) consist of only the three amino acids: Glu, Cys and Gly with the Glu, and Cys residues linked through a γ-carboxylamide bond. PCs form a family of structures with increasing repetitions of the γ-Glu-Cys dipeptide followed by a terminal Gly; $(\gamma\text{-Glu-Cys})_n$-Gly, where *n* has been reported as being as high as 11, but it is generally in the range of 2 to 5. PCs have been identified in a wide variety of plant species and in some microorganisms. They are structurally related to glutathione (GSH; γ-Glu-Cys-Gly) and were presumed to be the products of a biosynthetic pathway. In addition, a number of structural variants, for example (γ-Glu-Cys)$_n$-β-Ala, (γ-Glu-Cys)$_n$-Ser, and (γ-Glu-Cys)$_n$-Glu, have been identified in some plant species. Their synthesis is triggered by exposure to metal ions and catalyzed by the constitutively expressed enzyme, dipeptididyl transpeptidase, phytochelatin synthase (EC 2.3.2.15). PC synthase activity was found to be strictly dependent on the presence of metal ions in the assay buffer. Various metal ions and metalloids can activate phytochelatin synthase with Cd^{2+} ions being the strongest inducers. Other inducing ions include Pb^{2+}, Zn^{2+}, Cu^{2+}, Sb^{3+}, Ag^{+}, Hg^{2+} and As^{5+}. These metals also induce PC biosynthesis *in vivo* in plant cell cultures. In the presence of heavy metals, PC synthase is activated, followed by synthesis of PCs from reduced GSH. The PCs bind to heavy metal, for example cadmium, in the cytosol and form a complex, which is transported into the vacuole where it is associated with organic acids or as a high molecular weight PC-Cd complex (Cobbett 2000; Cobbett, Goldsbrough 2002; Hirata et al. 2005).

There are a number of mechanisms by which the PC biosynthetic pathway may be regulated. The first of these is the regulation of GSH biosynthesis. The formation of γ-glutamylcysteine (γ-EC) from glutamate and cysteine, a reaction catalyzed by γ-glutamylcysteine synthetase (γ-GCS, EC 6.3.2.2), is generally accepted as the rate-limiting step in the biosynthetic pathway of GSH because this enzyme is feedback-inhibited by GSH. In addition to γ-ECS, glutathione synthetase (GS, EC 6.3.2.3) and *O*-acetylserine(thiol)lyase (OASTL, EC 4.2.99.8) are also assumed to be involved in the regulation of GSH and PC synthesis. GSH synthesis is also regulated by oxidative stress. Exogenously applied and endogenously generated H_2O_2 increases the GSH levels in plants and cultured plant cells. In this model, heavy metal increases PC synthesis from GSH by activation of PC synthase and promotes the synthesis of GSH not only through transcriptional activation of the GSH biosynthetic pathway, but also through stimulation of endogenous generation of reactive oxygen species, for example H_2O_2 (Cobbett 2000; Beck et al. 2003; Hirata et al. 2005; Blum 2007).

Regulation of PC synthase activity is expected to be the primary point at which PC synthesis is regulated. Kinetic studies using plant cell cultures demonstrated that PC biosynthesis occurs within minutes of exposure to Cd and is independent of *de novo* protein synthesis, consistent with the observation of enzyme activation *in vitro*. The enzyme appears to be expressed independently of heavy metal exposure (Hirata et al. 2005).

3.1.3. Metallothioneins

Metallothioneins (MTs) are low molecular weight (4–14 kDa), cysteine-rich proteins found in animals, higher plants, eukaryotic microorganisms, and some prokaryotes. The biosynthesis of MTs is regulated at the transcriptional level and induced by several factors, such as hormones, cytotoxic agents, and metals. In different MTs, Cys residues were found to occur in metal-binding motifs, containing Cys-Cys, Cys-Xaa-Cys or Cys-Xaa-Xaa-Cys (where Xaa represents another amino acid), which furnished sulphydryl ligands for coordination of divalent metal ions. On the basis of the arrangement of Cys residues, plant MTs have been classified into type-I, II, III and IV (Klaassen et al. 1999; Cobbett, Goldsbrough 2002; Coyle et al. 2002). Although the precise physiological functions of MTs have not yet been fully elucidated, it is reasonable to propose the following: (a) participation in maintaining the homeostasis of essential transition metals; (b) sequestration of toxic metals, such as cadmium and mercury; and (c) protection against intracellular oxidative damage (Klaassen et al. 1999; Cobbett, Goldsbrough 2002; Coyle et al. 2002).

Type 1 MTs contain a total of six Cys-Xaa-Cys motifs that are distributed equally between two domains. In the majority of Type 1 MTs, the two domains are separated by approximately 40 amino acids, including aromatic amino acids. This large spacer is a common feature of plant MTs and contrasts with most other MTs in which cysteine-rich domains are separated by a spacer of less than 10 amino acids that do not include aromatic residues (Cobbett, Goldsbrough 2002).

Type 2 MTs also contain two cysteine-rich domains separated by a spacer of approximately 40 amino acid residues. However, the first pair of cysteines is present as a Cys-Cys motif in amino acid positions 3 and 4 of these proteins. A Cys-Gly-Gly-Cys motif is present at the end of the N-terminal cysteine-rich domain. Overall, the sequences of the N-terminal domain of Type 2 MTs are highly conserved (MSCCGGNCGCS). The C-terminal domain contains three Cys-Xaa-Cys motifs (Cobbett, Goldsbrough 2002).

Type 3 MTs contain only four Cys residues in the N-terminal domain. The consensus sequence for the first three is Cys-Gly-Asn-Cys-Asp-Cys. The fourth cysteine is not a part of pair of cysteines but it is contained within a highly conserved motif, Gln-Cys-Xaa-Lys-Lys-Gly. The six Cys residues in the C-terminal cysteine-rich domain are arranged in Cys-Xaa-Cys motifs. As with the majority of Type 1 and Type 2 plant MTs, the two domains are separated from each other by approximately 40 amino acid residues (Cobbett, Goldsbrough 2002).

Type 4 MTs differ from other plant MTs by having three cysteine-rich domains, each containing 5 or 6 conserved cysteine residues, which are separated by

10 to 15 residues. Most of the cysteines are present as Cys-Xaa-Cys motifs. Although a large number of Type 4 MTs have not been identified, compared to those from monocots, Type 4 MTs from dicots contain an additional 8 to 10 amino acids in the N-terminal domain before the first cysteine residue (Cobbett, Goldsbrough 2002).

Effects of various metals on MT expression have disclosed wide variation from species to species. Although it is believed that MTs could play a role in metal metabolism, their role in plants remains to be determined owing to the lack of information, and their precise function is not clear (Cobbett, Goldsbrough 2002; Hall 2002). For example, Class II MT was induced in *Arabidopsis thaliana* seedlings by Cu and Cd. In different plants, MT gene expression was strongly induced by Cu treatment followed by Cd and Zn (Usha et al. 2009; Xue et al. 2009).

3.2. Organic and amino acids

The presence of different concentrations of organic acids among various ecotypes of metal-tolerant plants in their natural habitat has deemed these substances as likely cellular chelators. Due to the reactivity of metal ions with S, N, and O, carboxylic acids and amino acids represent potential ligands. Carboxylic acid anions are abundant in the cells of terrestrial plants and form complexes with divalent and trivalent metal ions of reasonably high stability. In particular, carboxylates such as malate, aconitate, malonate, oxalate, tartrate, citrate, and isocitrate are common major charge-balancing anions present in the cell vacuoles of photosynthetic tissues. Citrate, malate and oxalate have been implicated in a range of processes, including differential metal tolerance, metal transport through xylem and vacuolar metal sequestration (Clemens 2001).

Citric acid has been hypothesized to be a major cadmium ligand at low Cd^{2+} concentration (Wagner 1993). It has been shown to form complexes with Ni+ in Ni-hyperaccumulating plants (Sagner et al. 1998). Exposure of plant cells of the cobalt hyperaccumulator *Crotalaria cobalticola* and non-accumulators *Raufolia serpentine* and *Silene cucubalus* to cobalt ions has resulted in an increase in both citrate and cysteine, suggesting that these two proteins are involved in cobalt ion complexation (Oven et al. 2002). Extracellular chelation by organic acids, such as citrate, oxalate, and malate, is important in aluminum tolerance. Malate is released from roots of Al-tolerant cultivars of wheat (*Triticum aestivum*); while, citrate is released from roots of Al-tolerant cultivars of snapbean (*Phaseolus vulgaris*), maize (*Zea mays*), *Cassia tora*, and soybean (*Glycine max*), whereas, oxalate is released from roots of buckwheat (*Fagopyrum esculentum*) and taro (*Colocasia esculenta*) (Ma, Miyasaka 1998; Ma et al. 2001; Yang et al. 2001). Some plant species, such as oat (*Avena sativa*), radish (*Raphanus sativus*), and rye (*Secale cereale*) release both malate and citrate (Zheng et al. 1998; Li et al. 2000; Ma et al. 2001).

Mathys (1977) found that malic acid levels were correlated with the degree of resistance to zinc, with far greater concentrations present in zinc-tolerant ecotypes. Similarly, the synthesis of mustard oils by *Thlaspi caerulescens* and of oxalate by *Silene vulgaris* was significantly greater in the Zn-resistant populations. On the basis of these findings, mechanism for zinc tolerance has been proposed, whereby Zn^{2+} ions are bound by malate upon uptake into the cytoplasm, and malate then serves as a carrier to transport Zn^{2+} ions to the vacuole. Zn^{2+} ions are then complexed by a terminal acceptor, possibly a sulfur-containing mustard oil in *Thlaspi caerulescens* and oxalate in *Silene cucubalus*, and the released malate is able to return to the cytoplasm where it is ready to transport more Zn^{2+} ions.

Although carboxylates are undoubtedly quantitatively important ligands for metal chelation in the vacuole, they tend to be present constitutively in the shoots of terrestrial plants and do not seem to account for either the metal specificity or species specificity of hyperaccumulation. Even though the concentrations of ligands such as malate and citrate can be higher in metal-treated plants, this may be a general metabolic response aimed to maintain charge balance by organic acid synthesis, rather than a specific one that accounts for tolerance toward a particular metal. The sulfur donor atom in organic ligands is a considerably better electron donor than oxygen and leads to complexes of very high stability with the first-row transition metals. Three classes of sulfur-containing ligands have been identified in plants that may play an important role in metal tolerance – glutathione, phytochelatins and metallothioneins (Clemens 2001; Cobbett, Goldsbrough 2002).

Organic ligands containing nitrogen donor centers (i.e. amino acids) also form complexes of high stability with the first-row transition metals, but with thermodynamic stability constants intermediate between those of the oxygen and those of sulfur donor ligands. Several studies proposed the involvement of either organic or amino acid chelation in enhancement of the rate of root-to-shoot transport of transition metal ions (Liao et al. 2000). Correlations were observed between the concentrations of copper and nicotianamine as well as those of copper and histidine in xylem sap. As a result, it has been suggested that both amino acids, nicotianamine and histidine, were involved in chelation of copper ions in the xylem sap. Some amino acids, particularly histidine and proline, are also involved in the chelation of metal ions both within plant cells and in the xylem sap (Rai 2002). Exposure of the hyperaccumulator *Alyssum lesbiacum* to nickel is known to result in a dose dependant increase in xylem sap concentrations of both nickel and the chelator-free histidine. It indicated that amino acids, together with carboxylic acids, could play a significant role in metal chelation in the xylem.

Many plants have been reported to accumulate proline (Pro) when exposed to heavy metals (Talanova et al. 2000; Siripornadulsil et al. 2002). It has been demonstrated that increased Pro levels provide enhanced protection against Cd in microalgae. It is interesting to note that Pro reduces Cd stress not by sequestering Cd, but by reducing Cd-induced free radical damage and maintaining a stringent reducing environment (higher GSH levels) within the cell.

Nicotianamine (NA), a non-proteinaceous amino acid, is ubiquitously present in higher plants, and it is known to be involved in chelation of metals. Nicotianamine aminotransferase catalyzes the amino group transfer of NA in the biosynthetic pathway of phytosiderophores, and it is essential for iron acquisition in graminaceous plants. In addition to its role in long-distance metal transport, NA is proposed to be involved in the regulation of metal transfer within plant cells (Takahashi et al. 2003). On the other hand, nicotianamine synthase is a critical enzyme for the biosynthesis of the mugineic acid family of phytosiderophores in graminaceous plants and for homeostasis of metal ions in nongraminaceous plants (Mizuno et al. 2003).

3.3. Heat shock proteins

Heat shock proteins (HSPs) characteristically show increased expression in a variety of organisms in response to temperatures above their optimal growth one. They are found in all groups of living organisms and are classified according to their molecular size. There are three classes of proteins that account for most HSPs, *viz.*, HSP90, HSP70 and low molecular weight proteins of 15–30 kDa. HSPs are now known to be expressed in response to a variety of environmental stress conditions. They act as molecular chaperones in normal protein folding and assembly, but may also function in the protection and repair of proteins under stress conditions. Limited evidence suggests that increased synthesis of some heat-shock proteins (HSPs) may be a general plant response to heavy metal stress, but the specific functions or structures protected by HSPs remain unidentified (Feder, Hofmann 1999; Wang et al. 2004).

It is known that chloroplast small HSPs (smHSPs) protect the photosynthetic electron transport (Ph_{et}) during heat, oxidative, and photoinhibitory stress, but it is not known if chloroplast smHSPs are synthesized during metal stress and protect photosynthesis. *Zea mays* (corn) plants were exposed to varying soil concentrations of Cu, Ni, Pb, and Zn to determine if chloroplast smHSPs are induced by heavy metals, if smHSPs protect Ph_{et}, and any effects on chloroplast smHSP and photosynthesis. Net photosynthetic electron transport (Ph_n) decreased upon exposure to all metals and the decrease was the stronger the higher the metal levels and the longer the exposures. The reduction in Ph_n resulted from damage to photosynthetic metabolism, including Ph_{et}. All metals increased chloroplast smHSP content, which increased with time of exposure. In vitro, Ph_{et} was protected from Pb (but not Ni) by purified chloroplast smHsp added to thylakoids. In vivo, Ph_n was protected from Ni and Pb by the increase in smHSP in a heat-tolerant *Agrostis stolonifera* selection genotype expressing additional chloroplast smHSPs compared to a near-isogenic heat-sensitive genotype. These results indicate that the chloroplast small HSP can protect photosynthesis during heavy metal stress. Interestingly, in corn, purified small HSP did not protect Ph_{et} from Ni in vitro, but in *Agrostis stolonifera*, protection of Ph_n was indicated, supporting the prediction that small HSP could protect other aspects of chloroplast function from heavy metals besides Ph_{et}, such as Calvin cycle enzymes (including rubisco), which are known to be readily damaged by excess heavy metals. At lower metal levels, chloroplast smHSP accumulation increases with metal accumulation in leaves, while at intermediate levels of metals, smHSP accumulation saturates, prior to declining at high levels of metals, that are so toxic that even smHSP production is inhibited. Analysis of all results indicates that the production of chloroplast small HSP is an early response to heavy metal accumulation in leaves and that the function of chloroplast small HSP is to limit the damage to photosynthesis, rather than to be involved in repair or recovery from heavy metal damage. The ability of chloroplast small HSP to protect photosynthesis from heavy metals was significant, both in vitro and in vivo, suggesting the potential utility of breeding, engineering, or selecting plants for increased production of chloroplast small HSP (e.g., either constitutive production, more rapid induction, or increased accumulation during stress) in improving plant tolerance to heavy metals (Heckathorn et al. 2004).

Neumann et al. (1994, 1995) observed that HSP17 is expressed in the roots of *Armeria maritima* plants grown on copper-rich soils. HSPs have also been shown to increase in cadmium-treated *Silene vulgaris* (e.g. HSP17) and *Lycopersicon peruvianum* (e.g. HSP17 and HSP70) cell cultures (Wollgiehn, Neumann 1999). HSP70 has been detected in the nucleus, cytoplasm and at the plasma membrane. It suggests that HSP70 could be involved in the protection of membranes against cadmium damage. It has also been reported that a short heat stress given prior to heavy metal stress induces a tolerance effect by preventing membrane damage. Clearly, more molecular evidence is required to support such an important repair or protective role.

4. Conclusion

Oxidative stress is associated with all kinds of environmental stresses (for example heavy metals action) in plants and poses a serious threat by disturbing their normal growth, development and physiology. Plants have developed various systems against essential metal ion uptake. Once metal ions enter the cell, they are bound by chelators and chaperones. Chelators contribute to metal detoxification by buffering cytosolic metal concentrations; while chaperones specifically deliver metal ions to organelles and metal-requiring proteins. There are several known metal-chelators in plants. These include phytochelatins, metallothioneins, organic acids, and amino acids. They play important roles in defense against oxidative stress and remain in focus for engineering to enhance resistance in plants. Oxidation of thiol groups in proteins especially the methionine and cysteine residues suggests that they are open and easy targets of oxidative attack. Heat shock proteins may also be involved in the protection and repair of protein under metal-stress.

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Mechanizmy detoksyfikacji metali ciężkich u roślin

Streszczenie

Zanieczyszczenia metalami ciężkimi obecnie jest jednym z najbardziej ważnych problemów środowiskowych. Większość metali jest łatwo absorbowana przez rośliny i akumulowana w różnorodnych organach. Metale ciężkie utrudniają wzrost roślinom poprzez zakłócenie procesów biochemicznych, fizjologicznych i metabolicznych. Wywołują zmiany w poziomie transkrypcji wielu genów kodujących białka aby posiadać zmiany ochronne przeciwko uszkodzeniom spowodowanym przez stresy. Ważnym mechanizmem związanym z toksycznością metali ciężkich jest ich zdolność do silnego przyłączenia się do atomu tlenu, azotu i siarki. Rośliny posiadają szereg potencjalnych mechanizmów na poziomie komórkowym, które mogą być włączone w detoksyfikację, a następnie tolerancję na stres metalami ciężkimi. Kiedy jony metalu przenikną do komórki zostaną związane przez chelatory lub chaperony. Chelatory przyczyniają się do detoksyfikacji metali poprzez zbuforowanie cytozolowego stężenia metalu, podczas gdy chaperony specyficznie dostarczają jony metali do organelli i białkom łączącym metale. Znanych jest kilka chelatorów w roślinach, tzn. fitochelatyny, metalotioneiny, kwasy organiczne i aminokwasy. Spośród ligandów wbudowujących metale ciężkie w komórkach roślinnych, najlepiej scharakteryzowane są fitochelatyny i metalotioneiny. W roślinach traktowanych metalami ciężkimi znaleziono również białka szoku termicznego. Jednakże specyficzne funkcje czy struktury HSP pozostają niezidentyfikowane.

Plant responses to wounding stress

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Abstract

Plants have developed mechanisms to increase their tolerance to stress conditions, including wounding that can be caused by both biotic and/or abiotic stress factors. Wounding of plants is caused by strong wind, rain, snow, by pathogen or insect attack. Damaged tissues are potential sites of bacterial or viruses infection and cause decrease in quality and yields. Plant responses to wounding have been extensively studied and have been divided into local and systemic. In a few minutes after wounding the reactive oxygen species are synthesised and cytoplasmic pool of calcium is increased. In response to wounding there are cell wall modifications: deposition of lignin and suberin and increase in cell-wall integrity. Deposition or synthesis of phenols, oligosaccharides, and alkaloids is observed. After tissue damage there is secretion of molecules like systemin and pathogenesis related proteins, hormones – especially jasmonic acid, salicylic acid, abscisic acid, ethylene, and traumatic acid. Both local and systemic responses to wounding activate many metabolic pathways causing changes in metabolism, photosynthesis and respiration rate. The pathway of plants responses is not plain and simple because of a number of molecules involved and different connections between them. This article presents various plant responses to wounding stress.

Key words: local response, systemic response, mechanical damage

1. Introduction

Wounding is a common stress which affects plant growth and metabolism. This stress is often caused by abiotic factors like wind, hail, strong rain or by biotic stress factors e.g. herbivores and insects feeding. A wounded area is an open way to infections caused by pathogens like fungi or bacteria and always limits viability of the organs where it occurs. Immediately after wounding chemical changes in the damaged area take place, such as synthesis or release (e.g. from vacuoles) of phenolic compounds, alkaloids and flavonoids. There are also cell wall modifications such as suberization of cells at the wound surface and increase in cell wall integrity (Howe 2004).

Plant responses are divided into local and systemic and include synthesis of molecules that stimulate regeneration, take part in signalling pathways and change gene expression (Fig. 1) (Knight, Knight 2001; Bruce, Pickett 2007). Different genes are expressed in response to wounding (Reymond et al. 2000; Lawrence et al. 2006). Molecular mechanism by which signals of wounding are perceived and transduced is poorly understood (Walley et al. 2007). Physiological mechanism of plants responses is a complex process involving a whole array of molecules which orchestrate this process.

2. Local response to wounding

Local response includes mechanisms which exist at the site of injury or close to this site. The wounded sites are easily accessible to penetration of pathogens thus this response occurs within a few minutes after wounding and involves wounded tissues regeneration, herbivore deterring defences, release of stored material and prevention of drastic loss of water (Maleck, Dietricj 1999; Howe 2004; Bruce, Pickett 2007; Denness et al. 2011). Early events after wounding start from ion imbalance and variations in membrane potentials, Ca^{2+} signalling, production of reactive oxygen species, phytohormones and kinase activities (Maffei et al. 2007). In local response, proteins and oligosaccharides are synthesized or released from a damaged cell wall. Oligogalacturonides (OGAs) are important in the local response as a signal molecule because of their limited mobility. OGAs modify genes expression (Fig. 1), especially polygalacturonase gene (Leon et al. 2001). Cell walls are thicker because of lignin and callose deposition (Denness et al. 2011). Improved synthesis of phenolic compounds has been also observed (Somssich, Hahlbrock

1998). Responses to wounding also include depolarisation of the membrane with the elevation of intracellular levels of calcium and protein phosphorylation (Chico et al. 2001; Leon et al. 2001; Zimmermann et al. 2009).

3. Systemic response to wounding

Systemic response to wounding occurs in undamaged leaves or plant organs in the distal site of attack (Leon et al. 2001). Different pathways induced by wound signals are needed to improve plant resistance. This kind of response includes metabolic changes and either induction or repression of genes expression. Recent DNA microarray studies have confirmed a central role of jasmonic acid in orchestrating genome-wide changes in genes expression (Fig. 1) (Rojo et al. 2003; Schilmiller, Howe 2005).

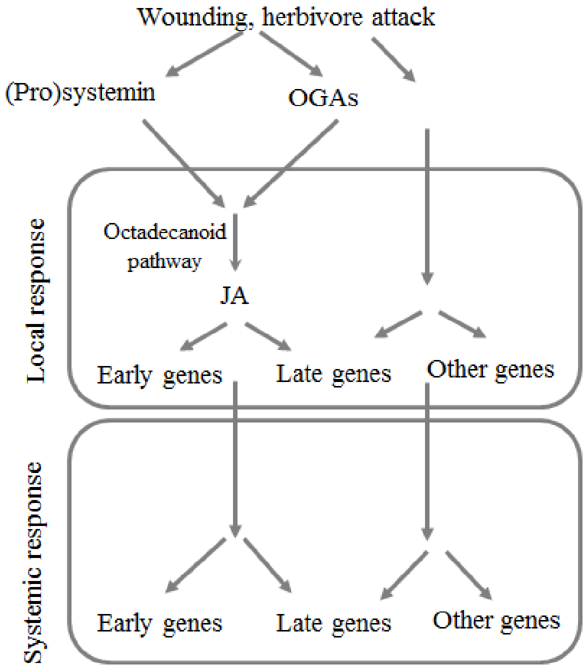

Figure 1. Model of local and systemic wounding signalling (Howe 2004, modified). JA – jasmonic acid, OGAs – oligogalacturonides

Rycina 1. Model odpowiedzi lokalnej i systemicznej o zranieniu (zmodyfikowane wg Howe 2004). JA – kwas jasmonowy, OGAs – oligogalakturonidy

Within two hours after an insect attack or mechanical wounding, proteinase inhibitors (PI) are inducted. Their function is to block digestive proteases in herbivore gut (Howe 2004). The signals that are involved in activation *PI* genes, include cell-wall delivered oligogalacturonides (OGAs) and systemin.

Systemin is a mobile signal for defence gene activation in the systemic wound response. Systemin is active even in subnanomolar concentrations. Nevertheless, systemin production is linked with *Solanaceae* plants, in *Arabidopsis* no functional homologue of systemin has been found so far (Leon et al. 2001; Rojo et al. 2003). This protein is synthesised from prosystemin in the wounding site and then it is translocated through vascular tissues to distal leaves where it activates the octadecanoid pathway and JA synthesis (Fig. 1) (Schilmiller, Howe 2005).

4. Hormones in the plant response pathway

It has been observed that the concentration of endogenous jasmonates increases after wounding or a pathogen attack (Koo, Howe 2009; Mielke et al. 2011). Kessler et al. (2004) observed that the silencing of octadecanoid pathway in tobacco contributes to greater vulnerability of plants to the pathogens specific of *Nicotiana attenuata* and such plants become attractive to novel herbivore species. Mechanical damage causes a 25-fold increase not only in JA but also in JA-amino acid conjugates, especially JA-Ile, JA-Leu, Ja-Val (Maffei et al. 2007; Koo, Howe 2009). Jasmonic acid is produced in peroxisome by the octadecanoid pathway in the lipid conversion from linoleic acid (Fig. 2) (Berger 2002; Schilmiller, Howe 2005; Koo, Howe 2009). All known jasmonate-mediated responses require COI1 (*Coronate Insensitive1*), a receptor, which is the F-box protein component of E_3 ubiquitin ligase SCF^{coi1} (Schilmiller, Howe 2005; Kazan, Manners 2008). This complex interacts with JAZ proteins (*Jasmonate Zim – domain proteins*) which are ubiquitinated, deliver transcription factors and allow gene expression (Fig. 2) (Koo, Howe 2009).

The timing and amplitude of JA accumulation is affected by the temporal and spatial patterns of leaf damage (Koo, Howe 2009). JA synthesis is also induced by compounds in insect oral secretions, the peptide systemin and oligosaccharides derived from cell-wall (Koo, Howe 2009). In *Arabidopsis* high level of JA is linked with the production of OGAs from a damaged cell-wall (Rojo et al. 2003). Jasmonates in damaged tissues improves the response by regulation of a wide range of defence-related processes, including synthesis of toxic secondary metabolites, production of morphological barriers, feeding deterrents (like PI) and volatiles

(Li et al. 2002; Lorenzo, Solano 2005). Large-scale changes in gene expression were observed, including induction of proteinase inhibitors genes, chalcone synthase genes and defensin genes (Koo, Howe 2009).

The octadecanoid pathway interacts with other signalling pathways such as salicylate and ethylene pathways (Rakwal, Agrawal 2003). Plants produce salicylic acid (SA) in response to wounding, but the biochemical pathways, leading to SA biosynthesis during the defence responses may differ between plant species (Hammond-Kosack, Jones 1996).

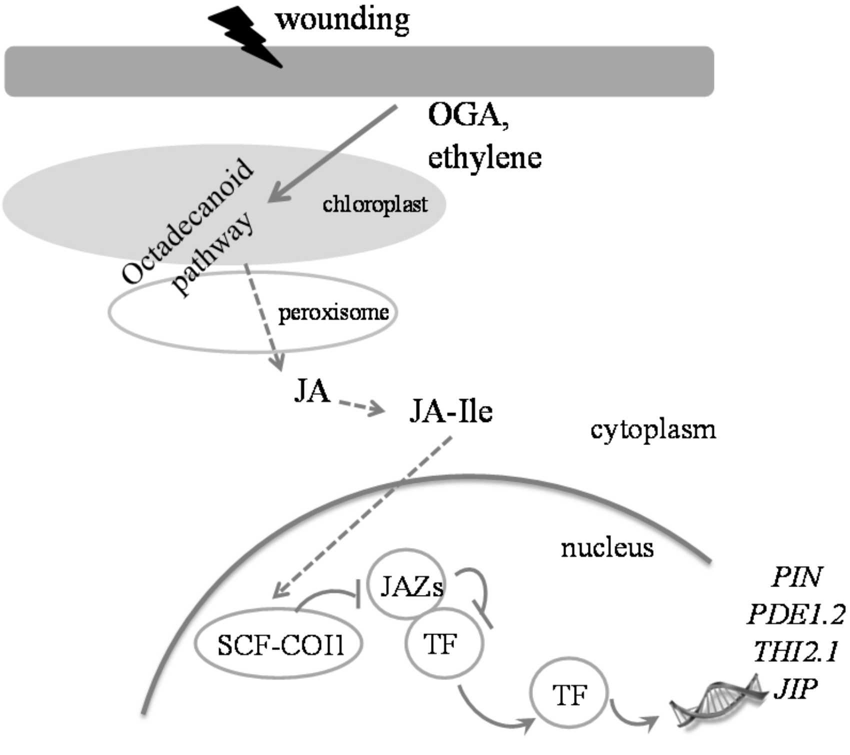

Figure 2. Jasmonates synthesis and signalling (Acosta, Farmer 2010, modified). JA – Jasmonic acid, JA-Ile – Jasmonoyl isoleucine, TF – Transcription factors, JAZs – Jasmonate Zim – domain proteins, SCF-COI1 – protein component of the E3 ubiquitin ligase, *PIN*, *PDE1.2*, *THI2.1*, *JIP* – *PR* genes

Rycina 2. Synteza i sygnalizacja o zranieniu za pośrednictwem kwasu jasmonowego (zmodyfikowane wg Acosta, Farmer 2010). JA – kwas jasmonowy, JA-Ile – jasmonian izoleucyny, TF – czynniki transktypcyjne, JAZs – białko JAZ, SCF-COI1 – specyficzna część białkowa ligazy ubikwitynowej, *PIN*, *PDE1.2*, *THI2.1*, *JIP* – geny *PR*

This hormone has antibacterial role and an elevated SA level inhibits wound-induced gene expressions by blocking JA biosynthesis and inhibiting catalase activity, thus resulting in an increased synthesis of ROS (Hammond-Kosack, Jones 1996). SA regulates many *PR* genes such as *PR1*or *BGL2*, which encode hydrolases targeted at fungal cell-wall (Reymond, Farmer 1998). An important component of SA signalling is gene *NPR1* (*Non-expressor of PR1*, in *Arabidopsis thaliana*). It has been observed that mutant *npr1* is insensitive to SA and unable to activate the expression of *PR* genes or disease resistance in response to pathogen attack (Sah 2003). The application of SA or its analogue stimulates the translocation of *NPR1* into the nucleus, which is required for the activation of downstream signalling. *NPR1* protein depolymerizes and forms monomers which migrate to the nucleus where the monomers associate with transcription factors that induce pathogen defence genes (Heidel, Baldwin 2004). The first way to activate expression of the pathogenesis-related genes by SA requires the *NPR1* gene. The second way is through *NPR1*-independent pathway with ethylene and JA signalling and it is supported by studies of various *Arabidopsis* constitutive-defence signalling mutants (Sah 2003). Penninckx et al. (1996) suggest that pathogen-induced expression of the plant defensin gene (*PDF1*) in *Arabidopsis* is independent of salicylic acid and requires components of the ethylene and jasmonic acid response.

Another major plant-specific hormone in defence against pathogens is ethylene (Gazzarrini, McCourt 2001; Frankowski et al. 2007). Ethylene is mainly connected with herbivores and pathogens infection and not to mechanical wounding. It has been shown that JA and ethylene signalling is required for resistance to pathogens, such as *Altenaria*, *Botrytis*, *Septoria*, *Phytium*, *Erwinia* etc. (Rojo et al. 2003). Ethylene comprises pathways with JA and salicylic acid leading to *PR* gene induction in tobacco (Dong 1998; Gazzarrini, McCourt 2001; Rojo et al. 2003; Adie et al. 2007). This hormone regulates multigene families involved in signal transduction, helps in production of xylem occlusions which block the xylem in prevention of pathogens spreading (Adie et al. 2007). The appearance of ethylene is probably also connected with synthesis of the cell wall-strengthening hydroxyproline-rich glycoproteins and some of phytoalexins (Adie et al. 2007). This hormone takes part in regulation of gene expressions of the pathogenesis-related proteins (Hammond-Kosack, Jones 1996). It has been observed that exogenous application of ethylene induces defence-related enzymes such as glucanases and chitinases as well as enzymes involved in phytoalexin synthesis (Penninckx et al. 1996).

Both jasmonic acid, traumatic acid (TA) and traumatin are products of the lipoxygenase pathway (Gardner 1998). Still little has been added to our knowledge about traumatic acid and traumatin. TA has growth-stimulating and woundhealing activity in plants and causes rise in activity of kinases and protein phosphatases in response to wounding (Kallenbach et al. 2011). Traumatin undergoes rapid modifications by diverse enzymatic and nonenzymatic reactions, generates multiple potential chemical signals (Kallenbach et al. 2011).

Abscisic acid (ABA) is another stress-respond hormone. It is produced *via* mevalonic pathway or indirectly from carotenoids. After wounding ABA is accumulated in a region close to damaged tissues (Leon et al. 2001). ABA plays a negative role in disease resistance, probably by an antagonistic effect on SA/JA/ethylene-mediated defence signalling (Mauch-Mani, Mauch 2005).

5. ROS in local and systemic response to wounding

To enhance plants' resistance, reactive oxygen species are synthesised (ROS) (Fig. 3). In low concentration they act as second messengers involved in cell signalling, in high concentration they are a part of direct defence (Maffei et al. 2007). Not only ROS produced take part in a local response, but they also induce a systemic response (Grant, Loake 2000; Ślesak et al. 2007). Wounding or pathogen elicitors stimulate ion flux into cytoplasm. H^+ and Ca^{2+} activate MAP kinases which are translocated into the nucleus where they activate genes of plant defence (Fig. 2, 3). The influx of Ca^{2+} activates the production of NADPH by NADPH-dependent oxidase, a cell wall peroxidase and apoplastic amine, diamine and polyamine oxidase-type enzymes – in effect there is an overproduction of ROS (Grant, Loake 2000; Jacobo-Velazquez et al. 2011). ROS are important in direct defence because of their toxicity to microbes. In local response H_2O_2 takes part in the formation of lignin polymers, in systemic response H_2O_2 activates synthesis of various compounds e.g. hormones (Fig. 3) (Hammond-Kosack, Jones 1996).

6. Pathogenesis-related proteins (PR proteins)

PR proteins are intra- and extracellular proteins that accumulate in intact plant tissue after pathogen attack or elicitor treatment (Hammond-Kosack, Jones 1996; Somssich, Hahlbrock 1998). These proteins include glucanases, chitinases, osmotin, proteinases, inhibitors of proteinases and peroxidases (Hammond-Kosack, Jones 1996; Pasternak, Sikorski 2002). SA and ethylene induce synthesis of PR proteins which are targeted in subcellular regions, mainly in vacuole and apoplastic space (Somssich, Hahlbrock 1998). These proteins are less likely to be components of a front line defence action but probably have their major effects after cellular decompartmentalization. Accumulation of PR proteins coincides with the plant's enhanced resistance to microbal pathogens. This phenomenon is known as systemic acquired resistance (SAR; Penninckx et al. 1996).

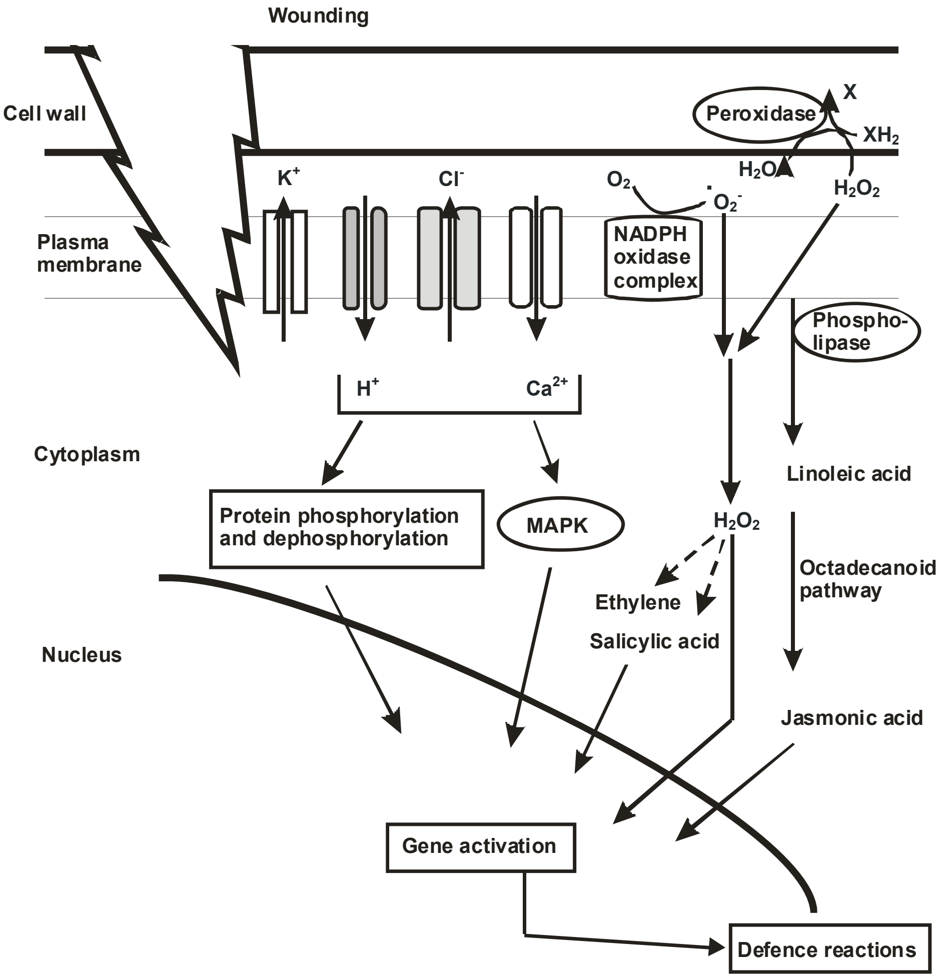

Figure 3. Signal transduction pathway in response to wounding (Hammond-Kosack, Jones 1996; Somssich, Hahlbrock 1998, modified)

Rycina 3. Szlak transdukcji sygnału w odpowiedzi na zranienie (zmodyfikowane wg Hammond-Kosack, Jones 1996; Somssich, Hahlbrock 1998)

7. MAP kinases in stress-responding pathway

Response to wounding is connected with mitogen-activated protein kinases (MAPK) (Fig. 1, 2) (Rakwal, Agrawal 2003; Fujita et al. 2006). Seo et al. (1995) observed that transcripts of the gene encoding a mitogen-activated protein kinase begin to accumulate in one minute after tobacco leaves wounding. MAPKs form a cascade composed of at least three sequentially activated protein kinases which are activated by dual phosphorylation on their threonine and tyrosine. However, this pathway remains complicated and elusive (Rakwal, Agrawal 2003). ROS or calcium ion influx activate MAPK, which are translocated to nucleus and activate transcriptor factors to change genes expression in response to a stress factor (Fig. 2) (Somssich, Hahlbrock 1998). Moreover, these systemic signalling pathways may interact with one another to optimize plants' responses to different types of wounding stress or during different stages of plant development (Koo, Howe 2009). It has been observed that MAP kinases are connected not only with Ca^{2+} or ROS changes but also with ethylene and abscisic acid pathways (Fujita et al. 2006). MAPKs are essential to jasmonic acid production and accumulation of wound-inducible gene transcripts. Calcium ions are the second messengers in wounding signalling pathways, thus calcium-dependent protein kinases (CDPKs) are also involved in wounding signalling pathways (Maffei et al. 2007).

8. Carbon metabolism after wounding treatment

Wounding or pathogen infection also influences the primary and secondary metabolism. Berger et al. (2004) have observed that photosynthetic gene (*RbcS*) expression decreases after inoculation as well as does photosynthetic activity in direct vicinity of the infected sites. Sugar-dependent repression of other photosynthetic genes expression has been also observed (Gazzarrini, McCourt 2001). Berger et al. (2004) have noted a decrease in soluble sugar content after infection; a decrease in sucrose was stronger than that in glucose and fructose. Consequently, wounding increases the respiration rate (Quilliam et al. 2006; Lafta, Fugate 2011). Concentration of ATP, NADPH, UTP decreased after wounding (Lafta, Fugate 2011). Nevertheless, the plants overcome restrictions in respiration by tricarboxylic cycle and glycolysis that allow carbon compounds to enter the metabolic pathway (Lafta, Fugate 2011). The most visible changes take place in activity of sucrose-hydrolysing enzymes, especially in that of cell wall invertases (Zhang et al. 1996;

Quilliam et al. 2006; Hawrylak, Wolska-Mitaszko 2007; Łukaszuk et al. 2011). Zhang et al. (1996) and Ahkami et al. (2008) have observed that mechanical wounding induce accumulation of cell-wall invertase mRNA in detached leaves, stems and roots. They have noted that accumulation of this type mRNA was also induced by abscisic and jasmonic acid. Berger et al. (2004) observed an increase in *Lin6* gene expression (the sink-specific extracellular invertase gene) in tomato leaves 24h after infection with *Botrytis cinerea* and *Pseudomonas syringae*.

9. Complexity of wound responding

Plant responses to wounding are not a result of isolated defence pathways but they are based on a complex network of interactions between different signals. Although it is known that several signals are involved in wound-induced systemic plant defence, little is known about their interaction. The type and strength of the interaction depends on plants species (e.g. *Arabidopsis* or tomato), developmental stage, nature of threat (e.g. wounding or insect feeding), and environmental conditions, even though it is caused by the same stress factor. However, plants are able to combine all available mechanisms and coordinate this response according to the type of attack so as the metabolic costs of plant defence are minimised.

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The notion of disturbances and progress in ecology

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Abstract

Assuming that one of the criteria of progress in ecology is increasing precision of the relevant notions, the currently used diversity of definitions and conceptions and imprecise terminology indicate that often the notion of disturbances is wrongly used. The interest of ecologists in disturbances has considerably increased in the last three decades, which is evidenced by a growing number of publications concerned with their observations and effects. The question is if these studies have brought about a considerable progress in formulation of the fundamentals of the theory of disturbances. Any scientific theory requires strong theoretical base and sound corroborating empirical investigation. This paper identifies differences in the approaches of different authors to the essence of this phenomenon.

Another problem considered is the imprecision of the terminology used leading to mistakes of treatment of basic population processes as a disturbance. Also, not all biotic interactions and ecological processes taking place in plant communities can be treated as disturbances. The ambiguity of notions and the lack of precise terminology increase the information noise and permits wide choice of possible interpretations of the phenomena observed depending on individual experience and knowledge. Therefore there is a strong need to arrange the operatively defined terminology and scientific methodology and specify which phenomena can and which cannot be treated as disturbances.

Key words: Concepts and definitions of disturbances, holistic or reductionistic approach, concepts of regeneration of tree stand, equilibrium/non-equilibrium theory

1. Introduction

Recently, much interest has been devoted to analysis of disturbances on many levels of the hierarchic structure of vegetation (an individual, a population, plant community, landscape) (Łaska 2001). Some authors treat disturbances as phenomena occurring on the level of a population, community, biocenose or ecosystem (Grubb 1985; Helmus et al 2010; Lee, Brown 2011). In the opinion of others, they are the outcome of the interactions between particular species (Denslow 1985; Wootton 1998; Kondoh 2001, Haddad et al 2008). In literature they have been analysed in the context of natural biotic and abiotic factors (Picket, White 1985a; Drever et al. 2006; Laughlin, Abella 2007; Fraver et al. 2009), anthropogenic factors (Bazzaz 1983; Bornkamm 1991; Łaska 2006), in variable space-time relations (Franceschi et al. 2000; Loehle 2000, Fraver 2004), on a local or global scale (Sheil, Burslem 2003). The concept of disturbances also includes natural processes taking place in communities, mainly regeneration of tree-stands in gaps formed as a result of treefalls and their role in shaping the structure and dynamics of communities (Battles, Fahey 2000; Brokaw, Busing 2000; Pecot 2001).

Many authors have discussed the relationships between science and progress and have evaluated how ecology can best improve progress (Thompson et al., 2001; Graham, Dayton 2002; Paine 2002; Salafsky et al. 2002; Osmond et al. 2004). If one of the criteria of progress in ecology is the increasing precision of notions (Shurin et al. 2001; Starzomski et al. 2004; Krebs 2006; Wołek 2008) then the diversity of definitions and conceptions and the lack of precision of the terminology applied indicates that what we call the theory of disturbances does not have be to be such a theory at all. In science a theory is a testable model of the manner of interaction of a set of natural phenomena capable of predicting future occurrences or observations of the same kind, and capable of being tested through experiment or otherwise verified through empirical observation (McIntosh 1985; Weiner 1995; Falińska 1996, 1998). In this paper I intend to show how different can be the approaches of authors to the question of disturbances.

2. Definitions and the concept of disturbances

Despite a considerable progress in formulation of the fundamentals of the theory of disturbances, different authors assume different approaches to the essential meaning of the term (Łaska 2001, 2003). The differences stem from alternative understanding of the term, assumption of the holistic or reductionistic concept of the study, consideration of different spatial scale of the patches forming as a result of disturbances, different conceptions of the renewal of tree stand in open space and different ideas as to the origins and effects of disturbances, implied by variation in the approaches to the notions of equilibrium and non-equilibrium in nature.

2.1. Definitions of disturbances

In recent years the state of knowledge about disturbances was has summed up (McCartly 2001; White, Jentsch 2001; Frelich 2002; Kuuluvainen 2002; Brassard, Chen 2006; Johnson, Miyanishi 2007). However, the most important are two different conceptions representing completely different paradigms in vegetation dynamics. In these two different conceptions the term disturbance is used in two main meanings. On the one hand, after Grime (1974, 1977, 1979) it is associated with the partial or total destruction of the plant biomass and arises from the activities of herbivores, pathogens, man (tramping, mowing, and ploughing), and from phenomena such as wind damage, frosts, desiccation, soil erosion, and fire. On the other hand, after White and Pickett (1985) the disturbance is any relatively discrete event in time that disrupts ecosystem, community, or population structure and changes resources, substrate availability, or the physical environment. This discrete event leads to different types decomposition of vegetation, characterised on the basis of the appearance of patches and gaps (Kransy, DiGregorio 2001; McCarthy 2001). Different approaches to disturbances impose different methods of their investigation, different conceptions of study and different problems of detailed concern.

2.2. Methods of studies of disturbances according to Grime

According to the concept of study proposed by Grime (1974, 1977, 1979) observations should be made on a level of an individual and the features of a given organism subjected to the effects of disturbances that should be considered are: height, structure, reproduction, dispersion, resistance to antagonistic correlations. These features determine the life history of species, types of reactions and adaptation to disturbances (Grime 1984, 1985, 1987, 1988; Łaska 1996a, b, 1997, 1998, 2001). The effect of disturbances on changes in the life strategies of species is determined experimentally in laboratories of the Botanic Garden of the Sheffield University, UK, where the influence of herbivores and predators is studied. Moreover, at the Buxton Climate Change Impacts Laboratory, in Derbyshire, along with the changing level of disturbing factors (mowing, grazing) the effect of climatic changes (temperature, moisture content, sunlight access or snow cover) is analysed (Grime 1995, 1996, 1998).

2.3. The concept of disturbances according to Grime

In the C-S-R theory of the life strategy of plants, Grime (1974, 1977, 1979) proposes a model in which disturbances (**R**), stress (S) and competition (C) are the phenomena that impose changes in the strategy of life of the species. The species of the ruderal strategy (**R**) growing in disturbed environment (fallow, arable land, heavily tramped paths) are characterised by low biomass, short life cycle, fast rate of growth and development, great reproduction effort and great fertility (MacArthur, Wilson 1967; Pianka 1970; Grime 1979, 1985). These species include mainly herbs (annuals and biennials) and bryophytes (Fig. 1).

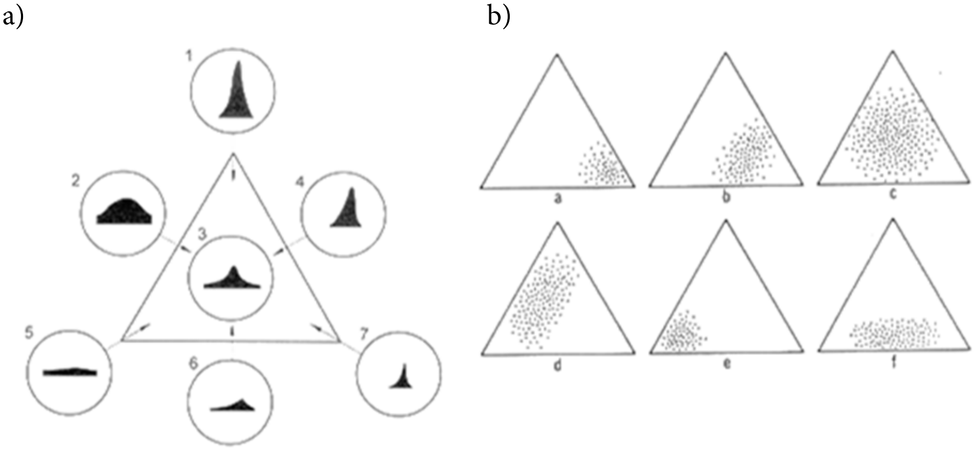

Figure 1. Model relating pattern of seasonal changes in shoot biomass **(a)** and the range of life forms **(b)** to life strategy (Source: Grime 1977, 1985): **a)** 1 – Competitor, 2 – Stress-tolerant competitor, 3 – C-S-R strategist, 4 – Competitive-ruderal, 5 – Stress-tolerator, 6 – Stress-tolerant ruderal, 7 – R – Ruderal; **b)** a – annual herbs, b – biennial herbs, c – perennial herbs and ferns, d – trees and shrubs, e – lichens, f – bryophytes

Rycina 1. Model określający sezonowe zmiany biomasy pędów **(a)** i prezentację form życiowych **(b)** w odniesieniu do strategii życia (Źródło: Grime 1977, 1985).

They are much different from the plants of the competitive strategy (C) characterised by large size and long life cycles and from the plants of the S (stress tolerator) strategy, showing high resistance to stress, small size, long lifetime and ability to use the resources in the short times of their availability. In temperate vegetation, seasonal changes in shoot biomass appear to be predictably related to the R, S, C equilibrium and deserve to be explored further as a potential basis for the recognition of plant strategies (Fig. 1).

However, as in many environments the vegetation is subjected to competition, stress and disturbances at a time, Grime (1979) distinguished four additional secondary strategies. Perennial species and ferns represent a wide range of life strategies (C-S-R type), adapted to the conditions in which competition is limited by medium level of disturbances and stress. In his model, Grime (1979, 1984, 1985) clearly shows the degree of adaptation of other taxonomic groups to changing environment. In the strategy of Ruderals he classifies mainly the annuals (Fig. 1).

2.4. Methods of investigation of disturbances according to White and Pickett

In the concept of White and Pickett (1985) the effect of disturbances is studied on higher levels of organisation of life, populations, communities and ecosystems (Frelich 2002; Harmon et al. 2002). Disturbances are considered as affecting different biomes (Bazzaz 1983) and trophic levels (Denslow 1985). The features of populations and communities that are mostly influenced by disturbances are structural elements, while in ecosystems the functional features such as the cycle of elements or energy relations in biocenoses. White and Picket (1985) have reduced the study on the effect of disturbances to analysis of the so-called structure of a system and the structure concerning the relation of biomass to the substrate (Fig. 2). This relation determines the effect of disturbances of a certain type on a given system and the effective threshold of their intensity. The effects of disturbances on populations, communities and ecosystems are studied by observations in the field in different spatial scales, taking into regard the regime of natural and anthropogenic disturbances (Seymour et al. 2002; Long 2009). The effects of disturbances on these levels are computer simulated in ecological modelling too, taking into account all types of disturbing events and processes depending on the scale of gaps and time.

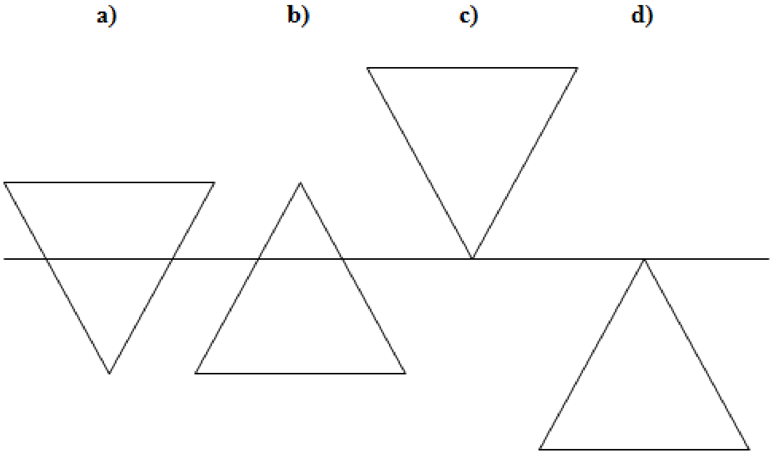

Figure 2. Diagram of four contrasting types of community structures showing disposition of biomass relative to the substrate and degree of attachment of the organisms to the substrate (Source: Pickett, White 1985b): **a)** shoot-biased community – disturbances disrupt the above ground portion of the community; **b)** a root-biased community – disturbances disrupt the within-substrate component; **c)** a surface-attached community – disturbances disrupt all biomass above the substrate (common among invertebrate animals); **d)** substrate contained – disturbances disrupt the underground portion of the community (communities of burrowing animals).

Rycina 2. Diagram czterech różnych struktur zbiorowiska określających związek biomasy z podłożem i stopień połączenia organizmów z podłożem (Źródło: Pickett, White 1985b)

2.5. White and Pickett conception of disturbances

According to the conception of White and Pickett (1985), disturbances lead to the appearance of empty spaces – "microhabitats" in which the process of colonisation of the disturbed area begins. Diversity of disturbances leads to the formation of gaps or transformed patches characterised by the size and shape or internal heterogeneity and variable spatial distribution. There are two concepts related to the appearance of transformed patches or gaps as a result of disturbances: the "disturbance regime" which is concerned with the temporal and spatial nature of appearance of free sites under the effect of different kinds of disturbances (Frelich 2002) and "patch dynamics" dealing with dynamics of changes in the vegetation in the patches and the surrounding environment (Pickett, White 1985b; Roxburgh et al. 2004).

3. Disturbances and the reductionistic or holistic conception of studies

Different approaches to the notion of disturbances arise from the assumption of either the holistic or reductionistic concept of study, which implies analysis of different effects of the same disturbance on different levels of the hierarchic structure of vegetation.

Assuming the reductionistic approach, the real object of study responding to disturbances is an individual organism. On this level of biological organisation the effects of disturbing factors are observed in the structural features as destruction of biomass of individuals. The response of individuals to a disturbance is different and the determination of the range of its variation is important for recognition of life history and is a criterion of division into different life strategies characterising the adaptation to disturbances (Grime 1974, 1977, 1979, 1984, 1985, 1988). Changes in the life strategies and modifications of development of species in disturbed communities can be indicators of the intensity of disturbance (Łaska 1996a, b, 1998, 2001).

According to the holistic point of view, the effects of different disturbances cannot be explained by analysing the fate of individuals. The effects of disturbances, apart from destruction of biomass, appear also on other features of the systems, with increasing structural complexity of subsequent biological levels (Tab. 1). On the level of populations, disturbances affect the spatial structure, size, age structure and genetic structure (Pickett et al. 1989). In the functional components the effect of disturbance can produce lability of developmental cycle of all individuals. The accumulated effect of a disturbance on all individuals is manifested on a level of population by modification of the life strategy, determining many properties of the population (Łaska 1996a, b, 1998, 2001).

The hierarchic structure of biological systems implies the necessity of considering the response of a given system to disturbances on the one hand as a separate unit, while on the other as a part of a larger structure (Picket et al. 1989; Rykiel 1995). On the level of a community, disturbances influence the floristic composition and determine the richness of species, species domination and community structure (Łaska 2001, 2006). Disturbances have significant effect on floristic composition as they influence the species of different types of life strategies on areas of different size and change the way of effective exploitation of the available resources. On the level of landscape the disturbances affect structural and functional features, ecological and evolution processes typical of a given level of integration.

Table 1. Disturbances analysed in term of reductionistic and holistic concept of study

Tabela 1. Definicje zaburzeń w ujęciu redukcjonistycznej i holistycznej koncepcji badań

Definition of disturbances	References
REDUCTIONISTINIC APPROACH	
“Disturbance – ...the mechanisms which limit the plant biomass by causing its partial or total destruction”	Grime 1979
“Disturbance defined by Grime (1979) is accepted in the present review, with the caveat that senescence of individual is excluded”	Grubb 1985
“Disturbances reduce the dominance of a site by established individuals and create openings for colonization and growth by new individuals”	Canham, Marks 1985
HOLISTIC APPROACH	
“A disturbance is any relatively discrete event in time that discrupts ecosystem, community, or population structure and changes resources, substrate availability, or the physical environment”	White, Pickett 1985 (adopted by Evans, Barkham 1992)
“Disturbance is any changes (natural and antropogenic) that discrupts population, community or biocenose structure”	Falińska 1996
COMPLEMENTARY APPROACH	
“Disturbance is a change in the minimal structure caused by a factor external to the level of interest”	Pickett et al. 1989
“Disturbance may affect each level of organisation addressed by ecologists, from individual to ecosystem and landscape, and the consequences and mechanisms of disturbance are diffrent at each hierarchical level”	Rykiel 1995 (after by Pickett et al. 1989)

Differences in interpretations of the disturbance effects can follow from the fact of considering different levels of biological organisation (individual, population, community, landscape) and application of the rules established for the specific level to the other levels of biological organisation (Tab. 1). The conclusion is that the effects of disturbances should always be considered and interpreted at a certain defined level of biological organisation, which permits drawing correct conclusions and correct discernment between disturbances and other events or phenomena (Picket et al. 1989; Łaska 2001, 2003).

4. Measures and classification of disturbances

Another problem leading to misunderstanding and ambiguity of terminology of the vegetation dynamics and the reasons and effects of disturbances is that of the measures and classification of disturbances.

4.1. Space and time in vegetation dynamics

The disturbed area is the notion related to the scale of activity of a given disturbing factor (micro- or macroscale). The greatest controversy is related to changes in vegetation analysed in the microscale, from the point of view of the causes and effects of disturbances or the vegetation dynamics. The process of overgrowing the gap caused by death of one or a few trees, that is the vegetation changes in a small scale (the areas of a few square meters) are treated as corresponding to the changes in the macroscale and referred to as succession, in analogy to the changes over large open areas (of a few hectares) taking place in a long time. Thus, many types of succession are distinguished with no respect of the differences between the dynamic processes taking place inside a community and those leading to transformation of communities, or sequence of successive communities.

The role of gaps in tree stands in the dynamics of communities and populations is doubtless (McCarthy 2001). The gaps offer the main or the only possibility of appearance of new individuals in many communities, especially those of compact vegetation cover. The size of the gap determines the size and species composition of the appearing patches. The gaps allowing the assess of sunlight initiate the sequence of events known as the regeneration of the gap phase.

However, it should be stressed that the appearance of gaps after death of single trees cannot always be treated as disturbance. The small gaps in tree-stands as a result of the physiological death of old trees which are replaced by younger generation of the same species or species of similar ecological requirements can be treated as fluctuations (Faliński 1998). The fluctuation is a process of continuous changes in the community, which have a mosaic type of appearance but do not affect the community as a whole entity. According to Faliński (1998, p. 30) “These changes should not be treated as disturbances, but only as a set of conditions necessary if the permanence of a community is to be assured”. Thus a fluctuation is a process which stabilises rather than disturbs a given community (Faliński 1998). However, the assumption that fluctuations maintain stability of communities implies the presence of a kind of equilibrium in nature.

4.2. Non-equilibrium in the nature

Many ecologists reject the idea of a dynamic equilibrium. They claim that in each system there are disturbances of different kinds, which prevent it from reaching any equilibrium (Pickett, White 1985a; Sommer, Worm 2002). The death of a single tree can also be treated as a disturbance because the change it implies on the level of resources can be drastic and the tree itself and the gap it leaves physically disturb the plants growing there earlier (Runkle 1985). On the other hand, the death of a single tree can also be treated as a natural population process determining – together with reproduction – the dynamics of plant populations. The question is if the within community changes following from the natural changes in the population size – reproduction and death leading to replacement of older individuals by young ones – can be treated as disturbances. The lack of any of these processes would be catastrophic for the population or community (Falińska 1996). The relations between the size of gaps and their distribution after the death of a tree and the mosaic of the undergrowth and its effective revival are dynamic in character but the inner bounds between the components of the community and its habitat are preserved.

5. Conclusions

The presented views on the equilibrium an non-equilibrium in the nature are good starting point for considering the dynamics of vegetation in the aspect of the effect of disturbing factors. In contrast to the approach of the advocates of the unequilibrium in the nature, the dynamics of vegetation does not have to be a dynamics of disturbances and the occurrence of disturbances does not shake the concept of stability but only points to lower universality and limited application of the theory of equilibrium to the effect of disturbing factors. In view of the above, empirical differentiation between the phenomena and events that are normal for the functioning and stability of communities and those that disturb them is of importance for development of the theory of disturbances and progress in ecology. According to Krebs (2006) "The key to increasing progress is to adopt a systems approach with explicit hypotheses, theoretical models, and field experiments on a scale defined by the problem. With continuous feedback between problems, possible solutions, relevant theory and experimental data we can achieve our scientific goals".

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Pojęcie zaburzeń a ocena postępu w badaniach ekologicznych

Streszczenie

Jeżeli jednym z kryteriów oceny postępu w ekologii jest progresja w doskonaleniu pojęć (Graham et al. 2002; Swihart et al. 2002; Starzomski et al. 2004; Krebs 2006; Wołek 2008), to różnorodność definicji i koncepcji oraz nieprecyzyjność w stosowanej terminologii świadczy o tym, że dotyczy to szczególnie zjawiska zaburzeń. Wieloznaczność pojęć i brak modelowych rozwiązań daje badaczom szerokie możliwości interpretacji zachodzących zjawisk pod wpływem zaburzeń, w dużej mierze zależnych od ich własnych doświadczeń i wiedzy. Teoria naukowa wymaga mocnych teoretycznych podstaw, a badania empiryczne silnego oparcia w teorii. Prezentowana w pracy problematyka ilustruje natomiast, jak różne mogą być podejścia poszczególnych autorów, co do rozumienia istoty zaburzeń, jak różne są zakresy znaczeniowe tego terminu oraz poglądy analizujące rolę tego zjawiska w badaniach dynamiki populacji i zbiorowisk roślinnych.

Analiza zjawiska zaburzeń wskazuje, że znaczącym osiągnięciem w tej dziedzinie jest przeprowadzenie wielu badań i opublikowanie licznych obserwacji. Przyjęcie holistycznej lub redukcjonistycznej koncepcji badań, uwzględnianie różnej skali przestrzennej i czasowej oddziaływania czynników zaburzeń, dowolna analiza przyczyn i efektów zaburzeń powoduje jednak, że analizując nawet te same dane różni badacze dochodzą do różnych wniosków.

Czy charakterystyczną cechą „ekologicznego myślenia" jest rozpatrywanie zawsze wielu kontrowersyjnych tez i poglądów oraz brak jednoznacznych odpowiedzi. Hierarchiczna struktura (wielopoziomowość) układów biologicznych narzuca konieczność osobnego rozpatrywania reakcji danego systemu na czynniki zaburzeń – z jednej strony osobno jako całości, z drugiej strony osobno jako elementu większej całości. Może więc odpowiedzią na to jest komplementarność pojęć i hierarchiczne traktowanie poziomów materii żywej, testowalność hipotez i prezentacja teoretycznych modeli, badania eksperymentalne i empiryczna odpowiedź na zadane pytania.

Pulsatilla patens (L.) Mill. in the Knyszyńska Forest on background of abiotic disorders

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Abstract

Abiotic factors are the most important elements influencing species distribution, plant growth and development. The aim of this study was to evaluate the influence of abiotic factors: air temperature, soil temperature and sunlight exposure on the number and condition of individuals of *Pulsatilla patens* (L.) Mill. in the Knyszyńska Forest. Using Statistica 9.0, the relationships between the number of individuals *Pulsatilla patens* (L.) Mill. and abiotic environmental factors were analysed.

Result of the present study show a positive reaction of *Pulsatilla patens* individuals to the rise of air temperature, soil temperature and sunlight intensity with decreasing distance from the road edge. The total number of individuals *P. patens* and the number of flowering individuals was the highest at the highest values of air and soil temperatures and insolation. Analysis of the effect of light conditions showed that the largest number of generative and juvenile individuals was located at distances up to 2 m from the road edge, and their number decreased with a marked reduction of availability light in the direction of compact canopy forest. Spearman's rank correlation coefficient indicates that there is a very strong negative correlation between increasing distance from the road and decreasing light intensity and the total number of individuals in the population. Statistically significant differences in the number of individuals in the generative and vegetative phase, depending on increasing distance from the road towards the compact hood forest and decreasing light intensity were indicated.

Key words: threatened plant, air temperature, soil temperature, sunlight exposure, plant growth and development

1. Introduction

Understanding of the effect of abiotic factors on the distribution of threatened plants enables us to estimate the drivers of a species distribution within a region (Kouba et al. 2011). A combination of these factors determines the organism's fundamental niche, defined as the range of conditions and resources within which individuals of a given species can persist. The borders of the fundamental niche are determined by physiological tolerances to abiotic factors; therefore, abiotic factors have been found to determine the growth of some plant species.

Plant growth and development are regulated by different abiotic factors, and temperature is the one of particular significance. In many plants, physiological and biochemical alterations occur after exposure to temperature for optimal growth. Temperature is a decisive factor regulating dormancy as well as seed germination. Low temperatures can induce dormancy and high temperatures can relieve dormancy (Brändel 2004). Temperature has significant effects on the onset, potential and rate of germination (Flores, Briones 2001).

Light is another important environmental abiotic factor influencing the species abundance (Elemans 2004; Whigham 2004; Bartemucci et al. 2006), diversity and composition (Jelaska et al. 2006). Light availability is critically important for plant growth, reproduction and distribution. Successful seed reproduction is closely associated with the seasonal timing of germination (Simons, Johnston 2006), and light, together with soil moisture and temperature, is among the most important environmental factors influencing the timing of seed germination (Grime et al. 1981). Specifically, the presence of a light requirement is one of the main elements of conservative germination strategies (Schütz 2002), and in the ability of seeds to postpone germination, i.e., to stay dormant in the soil and form seed banks (Kettenring et al. 2006), a trait posited as a "bet-hedging" strategy. Light signals are among the most important environmental cues regulating plant development (Franklin, Whitelam 2004).

Roads introduce micro and mesoclimatic changes, through variation of the sun radiation, wind regimes, moisture and temperature (Forman et al. 2002). Microclimate gradients across road edges influence many ecological processes and patterns. Abiotic gradients reaching the forest interior from the road may transform a large area of forest into a habitat suitable for plants (Goosem, Turton 2000). Floristic composition changes more or less suddenly along these gradients (Hansen, Clevenger 2005).

Understanding the influence of abiotic factors on endangered species is a major objective for explaining the reasons why populations of *Pulsatilla patens* (L.)

Mill. are threatened. In north-eastern Poland an important factor reducing the number of sites of this taxon is increasing instability of thermal conditions in the period of the species blooming, in March and April (Wójtowicz 2000). These unfavourable climatic conditions in growing season, might constrain seed development and seedling establishment or reduce seed mass. Therefore, the aim of this study was to determine the influence of abiotic factors on the number and condition of individuals of *P. patens* in the Knyszyńska Forest. The abiotic factors taken into regard were: a) air temperature, b) soil temperature, c) sunlight exposure, which were measured along transects perpendicular to the road.

2. Study area

The study of *P. patens* populations was conducted in north-eastern Poland, in the Supraśl Forest Division in the Knyszyńska Forest, specified within the Natura 2000 network (Łaska 2006, 2009) (Fig. 1).

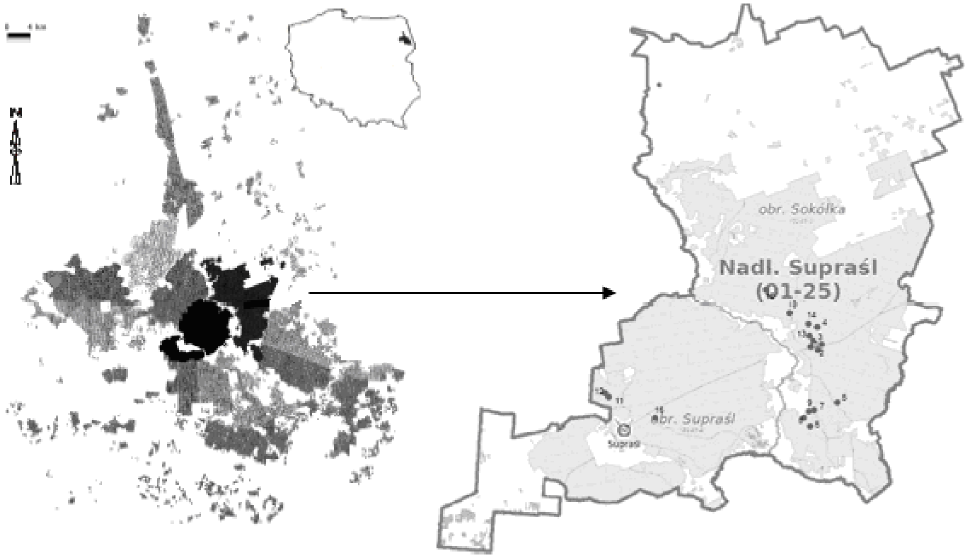

Figure 1. Location of *Pulsatilla patens* (L.) Mill. sites in the Knyszyńska Forest, Supraśl Forest Division (NE Poland) (Source: Łaska 2006)

Rycina 1. Rozmieszczenie stanowisk *Pulsatilla patens* (L.) Mill. w Puszczy Knyszyńskiej, w Nadleśnictwie Supraśl (NE Polska) (Źródło: Łaska 2006)

The vegetation of Knyszyńska Forest represents 23 forest and scrub associations and 835 species of vascular plants (Łaska 2006, 2010). The climate of the region is of continental type with long and cold winters (110 days) and long summers (90 days). The mean annual temperature is 7°C, and the mean annual rainfall reaches 610 mm. Snow cover is on average 10 cm thick for 85–90 days a year. Growing season (at 5°C threshold) lasts for about 200 days (Górniak 2000).

3. Study species

P. patens is found in central and middle-eastern Europe (Aichele, Schwegler 1957), with its northern limit of distribution at 66° northern latitude in Russia (Jalas, Suominen 1989). In Poland, most sites of *P. patens* comprising large numbers of individuals (tens to hundreds) are located in the north-eastern part of the country. In central and south-eastern parts of Poland, there are only a few, rather evenly scattered sites each of which with only a few individuals. In western and south-western Poland, *P. patens* is a rare species (Wójtowicz 2001).

Within Poland's territory *P. patens* favours well-sunlit locations with south-western and southern exposure, typically on fringes of boreal forests of the class *Vaccinio-Piceetea* (Matuszkiewicz 2001) or in slightly shady areas. It may also occur in ploughed sections of forests, forest glades and fire-protection forest belts or, sporadically, in xerothermic and psammophilus grasslands (Wójtowicz 2000; Łaska, Sienkiewicz 2010).

P. patens is a monoecious, long-lived (tens of years) hemicryptophyte with a vertically branching rhizome which can form several shoots and makes older plants form clumps (Rysina 1981; Pilt, Kukk 2002). In Poland *P. patens* flowers in early spring, from late March to mid May. Flowers are either pollinated by insects or by self-pollination (Jonsson et al. 1991). The seeds are dispersed by wind in June and July over short distances. The extent of the formation of leaf rosettes and of flowering and fruit bearing shoots depends on weather conditions such as winter temperatures, snow cover, autumn precipitation, temperature, and sunshine duration in spring (Wójtowicz 2000).

4. Material and methods

The effects of abiotic factors in the Knyszyńska Forest were studied in the growing season 2011. Air temperature, soil temperature and sunlight exposure were examined once at selected sites for 16 populations comprising from 1 to 165 individuals. Data were collected between 9:00 and 18:00, in the period with maximum values of sun radiation and temperature, minimum relative moisture, and the highest parameter stability. Abiotic data were measured every meter from the road edge towards the interior of the forest. On each transect, all individuals were recorded and classified according to life cycle stages: juvenile, vegetative, and flowering individuals.

Temperature was measured with a digital thermo-couple (Elmetron, Poland; error: ±1°C, precision: ±0.1°C) in the following layers: soil (10 cm depth of soil) and air (at a height of 5 cm above the forest ground), which measures the temperature in °C values in a scale from −50°C to 199.9°C. Sunlight exposure was estimated with a manual multifunction meter (ST–8820, made in China), which measures the intensity of light in Lux (lx) values in a scale from 20 to 20000. Illumination was given in kilolux (klx) = 10^{-3} lx. In variable light conditions the distance of individuals from the road and sunlight exposure at 1–2 cm above the forest ground were taken into account.

All statistical analyses were performed using Statistica 9.0 (Statsoft 2006). Correlations between the total number of individuals, number of juveniles, number of plants in vegetative stage, number of flowering individuals and distance from the road edge were analysed by Spearman rank order correlation. Correlation coefficient r_s was calculated at a significance level of $\alpha = 0.05$.

Statistical analysis of the differences between the numbers of individuals in different vegetative phases, the numbers of flowering individuals and the distances from the road edge were performed using multi-way arrays. Statistical relationships were determined by an Pearson's Chi-square test at a significance level of $\alpha = 0.01$. Juvenile specimens were excluded because of a too low expected value (<5). Statistical analysis was performed for the plants growing up to 6 m from the edge of the road because of the low number of individuals at distances from 7 to 9 m from this line.

5. Results

The temperatures measured at selected points showed overall decreasing trends from road edge to interior forest. In each transects, highly significant gradients of air and soil temperature were detected. Air temperature decreased towards the interior of the forest, when compared to that at the road edge. Mean (±S.D.) total air temperature was significantly higher closer to the edge (15.51±7.00), than in the forest interior (10.90±5.21), with the distance to the edge (Fig. 2). From the forest interior to the road edge, the air temperature increased by about 5°C. In the forest interior the air temperature probably become stable at 9 m from the road edge and did not change significantly over farther distances from the road. The soil temperature was also lower in the forest interior when compared to that at the road edge. Mean (±S.D.) total soil temperature was significantly higher closer to the edge (12.08±4.12), than in the forest interior (8.56±2.70), with the distance to the edge (Fig. 2). From the forest interior to road edge, the soil temperature increased by about 4°C.

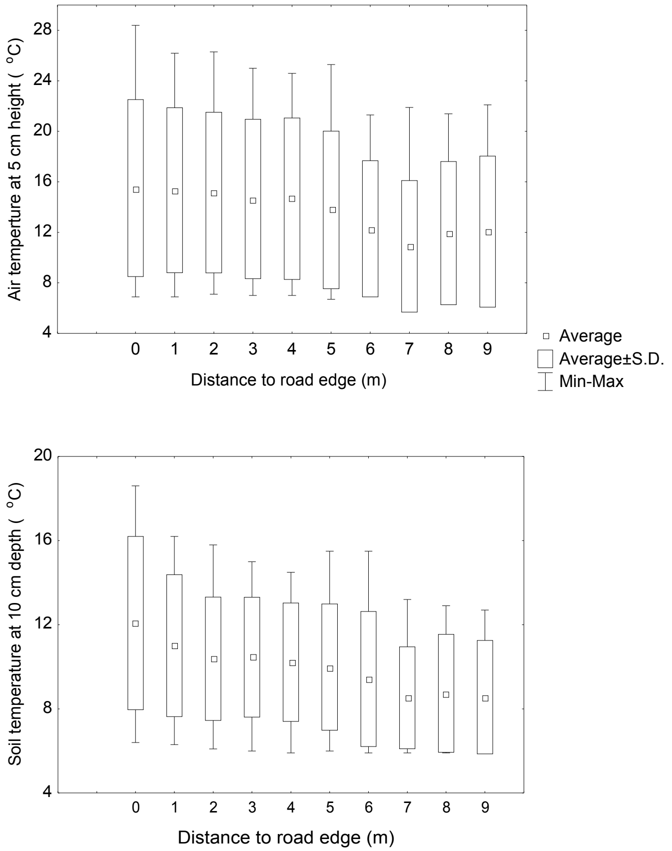

Figure 2. Variation in air and soil temperatures from the road edge to the forest interior

Rycina 2. Zmienność temperatury powietrza i temperatury gleby w odległości od krawędzi drogi do wnętrza lasu

Results of the present study show a positive reaction of *P. patens* individuals to the rise of air temperature, soil temperature and sunlight intensity with decreasing distance from the road edge. The total number of *P. patens* individuals and the number of flowering individuals were the highest for the highest air and soil temperatures. Analysis of the *P. patens* population revealed that the highest number of individuals (about 80%) was observed at the distances ranging between 0 and 5 m from the road, where the highest temperatures were recorded (Fig. 3). This effect is clearly visible at higher than at lower temperatures.

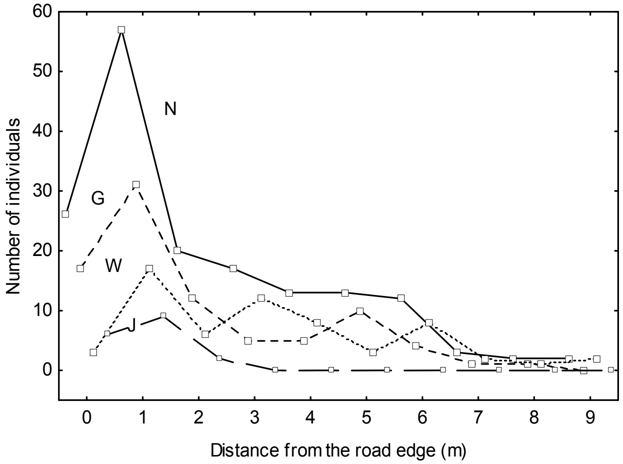

Figure 3. Relationship between the distance from the road edge and the number of individuals (N) and the number of individuals in flowering phase (G), vegetative phase (V), juvenile phase (J)

Rycina 3. Zależność pomiędzy odległością od krawędzi drogi i całkowitą liczbą osobników oraz liczbą osobników generatywnych, wegetatywnych oraz juwenilnych

The distribution and abundance of individuals in the population of *P. patens* were found to be closely correlated with the light intensity and distance from the road towards the compact canopy forest. It was established that with increasing distance from the road and increasingly shadowy conditions under dense tree canopies, the number of individuals at various stages of development decreased. The presence of individuals in generative phase was found only up to a distance of 8 m from the road, but the largest clusters were much closer to the edge of the road in good sunlight conditions, at distances up to 2 meters from the road ($y = 6.68–0.25 \cdot x$, $y = –0.80$, $p = 0.01$, $r^2 = 0.64$). This distance of 2 m from the road was the upper limit of occurrence of individuals in the juvenile stage, which similarly as the individuals in generative phase, need good lighting conditions for development. Most individuals in juvenile phase were growing at distances of no more than 2 m from the road edge, with a maximum at the distance of 1 m ($y = 5.67–0.69*x$; $r = –0.73$, $p = 0.02$; $r^2 = 0.53$). Individuals in the vegetative phase occurred over distances of up to 9 m from the road, and the greatest number of them was also noted also at distances within 1 m from the road ($y = 6.54–0.33*x$; $r = –0.56$; $p = 0.09$; $r^2 = 0.36$).

Spearman's rank correlation coefficient indicates that there is a very strong negative correlation between increasing distance from the road and decreasing light intensity and the total number of individuals in the population, particularly the number of individuals in juvenile and generative phases. These are the two most important phases determining the fate of the population, whose numbers of individuals is closely dependent on the good conditions of sun exposure.

Pearson Chi-square (Ch ^ 2 = 20.13, df = 6, $p = 0.01$) indicates that there are significant differences in the number of individuals in the generative and vegetative phase, depending on increasing distance from the road towards the compact hood forest and decreasing light intensity.

6. Discussion

Proper abiotic conditions are crucial for the growth and development of populations of the Eastern pasqueflower. Plants have an ideal temperature range for optimum absorption of mineral nutrients and at temperatures from this range their biomass production is greater (Hamlin et al. 1999), which is an important aspect of plant growth and development. This seems to be especially important for individuals *P. patens,* collecting maximum biomass and energy both for reproduction and high competitive skills. As follows from the results of this study, the average temperature 15/12 °C (air/soil) was the optimum temperature for the growth *P. patens.* Results of this study proved that these temperatures often coincide with increased flowering and faster growth of individuals. This indicates that lower temperatures have unfavourable effect on plant growth and development. Extreme temperatures are among the abiotic factors which also often inhibit this processes (Chaves et al. 2003) but the reaction to stressors is determined by plant genome and the interaction of the changed environmental conditions (Pastori, Foyer 2002).

According to Pluess et al. (2005) the weather conditions, such as temperature and growing season length, are important factors in determining the reproductive output of a single plant. Higher air/soil temperatures of plant growth enhanced the development of fruits *P. patens* more rapidly than lower ones. Besides, at higher air/soil temperatures, the seeds could faster become elevated in the zone of their dispersal. Kidson and Westoby (2000) have observed that increased seed mass within species correlated with higher seed germination, establishment success, growth rate and survival. However, De Frenne et al. (2009) have reported a contrasting effect of temperature on resource investment in reproduction. They concluded that the responses of the reproduction and population dynamics of forest herbs to temperature were primarily dependent upon phenology and distribution, but also that the response is to some extent species dependent.

In agreement with Wójtowicz study (2000), it was also found that flowers of pasqueflower are termonastic and helionastic which determines their reactions to different conditions of temperature and light. At high temperatures and strong insolation, the flowers of pasqueflower are wide open and mounted on an upright flower stalk, and at low temperatures and in shade, the flower petals are bell-like dropped and set on a flower stalk falling bent to the ground (Fig. 4).

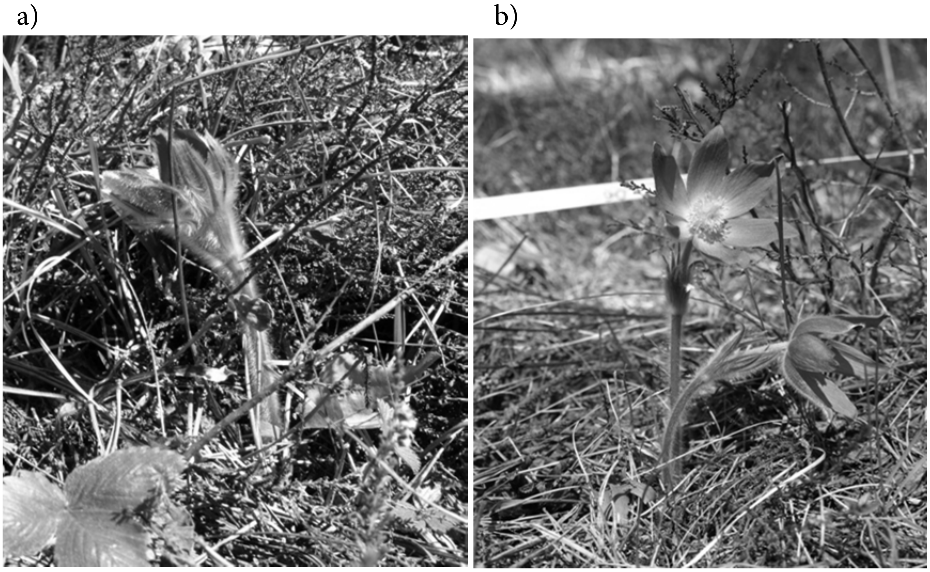

Figure 4. Thermonastic and helionastic flowers of *Pulsatilla patens* (L.) Mill. developed in response to the thermal conditions; **a)** low temperature and **b)** high temperature (Photo: Łaska, Sienkiewicz 18–19.04.2011)

Rycina 4. Reakcja termonastycznych i helionastycznych kwiatów *Pulsatilla patens* (L.) Mill. na warunki termiczne; **a)** niska temperatura i **b)** wysoka temperatura (Zdjęcie: Łaska, Sienkiewicz 18–19.04. 2011)

There is extensive research concerning the effects of light on the growth and abundance of plant populations. Wagner and Simons (2008) have observed that light germination responses are generally consistent with divergence in morphological, life-history and phenological traits: differentiation occurs among populations differing in environmental characteristics related to severity, such as mean growth season temperatures, and extreme maximum and minimum temperatures. According to Kalamees et al. (2005), important factors affecting germination and seedling establishment of *P. patens* are light intensity conditions.

High light intensity seems to be important for the flowering individuals of *P. patens* – the number of individuals and the number of flowers per individual were both highest in open sites. In addition, low light-grown plants were often smaller and reproduced less seeds than plants grown in higher insolation. Bartemucci et al. (2006) claim that light transmission was important for the cover and height of the understory vegetation, but it did not have strong influence on species composition and diversity. The results of this study confirm this idea, because plants growing in the open were generally more vigorous than those growing in heavy shade. This observation suggests that the ongoing processes of growth and development, and achievement of individual reproductive phase depends on good conditions of sun exposure. Hence, among other factors, light is responsible for the regeneration of this species in the nature.

However, Härdtle et al. (2003) have shown that the effects of light on the species richness of the understory depend on the type of the forest. In turn, soil conditions and topography were more decisive for understory vegetation than light in many cases (Augusto et al. 2003; Lenière, Houle 2006). Thomsen et al. (2005) found that understory species composition was primarily determined by indirect factors (such as light availability) of the overstory, but topographical, anthropogenic and spatial factors were similarly significant.

Consequently, the variation in abiotic factors with the distance from the road to the forest interior are the principal factors limiting distribution, plant growth and development. Higher concentration of individuals *P. patens* was recorded closer to the road edge, which can be explained by the highest air and soil temperatures at this location. Higher numbers of generative and juvenile individuals were observed at distances up to 2 m from the road edge, where no significant changes in the air and soil temperatures were observed. In the present study the optimum growth of the species studied took place in the favourable conditions. The air and soil temperatures as well as in sunlight intensity lower than the optimum values aggravate their effect.

In short, these results suggest that higher values of the abiotic factors considered have a positive influence on *P. patens*, whereas at lower values of the abiotic factors the plant development is restricted. Also important is the effect of local abiotic and biotic environmental factors, including temperature, on possible future shifts in vegetation ranges. Moreover, changes in climate and land use are of particular significance in predicting the outcome of a long-term relationship between plant life history (i.e. reproductive traits) and changes in both climate and soil (De Frenne et al. 2009). It seems interesting that the changes in air and soil temperatures in particular developmental phases are the key factors influencing the life cycle of the plants.

To get more reliable information the studies should be continued over larger area and longer time. It would be desirable to get representative results from the entire period of growth of *P. patens*. Another interesting point would be to investigate a relation between the minimum day temperatures and the condition of *P. patens* individuals.

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Pulsatilla patens (L.) MILL. w Puszczy Knyszyńskiej na tle zaburzeń abiotycznych

Streszczenie

Czynniki abiotyczne w głównym stopniu kształtują środowisko życia roślin, a niekorzystne zmiany warunków siedliskowych mogą prowadzić do zahamowania procesów ich wzrostu i rozwoju. Celem pracy jest ocena wpływu czynników abiotycznych: temperatury powietrza, temperatury gleby oraz natężenia światła słonecznego na osobniki sasanki otwartej Pulsatilla patens (L.) Mill. na obszarze Ostoi Knyszyńskiej. Przy użyciu analiz statystycznych w programie Statistica 9.0 dokonano określenia zależności pomiędzy liczbą osobników w populacji Pulsatilla patens (L.) Mill. a wybranymi abiotycznymi czynnikami środowiska przyrodniczego.

W badaniach stwierdzono pozytywną reakcję osobników na wzrost temperatury powietrza, temperatury gleby i natężenia światła słonecznego wraz z malejącą odległością od krawędzi drogi. Odnotowano, że całkowita liczba osobników Pulsatilla patens (L.) Mill. i liczba osobników kwitnących jest największa przy najwyższych temperaturach powietrza i gleby. Analiza warunków świetlnych wykazała, że największa liczba osobników generatywnych i juwenilnych sasanki otwartej występuje w odległości do 2 m od krawędzi drogi, a ich liczba maleje wraz z wyraźną redukcją dostępności światła w kierunku zwartego okapu lasu. Na podstawie analizy korelacji stwierdzono ujemną zależność pomiędzy rosnącą odległością od drogi i spadkiem natężenia światła słonecznego a całkowitą liczbą osobników w populacji Pulsatilla patens (L.). Wykazano też istotne statystycznie różnice w liczbie osobników w fazie generatywnej i wegetatywnej, w zależności od rosnącej odległości od drogi i malejącej intensywności światła słonecznego.

Rannoch rush *Scheuchzeria palustris* L. (*Scheuchzeriaceae*) as a threatened species in the Gorbacz Nature Reserve

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Abstract

Scheuchzeria palustris L. occurs in the Północnopodlaska Lowland only at a few sites and it is included among the endangered plant species in Poland. The paper presents results of studies on *Scheuchzeria palustris* population structure in the Gorbacz Nature Reserve (NE Poland). The size of the local population is estimated as several thousand of shoots (a few ares). *Scheuchzeria palustris* shows a distinct response to sunlight deficiency: in full light conditions it grows in large aggregations built by numerous high shoots, while in shaded sites shoots are relatively lower and occur in low density. Shading has a visible influence on the ability to form generative shoots. To protect stands of *Scheuchzeria palustris* against excessive shadowing, the succession process involving the invasion of trees and shrubs into open communities should be curbed. Methods of active protection of the species in this locality are recommended.

Key words: *Scheuchzeria palustris*, population structure, active protection, Lake Gorbacz

1. Introduction

Extinction of components of flora is one of the main problems of nature conservation in Poland. Progressing transformation of the environment has accelerated the process of extinction of species and their populations at the turn of the twentieth and twenty first centuries. The outcome of this process can be found in the regional lists/books of endangered and rare species (Żukowski, Jackowiak 1995; Jakubowska-Gabara, Kucharski 1999; Bernacki et al. 2000; Dajdok 2002; Głowacki et al. 2003; Kącki et al. 2003; Kucharczyk, Szukałowicz 2003; Mirek, Piękoś-Mirkowa 2008; Nowak et al. 2008). Mires are the most valuable areas in respect of preserving biological diversity and simultaneously they are the most threatened by man activities. Any changes in water relations caused by hydrotechnical works or constructions conducted in wetlands catchment areas affect negatively their flora. Adverse changes in habitats hydration are the main reason for the disappearance of species and plant communities of a narrow ecological amplitude, which usually belong to the most valuable elements of the local flora.

In old glacial landscape of Północnopodlaska Lowland, one of the most valuable natural objects is oligotrophic Lake Gorbacz surrounded by a complex of mires. The Gorbacz Nature Reserve is the place of occurrence of rare and endangered plants such as *Scheuchzeria palustris* L. and *Chamaedaphne calyculata* (L.) Moench (Czerwiński 1974; Sokołowski 1975). The main threat to the Gorbacz Nature Reserve is its dehydration, connected with industrial peat mining performed in "Imszar" mine – located only 800 metres west from the lake. Extensive system of deep drainage ditches drains not only the area of the mine but also its surroundings and disturbs the reserve hydrological conditions causing lowering of groundwater level. Desiccation of the peat deposit is manifested as a decreasing water level in the lake and its transformation into mainland (Banaszuk et al. 1994; Baranowski 2002; Zieliński et al. 2011). All communities in the Gorbacz Nature Reserve have suffered some transformations forced by dehydration of sites and numerous fires. In the areas most affected by dehydration in the eastern part of the reserve, there are only secondary and species-poor communities. A few non-forest communities have been undergoing changes in the process of secondary succession. A lot of rare flora species have become extinct (*Betula humilis* Schrank, *Carex chrdorrhiza* L. F., *C. dioica* L., *C. limosa* L.) or the number of their stands has diminished (*Chamaedaphne calyculata*). That is why monitoring of the changes in the population size of the most valuable remaining flora elements is so essential. The objective of the research reported in this paper is to estimate the condition and hazards of

Scheuchzeria palustris populations and recommend methods of active protection of this species and its biotope.

2. Study area

The research was conducted in the Gorbacz Nature Reserve located in Gródek-Michałowo Basin (the eastern part of Białystok Plateau). The basin with the reserve is filled with 5–6 metre thick peat deposits in the gyttja or directly in sands. The lake with the surrounded peat bog is situated in the vicinity of watershed of the rivers Narew and Supraśl. Over 40 hectares of catchment area of the lake are supplied mainly by water of precipitation origin. The peat bog is formed by peat and peat-marshy soils (Banaszuk et al. 1994).

Vegetation around the lake is arranged in zones. The first zone from open water side is formed by rushes and sedge swamps (*Phragmitetea*). On the west side there is sphagnum bog including *Carex rostrata* Stokes. community *Sphagno-Caricetum rostratae* Steffen 1931 em. Dierssen 1982 which occurs in three variants: with domination of *Carex rostata*, *Eriophorum angustifolium* Honck. and *E. vaginatum* L. From the west side the sphagnum bog neighbours the pine and birch brushwood, while from the north and the south it borders with coniferous bog forest *Vaccinio uliginosi-Pinetum* Kleist 1929. Sometimes on the border of two formations a narrow belt of sedge swamp *Caricetum lasiocarpae* Koch 1926 can be found. To the east of the lake the area of the reserve is grown by birch forests and alder forests together with willow bush *Salicetum pentandro-cinereae* (Almq. 1929) Pass. 1961, transformed to a different degree as a result of dehydration of the habitats. Open enclaves that spread between the forests and bushes are occupied by wet meadows and synanthropic herb communities.

Bog habitat degradation connected with draining reclamation is especially visible in the south and the west parts of the reserve adjoining to the areas occupied by “Imszar” peat mine. Extensive system of deep drainage ditches encircling the mining plots and the system of ditches directing water to Julianka and Rudnik watercourses (and further to the Narew River) cause advanced degradation of the habitats on the whole area of raised bog which practically means the area of the whole reserve. The reason for the degenerative changes in vegetation transformations was also the ditch dug along the east border of the reserve in the 1970s and cutting inflow of water flowing from the nearby hull supplying the peat bog and the lake (Baranowski 2002).

3. Object of study

Scheuchzeria palustris is a small geophyte. Its leaved stem, alternating up in the upper part grows to 15–20 cm. Narrow leaves of the same width are usually longer than the stem and they grow up to 40 centimetres. Inflorescence appears in a bunch consisting of 3–10 flowers growing on short peduncle. Inconspicuous flowers are built with six greenish-yellow perianths, not differentiated into calyx and corolla. The fruit of the species is a 5–7 mm long seed pod containing two big seeds. The plant flowers from May to August (Rutkowski 2004). *Scheuchzeria palustris* is wind-pollinated and animal-propagating species. However, the reproduction by seeds is relatively small and mostly through rhizomes.

Rannoch rush is a plant mainly connected with oligotrophic wetlands. It is mostly found on poor fens or raised bogs in sphagnum communities (*Oxycocco-Sphagnetea*). The species can be found only on very acidic peat soils (pH 3.0–4.0) with shallow groundwater. The optimum stage of development it reaches in patches of *Caricetum limosae* Br.-Bl. 1921 (*Scheuchzerio-Caricetea*), whose phytocoenosis develops in the form of a floating quaking bog on the lakeside of dystrophic lakes or after-peat pits forming the first vegetation zone in the vicinity of open waterside (Kucharski, Grzyl 1993; Dajdok 2002; Koczur et al. 2008).

Scheuchzeria palustris has a circumpolar distribution (Zając, Zając 2009). In Poland it can be mostly found in northern and eastern parts, mainly in Pomerania, Mazury Lake District, Polesie Lubelskie and Roztocze. Dispersed sites of Rannoch rush can be found in west Greater Poland, Lower and Upper Silesia, Mazovia, in Sandomierz Basin, rarer in the middle of Poland or in the Sudetes and Carpathians (Kucharski, Grzyl 1993; Zając, Zając 2001). On Północnopodlaska Lowland *Scheuchzeria palustris* can be found only at a few sites: Biebrzańska Valley (Pałczyński 1975; Werpachowski 2000), Knyszyńska Forest and in its vicinity (Karczmarz 1973; Stańko 2010), Gródek-Michałowo Basin (Karczmarz 1973; Czerwiński 1974; Sokołowski 1975).

4. Methods

Two sampling plots (4 m × 4 m quarters) were established in a locality where *Scheuchzeria palustris* could be found (southern part of the reserve). One was situated in an open bog, the other in a position shadowed by *Phragmites australis* (Cav.) Trin. ex Steud. and scattered shrub *Salix aurita* L. All vegetative and generative shoots in both surfaces were counted and measured. In order to determine the spatial structure of the population and the effect of shading on the condition of Rannoch rush, the distribution of shoots was charted against a background of coexisting species: the dominants, trees and bushes. Population study was conducted in 2007. In order to characterize the community in which *Scheuchzeria palustris* can be found, two phytosociological relevés covering the surface of 25 square metres were taken. They were repeated in 2011.

Phytosociological relevés from particular years which are juxtaposed in the table were used for estimation of changes in the floristic composition of the phytocoenoses containing *Scheuchzeria palustris*. The names of vascular plants are given according to Mirek et al. (2002), those of moss species according to Ochyra et al. (2003).

5. Results

Scheuchzeria palustris can be found in the southern part of the Gorbacz Nature Reserve in phytocoenoses of *Sphagno-Caricetum rostratae*. The community makes a narrow (about 50 metres) vegetation zone separating a reed community *Phragmitetum australis* (Gams 1927) Schmale 1939 (from open water side) from a bog coniferous forest *Vaccinio uliginosi-Pinetum* (from mainland site). In the phytocoenosis the dominant species are *Carex rostrata* and bog species such as *Eriophorum vaginatum, Oxycoccus palustris* Pers., *Sphagnum fallax* (H. Klinggr.) H. Klinggr. Closer to the lake reed appears, accompanied by *Pinus sylvestris* L., *Betula pubescens* Ehrh. and *Salix aurita*. *Scheuchzeria palustris* grows both on open bog and in shadowed places. Subsoil consists of strongly water saturated acid peat.

Over the last four years the cover of *Scheuchzeria palustris* has decreased, while that of *Carex rostrata* has increased. No further expansion of reed and bushes has taken place. Floristic composition of the community almost did not change (one new species appeared – *Eriophorum angustifolium*, Tab. 1).

Table 1. Changes in the species composition in *Sphagno-Caricetum rostratae* Steffen 1931 em. Dierssen 1982, A – sunny position, B – shady position

Tabela 1. Zmiany składu florystycznego w zespole *Sphagno-Caricetum rostratae* Steffen 1931 em. Dierssen 1982, A – stanowisko nasłonecznione, B – stanowisko zacienione

	A		B	
Year / Rok	2007	2011	2007	2011
Surface of record / Powierzchnia zdjęcia [m^2]	25	25	25	25
Cover of shrub layer / Pokrycie warstwy krzewów b	-	-	10	10
Cover of herb layer / Pokrycie warstwy ziół c	60	50	90	70
Cover of moss layer / Pokrycie warstwy mchów d	100	95	100	95
Number of species / Liczba gatunków	11	10	13	12
Cl. Oxycocco-Sphagnatea				
Sphagnum fallax	5	5	5	5
Oxycoccus palustris	1	1	3	2
Drosera rotundifolia	1	+	1	1
Eriophorum vaginatum	+	-	1	+
Politrychum strictum	+	+	1	-
Cl. Scheuchzerio-Caricetea				
Scheuchzeria palustris	3	1	2	1
Menyanthes trifoliata	+	-	2	1
Eriophorum angustifolium	-	-	-	+
Carex nigra	-	+	+	-
Cl. Phragmitetea				
Carex rostrata	+	3	2	3
Phragmites austalis	+	+	2	1
Accompanying species / Towarzyszące				
Pinus sylvestris b	-	-	1	1
Pinus sylvestris c	+	+	-	+
Betula pubescens b	-	-	+	+
Betula pubescens c	+	+	+	+
Salix aurita b	-	-	+	1

5.1. Abundance and spatial structure

The *Scheuchzeria palustris* population on the territory of the reserve is large, as regards both the area occupied (few ares) and the number of individuals. All population of the species is estimated as several thousand of shoots. The number of shoots in two sampling plots (32 square metres) reached 1030. The density of population ranges from 8 to 61 shoots/square metre (average 32.3 shoots/m^2). More than half of all shoots are vegetative (56%) and their density reaches 18 shoots/m^2 (generative – 14 shoots/m^2).

Spatial distribution of the population is of scattered and random character. Only in the areas shadowed by bushes, perennial individuals occurred in marginal concentrations of mainly vegetative shoots.

5.2. Size structure

Scheuchzeria palustris is a low perennial and its height does not exceed 50 cm (average 28.3). In the population analysed more than a half of the shoots grew up to 21–30 cm. The least numerous groups were the lowest and the highest shoots (with the height below 20 cm and above 40 cm, Fig. 1). Analogous height layout can be observed both in *Scheuchzeria palustris* growing in an open bog and in shadowed sites (Fig. 2).

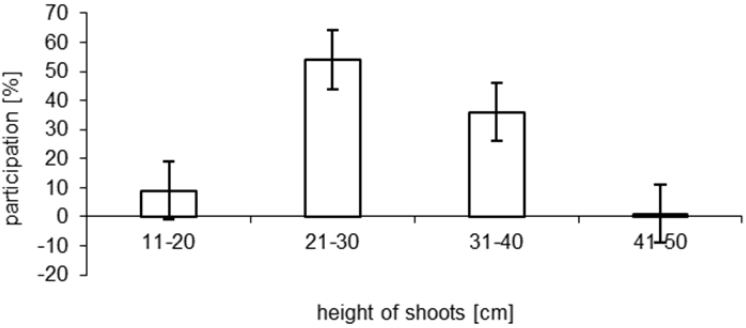

Figure 1. Size structure of shoots in *Scheuchzeria palustris* L. population in the Gorbacz Reserve

Rycina 1. Struktura wielkości pędów w populacji *Scheuchzeria palustris* L. w rezerwacie Gorbacz

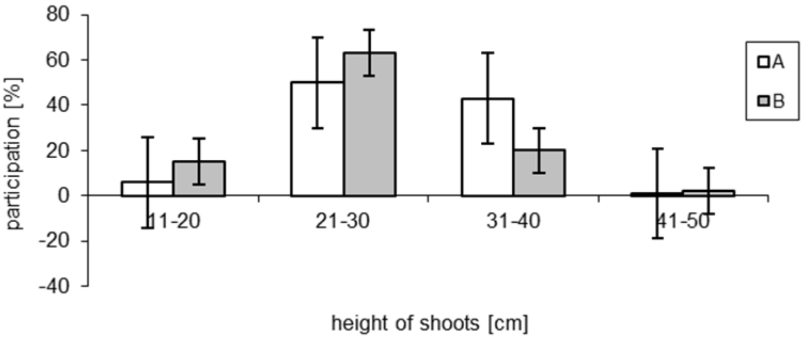

Figure 2. Size structure of shoots in *Scheuchzeria palustris* L. population in various conditions of sun exposure: A – sun exposure, B – shadow of shrubs and *Phragmites australis*

Rycina 2. Struktura wielkości pędów *Scheuchzeria palustris* L. w różnych warunkach nasłonecznienia: A – stanowisko nasłonecznione, B – stanowisko zacienione przez krzewy i trzcinę

5.3. The influence of shading on the structure of the population

In the vicinity of the *Scheuchzeria palustris* locality, a spontaneous secondary succession can be observed, expressed by the expansion of *Phragmites australis* and *Salix aurita*. Pine and European white birch are rarely found in the community. On the quaking bog, bushes can be found sporadically and their total coverage does not exceed 10%. The first rank in succession belongs to common reed which overgrows ¼ of the area studied (in 2011 the participation of this species diminished). In the site shadowed by reed and bushes, *Scheuchzeria palustris* population is less numerous (319 shoots, on average 19.9 shoots/m^2) than in open quaking bog (711 shoots, on average 44.4 shoots/m^2, Fig. 3).

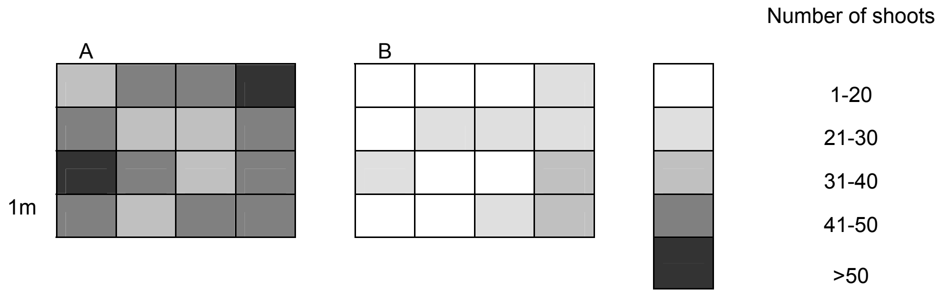

Figure 3. *Scheuchzeria palustris* L. population density in Gorbacz Reserve in the insolated stand (A) and in a shadowed stand (B)

Rycina 3. Zagęszczenie pędów bagnicy torfowej *Scheuchzeria palustris* L. na stanowisku nasłonecznionym (A) i zacienionym (B).

Shading has a distinct influence on the ability to form generative shoots. In full light conditions, the contributions of vegetative and generative shoots and their density were almost the same (22.6 and 21.9 shoots/m^2). In shaded conditions, the contribution of generative shoots (when compared to that of vegetative ones) was twice lower, similarly almost twice lower was the density (13.4 and 6.5 shoots/m^2, Fig. 4). The coverage of the other helophyte *Carex rostrata* increased immensely over the four years of observation, which is an additional factor reducing the growth of *Scheuchzeria palustris* (Tab. 1).

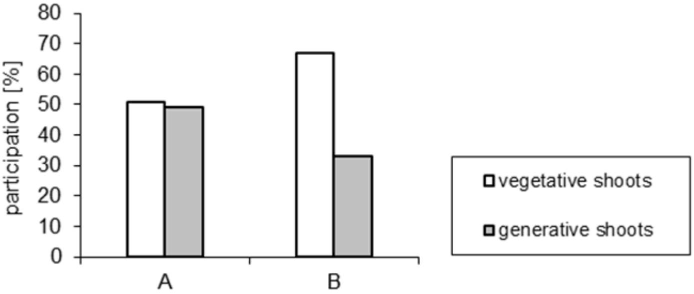

Figure 4. Participation of vegetative and generative *Scheuchzeria palustris* L. shoots growing in various conditions of sun exposure; A – full sun exposure, B – shadow of shrubs and *Phragmites australis*

Rycina 4. Udział pędów wegetatywnych i generatywnych *Scheuchzeria palustris* L. rosnących w różnych warukach nasłonecznienia; A – pełne nasłonecznienie, B – zacienienie przez krzewy i trzcinę

In the site shadowed by reed and bushes, *Scheuchzeria palustris* had smaller height (average height 26.3 cm) than in the insolated site (average height 29.2 cm). In the shadowed quaking bog the number of the lowest shoots (15%) was twice greater, while that of shoots of 31–40 cm in height (20%) was twice lower than in insolated areas. An increased contribution of the highest shoots was also observed. Both vegetative and generative shoots are higher in sites of full insolation. The average height of *Scheuchzeria palustris* vegetative shoots in the open quaking bog reached 29.1 cm and in a shadowed site – 25.5 cm. The average height of generative shoots shows little variation depending on light conditions (28 cm in shadow and 29.4 cm in full light). Relevance of the differences described was confirmed by a statistical test t-Student (at $p<0.05$).

6. Discussion

In Poland *Scheuchzeria palustris* is considered an endangered species (E category, Zarzycki, Szeląg 2006). In Podlaskie Voivodeship it is found in numerous localities mostly in the northern part – Wschodniosuwalskie Lake Distict and Augustowska Plain (Zając, Zając 2001). The further to the south the rarer it is found. The population of *Scheuchzeria palustris* in the Gorbacz Nature Reserve belongs to the greatest and the best preserved in the region and plays the vital role for saving of the species in the area between the Biebrza River and the Bug River.

The number of habitats of *Scheuchzeria palustris* and other rare peat species has decreased as a result of exploitation and drainage of mires. In Pomorze, where *Scheuchzeria palustris* is often found, the number of subfossil sites of this species by a few times outnumbers the number of existing sites (Jasnowska, Jasnowski 1977). The disturbance of hydrological conditions is the reason behind the bush and rush species accelerating succession in the area of mires and it can lead to changes in vegetation formations. Excessive shading of *Scheuchzeria palustris* which is heliophyte, leads to condition impairment or population disappearance.

According to the research conducted in the Gorbacz Nature Reserve, the light accessibility determines not only the species population density but also the size and contribution of generative forms. *Scheuchzeria palustris* growing in the open quaking bog is more numerous (on average 44.4 shoots/m^2), plants are higher (for vegetative shoots the difference is statistically important) and the contribution of generative shoots and their density are twice bigger when compared to those of *Scheuchzeria palustris* growing in the shadowed sites. The species avoids shading especially that caused by bushes. Loosely growing reed is less competitive to this species although when it occurs in greater abundances it can also cause population disappearance (Dajdok 2002; Koczur et al. 2008). The possibility of dispersal of *Scheuchzeria palustris* to larger area is dependent on the accessibility of light. In the Gorbacz Nature Reserve there are plenty of sites offering the optimal light conditions and available for occupancy. Large areas of sphagnum bog which neighbours the lake to the north and west side are potential habitats of this species. A number of factors influencing simultaneously the population of the species studied adds up making a vital threat to preservation of the present locality of this species – proceeding succession of bushes and reed on the open surface of sphagnum bog combined with changes in hydration and fertility of the habitat.

The alarming phenomenon which was noted in the reserve is the disappearance of populations of other valuable species, i.e. lake hydrophytes and rare sedges formerly coexisting with *Scheuchzeria palustris* (*Carex limosa* and *C. chordorrhiza*, Baranowski 2002). Lowering of the level of water has led to replacement of communities of litoral lake zone, primarily built by *Carex limosa* and *Carex lasiocarpa* by aggregation of *Carex rostrata*, *Phragmites australis* or *Typha latifolia*. The share of *Typhetum latifoliae* Soó 1927 in the direct vicinity of the lake has diminished significantly over last years. Disappearance of *Carex limosa* and its replacement in phytoceonosis by *Carex rostrata* is probably correlated with the lake water fertility increase (Gąbka et al. 2007; Zieliński et al. 2011) and transformations of vegetation in coastal zone. The appearance of a competitive helophyte (usually of one species) and thus deposition of large amounts of biomass restricts the growth of plants of the species studied because of a too thick litter layer. In the course of time the dominant slowly recedes and its place is taken by mosses (*Sphagnum sp.*) when the level of water is high or by vascular plants i.e. *Carex rostrata* when the water level is low (Bragazza 2006). .

To protect stands of *Scheuchzeria palustris* against excessive shadowing the succession process involving the invasion of trees and shrubs into open communities should be stopped. Procedures of active protection should include removal of shrubs (willows and birch) in the five-metre-radius around *Scheuchzeria palustris* sites, repeated in three-year-intervals. Such procedures should be conducted during winter with frozen subsoil and thick snow layer. Active protection procedures involving cutting out bushes around *Betula humilis* sites were performed in Wiejki Lake Reserve located nearby on the territory of Gródek-Michałowo Basin. Usually abandonment of such active measures would result in disappearance of *Salix lapponum* L. in a given area (Kołos, Tarasewicz 2005)..

Radical action should be performed in the reserve, aimed at protection of water relations of Lake Gorbacz and surrounding mires. It is necessary to block water outflow made by drainage ditches and especially by the main ditch running along the east border of the preserve and its branches, which was already postulated in the 1990s (Banaszuk et al. 1994). Another recommendation is control of the condition of technical state of blockades made once on drainage ditches situated on the territory of the Imszar peat mine, as they had been damaged many times in the past (Baranowski 2002). Enlargement of the reserve by new areas located to the west and south of the lake will allow hydrotechnical procedures (including building penstocks and maintaining blockades) in ditches draining the bog in the west part of the reserve. The fact of great significance is that the ditches collecting water from exploitative fields of peat mine and Julianka watercourse contribute to bog deterioration in the southern part of the reserve, where *Scheuchzeria palustris* population is located. Only total abandonment of peat excavation (mine closing) and reclamation of the whole after-exploitative area will allow preservation of Lake Gorbacz in Gródek-Michałowo Basin.

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Bagnica torfowa *Scheuchzeria palustris* L. (Scheuchzeriaceae) zagrożona wyginięciem w rezerwacie Gorbacz

Streszczenie

Bagnica torfowa *Scheuchzeria palustris* L. występuje w południowej części rezerwatu Gorbacz na ple jeziornym w płatach zespołu *Sphagno-Caricetum rostratae*. Zbiorowisko tworzy wąski około 50. metrowy pas roślinności rozdzielający szuwar trzcinowy (od strony toni wodnej) i bór bagienny (od strony lądu). W pobliżu jeziora w zbiorowisku pojawia się trzcina, której miejscami towarzyszą sosna, brzoza omszona i wierzba uszata. Bagnica rośnie na otwartym ple, jak również w miejscach zacienionych. Populacja bagnicy torfowej na terenie rezerwatu jest duża, zarówno pod względem zajmowanej powierzchni (kilka arów), jak i liczebności

(kilka-kilkanaście tysięcy pędów), tym samym należy do największych i najlepiej zachowanych w regionie i pełni kluczową rolę dla zachowania gatunku na Nizinie Północnopodlaskiej.

W ciągu czterech lat (2007–2011) wyraźnie zmniejszyło się w zbiorowisku pokrycie bagnicy torfowej i znacznie wzrosło pokrycie turzycy dzióbkowatej. Nie nastąpiła dalsza ekspansja trzciny i krzewów, a skład florystyczny zbiorowiska prawie się nie zmienił.

Badania przeprowadzone w rezerwacie Gorbacz wskazują, że dostępność światła jest czynnikiem determinującym nie tylko zagęszczenie, ale również wysokość pędów i udział form generatywnych. Bagnica rosnąca na otwartym ple występuje bardziej licznie, rośliny są wyższe (w przypadku pędów wegetatywnych jest to różnica istotna statystycznie), a udział pędów generatywnych i ich zagęszczenie jest dwukrotnie większe niż bagnicy rosnącej na stanowisku zacienionym. Roślina unika zacienienia pochodzącego zwłaszcza od krzewów. Luźno rosnąca trzcina jest dla gatunku znacznie mniej konkurencyjna. Istotne zagrożenie dla utrzymania obecnego stanowiska stwarza kilka czynników oddziałujących na populację jednocześnie – postępująca sukcesja krzewów i trzciny na otwartą powierzchnię mszaru połączona ze zmianą uwodnienia i trofizmu siedliska, o czym świadczy zwiększony udział *Carex rostrata* w zbiorowisku.

Aby ochronić stanowisko *Scheuchzeria palustris* przed nadmiernym zacienieniem należy zatrzymać proces sukcesji polegający na wkraczaniu drzew i krzewów do otwartych zbiorowisk. Zabiegi ochrony czynnej powinny polegać na usunięciu krzewów i drzew (wierzb i brzóz) w promieniu 5 m wokół stanowiska bagnicy i powinny być powtarzane co 3–4 lata. W rezerwacie należy podjąć radykalne działania mające na celu ochronę stosunków wodnych jeziora Gorbacz i torfowisk otaczających akwen.

Population history and genetic variation of *Betula humilis* Schrk. in Poland

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Abstract

The shrub birch *Betula humilis* is widely distributed in eastern Europe, but it is an endangered glacial relict in the central part of the continent. The main factors responsible for the shrub birch population decline are: drainage of peatlands, intensive use of meadows and overgrowing by forest and brushwood vegetation. Polish populations of the shrub birch make the south-western margin of its continuous range. The population history of *B. humilis* on the territory of Poland dates back to almost a million years ago, because the oldest fossil shrub birch remains in Poland are dated to the Augustovian interglacial. *B. humilis* appeared later during the Mazovian and Eemian interglacials, and it is possible that the species survived the Vistulian glaciation in the Carpathians and their northern foreland. Palaeobotanical studies suggested that postglacial expansion has rapidly gone, which was confirmed by chloroplast DNA analysis. Generally, nuclear microsatellite investigation has shown relatively high genetic variation in most of the shrub birch populations. The maintenance of substantial genetic variation in the fragmented distribution of *B. humilis* in Poland could be explained by either too short time for the genetic decline to occur or effective generative reproduction. The substantial genetic diversity in north-eastern Polish localities is likely a consequence of admixture of phylogenetic lineages. However, in the smallest and most isolated localities of *B. humilis,* the process of genetic erosion and differentiation has started. The chromosome analysis has revealed a high contribution of aneuploid individuals in six populations. Aneuploidy could result from hybridisation with closely related species.

Key words: cpDNA, habitat fragmentation, nuclear microsatellite, palaeobotany, suture zone

1. Introduction

The shrub birch *Betula humilis* Schrk. is a cold-tolerant plant occurring in eastern Europe, Siberia and north-western Mongolia. The species reaches the south-western border of its continuous European range in Poland, but some remnant populations still exist in the Alps, the Carpathians, and in northern Germany, suggesting that *B. humilis* had been more widespread in the past (Hultén, Fries 1986; Załuski et al. 2001). Polish populations of the shrub birch are grouped mainly in the West Pomerania, Masurian Lakeland, Podlasie and Polesie regions (Fig. 1). The shrub birch is recognized as a glacial relict in central Europe.

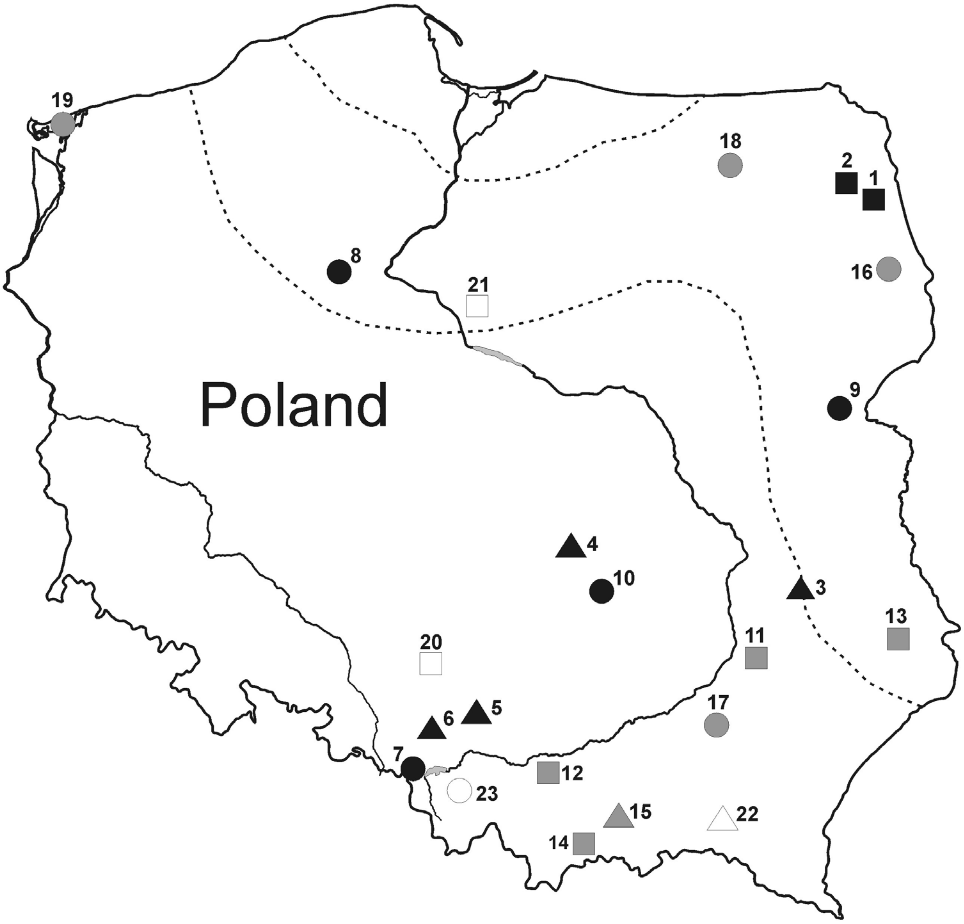

Figure 1. Present range and location of historic populations of *B. humilis* in Poland. Black squares – the Augustovian interglacial sites, black triangles – the Mazovian interglacial sites, black circles – the Eemian interglacial sites, grey squares – the Early Vistulian sites, grey triangle – the Middle Pleniglacial site, grey circles – the Allerød sites, open squares – the Younger Dryas sites, open triangle – the Preboreal site, open circle – the Boreal site. Dashed lines indicate the present range of *B. humilis* in Poland.

Populations: 1 – Czarnucha, 2 – Żarnowo (Stachowicz-Rybka 2011), 3 – Nowiny Żukowskie (Velichkevich, Mamakowa 2003), 4 – Olszewice, 5 – Stanowice, 6 – Gościęcin (Velichkevich et al. 2004), 7 – Golasowice (Granoszewski 1993), 8 – Nakło (Noryśkiewicz 1978), 9 – Horoszki Duże (Velichkevich, Granoszewski 1996), 10 – Bedlno (Velichkevich et al. 2005), 11 – Łążek, 12 – Ściejowice, 13 – Tarzymiechy, 14 – Brzeziny, 15 – Dobra (Velichkevich, Mamakowa 1999), 16 – Taboły mire (Drzymulska 2010), 17 – Rzochów (Velichkevich, Mamakowa 1999), 18 – Miłkowskie Lake (Wacnik 2009), 19 – Wolin Island (Latałowa 1999), 20 – Jezioro Lake (Nita, Szymczyk 2010), 21 – Steklin Lake (Noryśkiewicz 1982), 22 – Jasło (Harmata 1995), 23 – Zapadź peat-bog (Łopatka, Gałka 2009).

Rycina 1. Obecny zasięg występowania i lokalizacja historycznych populacji *B. humilis* w Polsce. Czarne kwadraty – populacje z okresu interglacjału augustowskiego, czarne trójkąty – populacje z okresu interglacjału mazowieckiego, czarne kółka – populacje z okresu interglacjału eemskiego, szare kwadraty – populacje z okresu wczesnego zlodowacenia Wisły, szary trójkąt – populacja ze środkowego pleniglacjału, szare kółka – populacje z interstadiału Allerød, białe kwadraty – populacje ze stadiału Młodszy Dryas, biały trójkąt – populacja z okresu preborealnego holocenu, białe kółko – populacja z okresu borealnego holocenu. Linie przerywane oznaczają obecny obszar występowania *B. humilis* w Polsce.

Populacje: 1 – Czarnucha, 2 – Żarnowo (Stachowicz-Rybka 2011), 3 – Nowiny Żukowskie (Velichkevich, Mamakowa 2003), 4 – Olszewice, 5 – Stanowice, 6 – Gościęcin (Velichkevich et al. 2004), 7 – Golasowice (Granoszewski 1993), 8 – Nakło (Noryśkiewicz 1978), 9 – Horoszki Duże (Velichkevich, Granoszewski 1996), 10 – Bedlno (Velichkevich et al. 2005), 11 – Łążek, 12 – Ściejowice, 13 – Tarzymiechy, 14 – Brzeziny, 15 – Dobra (Velichkevich, Mamakowa 1999), 16 – Taboły mire (Drzymulska 2010), 17 – Rzochów (Velichkevich, Mamakowa 1999), 18 – Miłkowskie Lake (Wacnik 2009), 19 – Wolin Island (Latałowa 1999), 20 – Jezioro Lake (Nita, Szymczyk 2010), 21 – Steklin Lake (Noryśkiewicz 1982), 22 – Jasło (Harmata 1995), 23 – Zapadź peat-bog (Łopatka, Gałka 2009).

B. humilis is much-branched shrub with dark-brown bark. Young shoots are downy and covered with numerous white, resin glands (Kłosowski, Kłosowski 2001). Like other birches, this plant is very sensitive to light conditions and its growth depends on the intensity of solar radiation. Typically, the shrub birch is not higher than 1–2 m, but it can reach a height of 3–4 m when growing on heavily shaded position (Matowicka, Jabłońska 2008). The shape and size of *B. humilis* leaves depend on both the kind of shoots at which leaves develop (fruiting short, vegetative short and long shoots) and light conditions (Staszkiewicz et al. 1991a; Jabłońska 2009). The influence of hybridisation with closely related taxa on morphological variation of the shrub birch cannot be excluded, either (Staszkiewicz et al. 1993b; Jabłońska 2009). The most common are ovate or obovate leaves with cuneate or rounded base, acute or subacute apex and serrate or crenate margin (Staszkiewicz et al. 1991a). The morphological variation of the shrub birch resulted in recognition of many intraspecific varieties in the past; however, detailed biometric analyses of leaves, fruits and scales showed continuous variability of characters and no correlation between variability of the leaves and generative organs (Staszkiewicz et al. 1991a, b; 1993a). Morphometric analyses also contradicted that the intraspecific variation of *B. humilis* leaves could result from different ground conditions (Staszkiewicz et al. 1991a).

The shrub birch is a monoecious, wind-pollinated and wind-dispersed species. *B. humilis* flowers form catkin (inflorescences) sets on very short petioles (Staszkiewicz et al. 1991b). Male catkins are placed apically on long shoots, female inflorescences develop on short generative shoots (Kłosowski, Kłosowski 2001). The male catkins develop between July and September, but pollen is released during the following April or May. The female flowers overwinter as primordia and appear at the time of bud burst. Fertilization and development of seeds take place in the second summer followed by autumn and winter seed fall.

B. humilis is associated with different kinds of habitats: natural and drained fens, transitional mires and wet meadows. The populations of the shrub birch inhabiting natural fens in north-eastern Poland and drained fens in south-eastern Poland are comparatively abundant. Detailed studies on the hydrology and chemistry of different environments populated by *B. humilis* have revealed that the main factors influencing the abundance of the plant are low $P\text{-}PO_4^{3-}$ concentration and water level equal to the ground level (Jabłońska 2006). On calcium-rich soils in south-eastern Poland, the $P\text{-}PO_4^{3-}$ concentration is low due to the absorption of phosphate by Ca-hydroxides (Jabłońska 2006). In these conditions, the development of competitive species such as tall sedges, reeds and trees is inhibited, and then the shrub birch can spread (Jabłońska 2009). A high enough water level also prevents the overgrowing of fens with *B. humilis* by forest plants (Jabłońska 2009).

At present, about 70 localities with shrub birch are known in Poland, but their number was five times greater at the beginning of 20th century (Załuski et al. 2001). The abundance of *B. humilis* has decreased at an alarming rate in central Europe due to the lowering of groundwater levels and a decline in the use of wet meadows, hence the species has been classified as endangered (EN) in Red Data Books of Plants in Poland, Germany, Ukraine and the Kaliningrad Oblast of north-western Russia (Załuski et al. 2001). The declining of shrub birch populations is distressing, because its natural habitats – peatlands are often formed by relatively low number of species; thus, extinction of one species could result in irreversible changes in the environment (Minayeva et al. 2009). Undisturbed peatlands are extremely important terrestrial ecosystems, because they mitigate the global climate via absorbing carbon; however, they are the most threatened ones. The above reason is a strong argument for urgent protection of *B. humilis*. To protect endangered species, it is necessary to estimate their genetic diversity within populations and genetic differentiation among populations (Ellstrand, Elam 1993). Thus, genetic studies have been carried out in Polish localities of *B. humilis* for several years in order to establish which populations represented the bulk of the species' genetic resources as they are of the highest conservation priority. In the present paper, a synthesis of genetic diversity research conducted within and between Polish populations of the shrub birch as well as population history of the species are reviewed.

2. Genetic variation of Polish populations of *B. humilis*

The central–marginal model predicts that the most of genetic diversity is concentrated in the centre of the species range, where a mosaic of favourable habitats allows the existence of many different genotypes (Eckert et al. 2008). On the other hand, the genetic drift and limited gene flow from the core populations reduce genetic diversity at the margins of the species range (Eckert et al. 2008). On the basis of the central-marginal model, it was expected that the level of genetic variation should be lowered in marginal south-western localities of *B. humilis* in Poland when compared to that in the subcentral populations in Belarus (Jadwiszczak et al. 2011a). However, nuclear microsatellite investigation conducted in 16 Polish populations of *B. humilis* revealed relatively substantial genetic variation in most of the localities studied (Jadwiszczak et al. 2011a, b). Parameters of genetic diversity (mean number of alleles per locus $A = 8.2$, mean observed heterozygosity $H_O = 0.60$, mean expected heterozygosity $H_E = 0.65$) at the *B. humilis* range edge were the same as in the subcentral populations of the species (Jadwiszczak et al. 2011a) and in the long-lived, widespread perennials (Nybom 2004).

The maintenance of quite a substantial level of genetic variation in the fragmented distribution of the shrub birch in Poland could be explained in two ways. The first is that the fragmentation process is too recent in order to influence the genetic variation in *B. humilis* populations (Jadwiszczak et al. 2011b). Disappearance of the shrub birch has been noted for several decades in Poland (Załuski et al. 2001); thus, this period of time may be too short for genetic erosion, because

B. humilis can reach 20 years of age. The second explanation is that the outcrossing breeding system could impede the negative consequences of small population sizes and reduced gene flow (Jadwiszczak et al. 2011b). Nuclear microsatellite studies carried out in the Polish populations of *Betula nana* located in the Sudety Mts. showed considerably lower values of genetic diversity compared to *B. humilis*, which could result, among other reasons, from the lack of generative reproduction of *B. nana* individuals (Jadwiszczak et al. 2012b).

The highest molecular variation was observed in some populations of the shrub birch situated in north-eastern Poland. These were Czerwone Bagno and Szuszalewo in the Biebrza National Park, Magdzie Bagno in the Suwałki Lake District, and one locality from the Białowieża National Park (Jadwiszczak et al. 2011a, b; 2012a). This result was surprising because the Magdzie Bagno locality is placed in the area covered by the ice-sheet during the Last Glacial Maximum (LGM), and other populations occupied the territory which was in very close proximity to the front of the Vistulian glacier. Taking into account the locations of Czerwone Bagno, Szuszalewo, Magdzie Bagno and the Białowieża National Park, one could suppose that these populations have appeared not earlier than at the Late Glacial. Generally, the areas recolonised after the retreat of ice sheet are expected to represent low genetic variation in relation to the old refugial populations, as the effect of repeated founding events during postglacial range expansion (Hampe, Petit 2005). Jadwiszczak et al. (2011a) supposed that substantial genetic variation in *B. humilis* populations situated in north-eastern Poland could result from a rapid recolonisation process starting at high latitude glacial refugia and the admixture of phylogenetic lineages. Genetic evidence supporting the existence of glacial isolates at higher latitudes is the lack of isolation by distance (isolation by distance – geographically adjacent populations are genetically more similar than distant populations). Both molecular markers, nuclear microsatellites and chloroplast DNA (cpDNA), exhibited no isolation by distance among the shrub birch populations, which supports the idea of *B. humilis* survival in central Europe (Jadwiszczak et al. 2011a, b; 2012a).

Analysis of cpDNA variation indicated that two phylogenetic lineages of *B. humilis* had mixed in the territory of Poland, thus the high genetic diversity in north-eastern Poland is also likely to be a consequence of suture zone formation (Jadwiszczak et al. 2012a). The suture zone is an area where waves of migrations from distinct glacial refugia came into the contact. High haplotypic richness (which is equivalent to v_T) accompanied by $h_T \leq v_T$ (h_T – total diversity) is the evidence of the suture zone (Petit et al. 2002; Provan, Bennett 2008). In northern and north-eastern Polish populations of the shrub birch, the high haplotypic richness was noted and the value of v_T was slightly higher than h_T, which strongly suggests that the shrub birch suture zone is spread through the country (Jadwiszczak et al. 2012a). Moreover, within the area studied, haplotypic diversity was related to both phylogenetic lineages, which is additional proof for the suture zone concept (Jadwiszczak et al. 2012a). In refugial populations, a high haplotypic variation is confined to one lineage only (Petit et al. 2002).

Unfortunately, not all populations of the shrub birch have retained a high level of genetic variation. In the most isolated and the smallest populations – Góra Perkuć in north-eastern Poland and Jezioro Mętne in northern part of the country, lower values of genetic diversity parameters were noted (Jadwiszczak et al. 2011a, b). In the same time, the pairwise comparisons of genetic differentiation between populations (F_{ST}) showed statistically significant differences between Jezioro Mętne and Torfowisko Mieleńskie localities from northern Poland and all other populations (Jadwiszczak et al. 2011a). Such a result strongly implies that low number of individuals and limited gene flow can induce genetic differentiation.

Hybridisation with congeneric species is another threat to the persistence of *B. humilis.* The hybridisation is a commonly observed phenomenon in the genus *Betula*, and it was also suggested for *B. humilis* (Staszkiewicz et al. 1991a; 1993b). Morphometric analyses revealed that ca. 45% of individuals in *B. humilis* populations were hybrids and introgressive forms (Staszkiewicz et al. 1993b). Cytogenetical investigation conducted in six Polish populations of the species showed 19–60% of aneuploid individuals, whereas typical karyotype of *B. humilis* is diploid (2n = 28) (Jadwiszczak et al. 2011c). Jadwiszczak et al. (2011c) suspected that aneuploidy could result from hybridisation of the shrub birch with congeneric species. It seems likely that, due to a change of selection pressure in fragmented south-western part of the shrub birch range, atypical karyotypes of aneuploids could be preferred leading to the expansion of hybrids. It was found that the triploid interspecies hybrids between *Betula pubescens* and *B. nana* often occupied edges of birch populations, which makes them successful pioneer individuals (Anamthawat-Jónsson, Thórsson 2003). Extensive hybridisation in *B. humilis* populations was also revealed by molecular studies. On the one hand, the analysis of molecular variance (AMOVA) showed no genetic differences between the shrub birch and congeneric trees (F_{CT} = –0.031, P = 0.563) indicating no diagnostic molecular marker for the shrub birch (Jadwiszczak et al. 2012a). Hybridisation between *B. humilis* and the tree birches was evidenced by the value of introgression ratio (IG = 0.71), which confirmed a considerable interspecies gene flow (Jadwiszczak et al. 2012a). The introgression ratio reflects the proportion of locally shared haplotypes.

On the basis of the results of molecular investigations, Jadwiszczak et al. (2011a; 2012a) suggested that the shrub birch populations from northern and north-eastern Poland represented the highest conservation worth as they are the most genetically variable among all Polish localities. The northern populations are the most unique as they comprise specific cpDNA haplotypes and particular frequency of genotypes, which make them significantly different from other populations of the species (Jadwiszczak et al. 2011a; 2012a).

3. Population history of *B. humilis* inferred from palaeobotanical and molecular studies

The Pleistocene stratigraphic scheme of Poland includes eight glaciations (Narevian, Nidanian, Sanian 1, Sanian 2, Livecian, Krznanian, Wartanian, Vistulian; Lindner et al. 2004) and seven interglacial periods (Augustovian, Małopolanian, Ferdynandovian, Mazovian, Zbójnian, Lubavian, Eemian; Ber 2005). A treeless tundra and park tundra containing birch species developed in the periglacial zone of the Scandinavian ice-sheets during the last glaciation. Inferences on the possible glacial refugia and routes of recolonisation of birches after climate warming are difficult and depend mainly on the availability of suitable palaeoecological data (Lascoux et al. 2004). Description of glacial and postglacial history looks to be especially difficult for *B. humilis*, the species with scarce macrofossils and great morphological similarity of scales and pollen grains to these of *B. nana* (Staszkiewicz et al. 1991b; Blyakharchuk et al. 2004). However, despite of the difficulties in identifying the fossil remains of the shrub birch, the evidence found at Czarnucha and Żarnowo localities (north-eastern Poland; Fig. 1) and dated to the Augustovian interglaciation (Stachowicz-Rybka 2011) revealed that the population history of *B. humilis* on the territory of Poland dates back to almost a million years ago. The species is recognized in several interglacial assemblages because its appearance was associated with climate warming (Środoń 1979). At the Czarnucha and Żarnowo localities the shrub birch survived till the Nidanian glaciation (Stachowicz-Rybka 2011). Further chronological data came from eastern (Nowiny Żukowskie site; Velichkevich, Mamakowa 2003) and southern (Olszewice, Stanowice, Gościęcin; Velichkevich et al. 2004) Poland and refer to the Mazovian interglacial (Fig. 1). During the Eemian interglacial period, *B. humilis* occurred in northern (Nakło on the river Noteć locality; Noryśkiewicz 1978), eastern (Horoszki Duże; Velichkevich, Granoszewski 1996) and southern (Golasowice, Bedlno; Granoszewski 1993; Velichkevich et al. 2005) parts of the country. At the Horoszki Duże site, the species was also revealed in the Early Vistulian deposits (Velichkevich, Granoszewski 1996). Moreover, the Early Vistulian records of the shrub birch were found at Łążek and Tarzymiechy sites (eastern Poland) as well as in Ściejowice and Brzeziny (southern Poland; Fig. 1) (Velichkevich, Mamakowa 1999).

It is likely that *B. humilis* survived LGM in the Carpathians and their northern foreland (Ralska-Jasiewiczowa et al. 2004). This supposition could be deduced on the basis of the presence of the shrub birch fruits and scales dated to the Middle Pleniglacial at the Dobra locality (Velichkevich, Mamakowa 1999). It was hypothesised that another glacial refugium of *B. humilis* could exist in the Polesie region in south-eastern Poland (Środoń 1979), but no palaeobotanical evidence was found to prove continuous *in situ* existence of the species there (Mamakowa, Latałowa 2003). To test the hypothesis about glacial isolate in south-eastern Poland during the Vistulian, nuclear microsatellite and cpDNA analyses were conducted (Jadwiszczak et al. 2011a, 2012a). Nuclear microsatellite investigation revealed ca. 95% of genetic variation within the populations and 4% only at the interpopulation level (Jadwiszczak et al. 2011a). This result is opposed to the distribution of genetic diversity in the area of a putative refugium, because populations inhabiting glacial isolates usually show low genetic variation at the population level and disproportionally high levels of regional genetic differentiation (Hampe, Petit 2005). Analysis of cpDNA also contradicts the thesis that the shrub birch could have populated the Polesie region during LGM. Only two cpDNA haplotypes were detected in south-eastern Poland, which resulted in a low value of genetic differentiation among populations ($G_{ST} = 0.281$; Jadwiszczak et al. 2012a). Potential shrub birch populations from refugial areas should be characterised by many different haplotypes, and hence by high G_{ST} parameters. This is because the glacial populations are much older than those occupying the newly colonised regions, and selection for local adaptations in association with reduced gene flow could have led to high haplotypic richness (Hampe, Petit 2005; and references therein). For example, 12 cpDNA haplotypes were found in each of the south European glacial refugia of *Quercus* species – the north Balkans and the Iberian Peninsula, accompanied by high measures of G_{ST} – 0.773 and 0.889, respectively (Petit et al. 2002).

Comparison of cpDNA haplotypes within the marginal Polish and subcentral Belarusian populations of *B. humilis* showed significantly higher value of N_{ST} [differentiation among populations for ordered alleles (both the frequency of haplotypes and number of mutation between particular haplotypes are taken into account)] than G_{ST} [differentiation among populations for unordered alleles (the frequency of particular haplotype is considered only)] (Jadwiszczak et al. 2012a). $N_{ST} > G_{ST}$ indicates a phylogeographic structure in the study area, which could be a consequence of recolonisation from distinct refugia (Pons, Petit 1996; Fussi et al.

2010). Therefore, the molecular studies suggested that Poland was recolonised by the shrub birch from at least two disconnected glacial isolates at the end of the Vistulian glaciation (Jadwiszczak et al. 2012a).

It is likely that the recolonisation could proceed rapidly, because *B. humilis* pollen and macrofossils were found on the Wolin Island (the most north-western margin of Poland; Fig. 1) as early as in the Allerød interglacial period of the Late Vistulian (Latałowa 1999). During the Allerød, the species populated also north-eastern (Miłkowskie Lake; Wacnik 2009; the Taboły mire; Drzymulska 2010) and south-eastern (Rzochów; Velichkevich, Mamakowa 1999) parts of Poland. Since the Younger Dryas stadial of the Late Glacial to the Atlantic period of the Holocene, *B. humilis* has grown in southern Poland (Jezioro Lake; Nita, Szymczyk 2010). The shrub birch was also present in the Steklin Lake surroundings in northern Poland in the Younger Dryas (Noryśkiewicz 1982). The oldest Holocene records are referred to the Preboreal (Jasło site in southern Poland; Harmata 1995) and Boreal periods ("Zapadź" peat-bog in the southern part of the country; Łopatka, Gałka 2009) (Fig. 1).

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Historia populacji i zmienność genetyczna *Betula humilis* Schrk. w Polsce

Streszczenie

Brzoza niska *Betula humilis* Schrk. jest gatunkiem szeroko rozprzestrzenionym w Europie Wschodniej, ale zagrożonym wyginięciem w centralnej części kontynentu. Ginięcie tego gatunku związane jest z: osuszaniem torfowisk, nieprawidłowym użytkowaniem łąk oraz zarastaniem przez roślinność leśną i zaroślową. Polskie populacje brzozy niskiej stanowią południowo-zachodni kraniec ciągłego zasięgu gatunku w Europie. Historia populacji *B. humilis* na obszarze Polski sięga prawie miliona lat wstecz, gdyż najstarsze szczątki kopalne tego gatunku są datowane na interglacjał augustowski. Brzoza niska pojawiała się także później, w czasie integlacjałów mazowieckiego i eemskiego. Prawdopodobnie gatunek ten mógł przetrwać zlodowacenie Wisły m.in. w polskiej części Karpat i na ich przedgórzu. Badania z zakresu paleobotaniki sugerują, że rekolonizacja brzozy niskiej mogła zachodzić gwałtownie, co zostało potwierdzone przez analizy chloroplastowego DNA.

Generalnie, analiza loci jądrowego mikrosatelitarnego DNA wykazała stosunkowo wysoki poziom zmienności genetycznej w polskich populacjach *B. humilis*. Utrzymywanie się tej zmienności w coraz bardziej pofragmentowanej części zasięgu może wynikać ze zbyt krótkiego czasu jaki minął od początku procesu zanikania populacji oraz z efektywnego rozrodu generatywnego. Znaczący poziom zmienności genetycznej brzozy niskiej w północno-wschodniej Polsce jest prawdopodobnie następstwem zmieszania się różnych linii filogenetycznych. Jednak nie wszystkie polskie populacje charakteryzują się wysoką zmiennością genetyczną. W najmniejszych liczebnie i najbardziej izolowanych populacjach *B. humilis* rozpoczął się proces utraty zmienności i ich różnicowania. Analiza chromosomowa, przeprowadzona w sześciu populacjach wykazała, że niektóre osobniki są aneuploidalne. Aneuploidia może być następstwem hybrydyzacji brzozy niskiej z blisko spokrewnionymi gatunkami.

Changes in flora and vegetation of the Knyszynska Forest mires since the last glaciation

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Abstract

Mires are excellent source of information about past vegetation. One of the territories very rich in mires is the Knyszyńska Forest, located at the North Podlasie Lowland. Analysis of plant macroscopic remains contained in peat and lacustrine sediments has permitted identification of plant species present there in the past but absent nowadays. They included *Cladium mariscus*, *Betula nana*, *Scheuchzeria palustris*, a few species of *Potamogeton*, and such mosses like *Meesia triquetra* and *Scorpidium scrorpioides*. Subfossil plant communities were reconstructed, some of them are no longer met in Poland, like *Menyantho trifoliatae-Sphagnetum teretis*, *Sphagnetum betulo-pinosum eriophoreto fruticuletosum*, *Caricetum rostratae sphagnetosum fallacis*, community of *Scorpidium scorpioides*, and sedge-brown moss and brown moss community with scrubby birches. These phytocenoses occur nowadays in Western Europe or/and in North-Western Russia and Western Siberia.

Key words: Late Glacial, Holocene, peat, plant macrofossil remains, subfossil plant communities

1. Introduction

Mires make one of the most valuable components of landscape. They are often preserved in almost undisturbed state, being refuges of anthropopressure-sensitive species. Their role in water retention, extension of the water outflow time and maintenance of high level of surface and underground water table has been well established (Okruszko 1995). Much less attention has been devoted to mires as the sources of information on changes in the vegetation and environment in the past. However, recently this role of mires has begun to be appreciated by both naturalist, archaeologists and historians (Tobolski 2000). Biogenic sediments making peat beds, first of all peat, are one of the most important archives of data on the history of the natural environment. Peat archives contain micro- and macrofossils which give a comprehensive picture of peatland development (Rydin, Jeglum 2008). They provide information on autogenic and allogenic changes in peatlands (Charman 2002), which permits reconstruction of subfossil plant communities that had occurred in a given area. Sometimes the phytocenoses discovered are no longer represented as the climate changes that took place after the last glaciation, in the Holocene, had forced the shifts of certain plant communities from the south to the north and from the west to the east.

The area of our study was the Knyszyńska Forest, which is characterised by a high, close to 10% contribution of wetlands (Okruszko 1995). A few mires from this area (Machnacz, Stare Biele, Taboły, Borki, Kładkowe Bagno) were subjected to paleoecological study by the methods of pollen analysis and macroscopic plant remains analysis (Dembek 1989; Żurek 1992; Kupryjanowicz 2000; 2004; Drzymulska 2006; 2008; 2011).

In this paper we concentrate on plant species and communities recognized in the history of some mires in the past but not present contemporarily. We want to show when and in which conditions they functioned. We also try to connect subfossil communities with contemporary phytocoenoses known from different regions of Europe.

2. Study site

According to physical-geographical division of Poland (Kondracki 1998), the Knyszyńska Forest is located in the eastern part of the North Podlasie Lowland. The relief of the area was formed by the Saalian glaciation (Mojski 1972; Musiał

1992) that produced a number of fluvioglacial features, including kames, kame terraces and numerous melt water forms. During the last glaciation this territory was located in the periglacial zone. Ice-front of the Vistula glaciation was situated only 60 km north from Białystok (Pawłowska, Miodek 1993).

Climate of the Knyszyńska Forest is temperate transitional (Górniak 1999). Its most characteristic features are: long winter (110 days), long summer (90 days), and shorter, than in central and western Poland, spring and autumn. Mean annual temperature achieves only 7°C, but the annual amplitude of temperatures achieves even 22°C. Annual precipitation is 570 mm (mean value for 1988–1992) (Sasinowski 1995).

In accordance with geobotanical position of the Knyszyńska Forest (Matuszkiewicz 2008), the most characteristic features of this area are the dominance of spruce, the lack of beech in tree stand, and the presence of numerous boreal species. In total, 837 species of vascular plants (Sokołowski 1995), 179 species of bryophytes (Karczmarz, Sokołowski 1995) and 341 species of lichens (Bystrek, Kolanko 2000) have been described. Many of them are protected. About 80% of the Knyszyńska Forest area is covered by forests. *Pinus sylvestris* L. and *Picea abies* (L.) Karsten are basic species there (Żarska 1993).

The Knyszyńska Forest is one of the most valuable forest complexes in Poland. The Landscape Park named after Prof. Witold Sławiński was established there in 1988. The area of the Park is 73 095 ha, and 50 405 ha is occupied by protected zone (Fig. 1).

3. Methods

Macroscopic plant remains analysis (vegetative and generative remains) was performed in the samples of biogenic sediments (peat and gyttja) collected from Taboły, Kładkowe Bagno and Borki mires (Fig. 1).

Details of field and laboratory works have been described by Drzymulska in other works (2006; 2008; 2011).

The plant remains were identified with the help of Mauquoy, van Geel (2007), Hedenäs (2003), Rybníček, Rybníčková (1974), Katz et al. (1965) and the collection of macroscopic plant remains at the Institute of Biology, University of Białystok, and at the Institute of Botany, Polish Academy of Sciences in Kraków. Recognition of botanical composition of peat and gyttja allowed reconstruction of plant subfossil communities which occurred in different periods of the mires history. Identification of subfossil syntaxa was based on a combination of plant remains. The criteria established for contemporary plant phytocoenology were adapted after (Oświt 1973, Pałczyński 1975). In this reconstruction we made references to contemporary communities of Central, Western and Eastern Europe (Matuszkiewicz 2001; Dierssen 1982; Rybniček 1973; Liss, Bjerjesina 1981; Botsch, Smagin 1993).

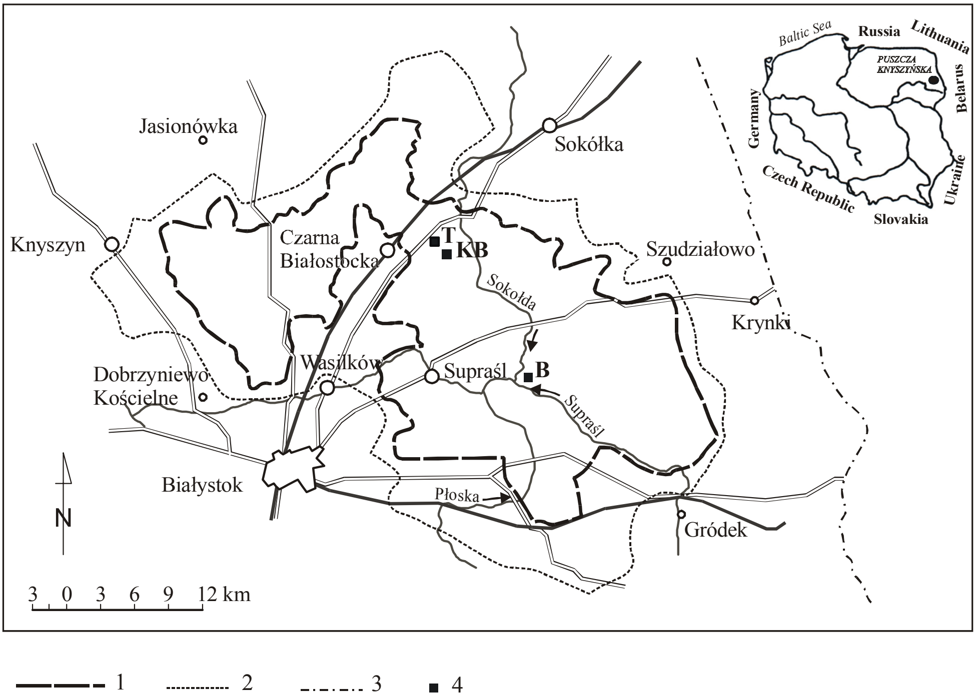

Figure 1. Location of the mires studied in the Puszcza Knyszyńska Forest. 1 – border of the landscape park, 2 – border of the protected zone, 3 – the state border, 4 – mire under study

Rycina 1. Mapa Polski i Puszcza Knyszyńska. 1 – granica parku krajobrazowego, 2 – granica otuliny, 3 – granica państwa, 4 – badane torfowisko

Selected samples of peat were dated using the radiocarbon dating method at the Poznań Radiocarbon Laboratory (Poznań, Poland) (AMS method), and in the Radioanalytical Laboratory of the Institute of Hygiene and Medical Ecology in Kiev (Kiev, the Ukraine). The radiocarbon age of the samples was calibrated with CalPal–2007 ver. 1.5 online software (Danzeglocke 2011). Chronology for the Holocene was presented according to Mangerud et al. (1974), with calibration of chronozone boundaries (Walanus, Nalepka 2010). Periodization of the Late Glacial by Litt et al. (2001) was used.

4. Results

Remains of 116 different plant taxa (species, section, genus, family) were identified in the investigated peat sediments. Among them were the following taxa, characteristic of 9 vegetation classes: *Scheuchzerio-Caricetea nigrae* – 31, *Phragmitetea* – 13, *Oxycocco-Sphagnetea* – 13, *Potametea* – 9, *Alnetea glutinosae* – 4, *Bidentetea tripartiti* – 2, *Charetea* – 2, *Molinio-Arrenatheretea* – 2, *Litorelletea* – 1. Quantitative representation of major plant types was as follows: trees and shrubs (10 taxa), dwarf shrubs (4), herbs (56), pteridophytes (2), peat-mosses (16), brown mosses (23), and algae (5).

Sixteen of the taxa identified are not found in the Knyszyńska Forest region at present:

a) vascular plants: *Betula nana* L. (Fig. 2), *Ranunculus reptans* L., *Myriophyllum alternifolium* DC., *Scheuchzeria palustris* L., *Potamogeton filiformis* Pers., *Potamogeton panormitanus* Biv., *Potamogeton friesii* Rupr., *Cladium mariscus* (L.) Pohl, *Hippuris vulgaris* L.

b) peat mosses: *Sphagnum platyphyllum* (Lindb.) Warnst., *Sphagnum angustifolium* (C.E.O. Jensen ex Russow) C.E.O. Jensen, *Sphagnum centrale* C. Jens. in Arnell & C. Jens.

c) brown mosses: *Meesia triquetra* (Richt.) Ångstr., *Warnstorfia fluitans* (Hedw.) Loeske , *Drepanocladus Sendtneri* (Schimp. ex H.Müll.) Warnst., *Scorpidium scorpioides* Limpr. (Fig. 3)

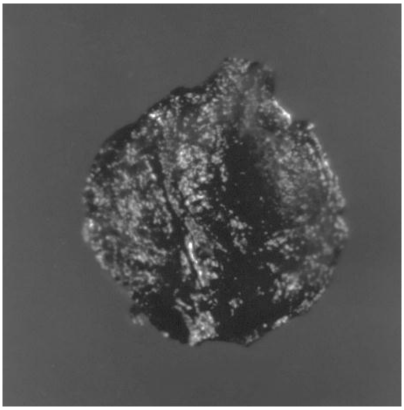

Figure 2. *Betula nana* – nut, x 72; photo by J. Kupryjanowicz

Rycina 2. *Betula nana* – orzeszek, x 72; fot. J. Kupryjanowicz

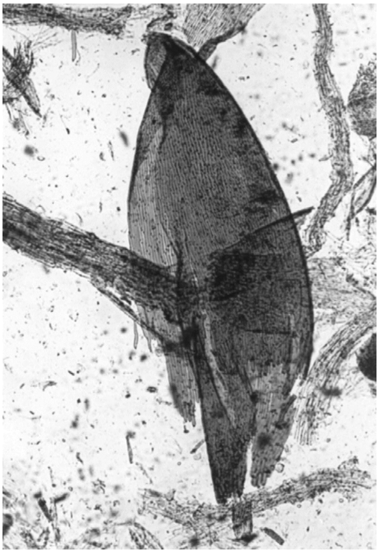

Figure 3. *Scorpidium scorpioides*, x 74; photo by D. Drzymulska

Rycina 3. *Scorpidium scorpioides*, x 74; fot. D. Drzymulska

Table 1 presents occurrence of selected taxa (from the above mentioned 16 taxa) in the past, in the mires studied.

Table 1. Occurrence of selected taxa in the mires studied in the past

Tabela 1. Występowanie wybranych taksonów na badanych torfowiskach w przeszłości

Taxon	Mire	Chronostratigraphy
Betula nana	Taboły	Younger Dryas, Preboreal, Boreal
Cladium mariscus	Taboły	Boreal, Atlantic
Scheuchzeria palustris	Taboły	Subatlantic
	Borki	Boreal, Atlantic, Subboreal, Subatlantic?
Potamogeton filiformis	Taboły	Late Glacial
Potamogeton friesii	Taboły	Late Glacial
Potamogeton panormitanus	Taboły	Late Glacial
Myriophyllum alternifolium	Taboły	Late Glacial
Scorpidium scorpioides	Taboły	Older Dryas
Meesia triquetra	Taboły	Late Glacial, Preboreal, Boreal/Atlantic
	Borki	Boreal/Atlantic, Subatlantic

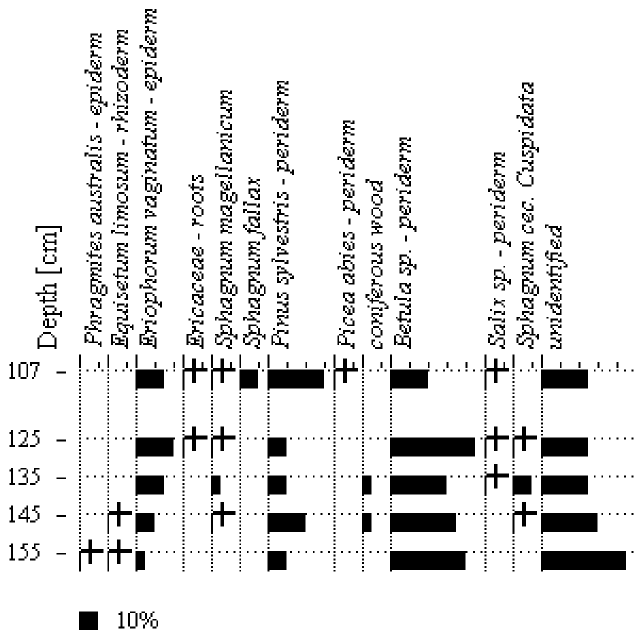

Figure 4. Macrofossil record of subfossil community *Sphagnetum betulo-pinosum eriophoreto fruticuletosum* in the Kładkowe Bagno mire

Rycina 4. Zapis makroszczątkowy subfosylnego zbiorowiska w typie *Sphagnetum betulo-pinosum eriophoreto fruticuletosum* z torfowiska Kładkowe Bagno

A few from among the subfossil plant communities identified are not present nowadays in Poland. Some of them seem to be quite similar to the contemporary *Menyantho trifoliatae-Sphagnetum teretis*, *Sphagnetum betulo-pinosum eriophoreto fruticuletosum* (Fig. 4), *Caricetum rostratae sphagnetosum fallacis*. We described also sedge-brown moss and brown moss community with scrubby birches, and the community of *Scorpidium scorpioides*.

5. Discussion

Palaeobotanical studies conducted in the Knyszyńska Forest delivered interesting data about subfossil flora and vegetation. Among the examples of subfossil flora there are species of aquatic plants, like *Myriophyllum alternifolium*, which occurred in the Late Glacial, in water body at Taboły. This species, connected now with mild maritime climate (Podbielkowski, Tomaszewicz 1982), is considered as a rare relict boreal-atlantic species (Dąmbska 1965). According to Mikulski (1974), *Myriophyllum alternifolium* is negative temperatures sensitive.

This indicates that the species had been present in lakes during one of the interstadials, most probably in the Bölling, when the climate was warmer. The second, mild climate species is *Potamogeton friessi*, also identified in this water body at the same time. An indicator of cool climate in the beginning phase of existence of the north part of the reservoir functioning in Taboły was *Potamogeton filiformis* (see Tobolski 1998). The age of this sediment was determined as 13016–12776 cal. BP (Ki–10401), so to the Alleröd/Younger Dryas decline.

One of the most interesting species of subfossil flora was *Betula nana.* Dwarf birch occurred at Taboły mire since the Younger Dryas probably till the Boral period (Drzymulska 2006), and at Kładkowe Bagno – since the Younger Dryas till the beginning of the Atlantic period (Kupryjanowicz 2004). Earlier, in the Older Dryas, this species was present at Stare Biele mire, which was confirmed by pollen analysis (Kupryjanowicz 2000). In Taboły, *Betula nana* formed quite a well-recognized plant community with *Betula humilis*, sedges and brown mosses. Species of different vegetation classes were identified in this subfossil phytocoenosis: *Betula nana* (*Oxycocco-Sphagnetea*, *Oxycocco-Empetrion*), *Carex vesicaria* and *Carex pseudocyperus* (*Phragmitetea*, *Magnocaricion*), *Betula humilis* and *Thelypteris palustris* (*Alnetea glutinosae*), *Menyanthes trifoliata*, *Calliergon* cf. *giganteum* and *Drepanocladus* sp. (*Scheuchzerio-Caricetea nigrae*). This sedge-brown moss and brown moss community with scrubby birches could be probably identified with the shrubs-sedges-brown mosses associations described by Liss and Bjerjesina (1981) in the Western Siberia territory. There are also some references to contemporary *Betuletum humilis* Fijałkowski 1959 (see Pałczyński 1975; Botsch, Smagin 1993). According to Botsch, Smagin (1993), *Betula nana* is a component of this association in North-Western Russia.

A separate subfossil community *Menyantho trifoliatae-Sphagnetum teretis*-type, with *Sphagnum teres* as a dominant, was described at Taboły mire. It functioned there in the Boreal/Atlantic period. At present *Menyantho trifoliatae-Sphagnetum teretis* Warén 1926 occurs in North-Western Europe and is connected with *Caricetalia nigrae* order (Dierssen 1982). In a different manner *Sphagnum teres* has been ordered by Matuszkiewicz (2001), who placed this species in *Caricion lasiocarpae* assemblage (*Scheuchzerietalia palustris* order), as a characteristic species. An analogue of subfossil *Menyantho trifoliatae-Sphagnetum teretis*-type occurs nowadays also in Western Siberia, where *Menyanthes trifoliata-Sphagnum* *teres* phytocoenosis was described (Liss, Bjerjesina 1981). There are characteristic admixtures of elements indicating poor habitats, like Ericaceae dwarf shrubs, *Carex limosa*, and peat mosses of *Acutifolia* section. These components were also found in the subfossil community at Taboły.

Scorpidium scorpioides is another example of subfossil flora of the Knyszyńska Forest. This brown moss formed community at Taboły, in the Older Dryas [13926–13636 cal. BP (Poz–2885)] (Drzymulska 2011). This phytocenosis seems to be connected with *Caricion davallianae* Klika 1934 assemblage, which was pointed out by Jasnowski (1959). The absence of identified sedge species impedes a connection of this community from Taboły deposit with others reported in literature, like subfossil *Carex rostrata-Scorpidium scorpioides* (Rybniček 1973) described in Central Europe and in the present-day occurring in Scandinavia and Scotland. It is easier to relate this community to the *Scorpidium scorpioides* subfossil phytocoenosis described by Oświt (1973, 1991) in the Lower Biebrza Basin, and at Rabinówka mire (Drzymulska 2004). Moreover, all communities existed in the Late Glacial. *Scorpidium scorpioides* is also known as an initiator of peat-forming process in the Ilmen Lowland (Bogdanovskaja-Gijenef 1969). Nowadays this brown moss occurs in moss-lichens tundra and willow-birch dwarf shrubs in Central Siberia (Katz 1975).

Sphagnetum betulo-pinosum eriophoreto fruticuletosum-type is a subfossil community reconstructed in Kładkowe Bagno bog, where it functioned in the Subboreal and Subatlantic periods. A contemporary analogue – *Sphagnetum betulo-pinosum* Filatov et Yurev 1913 association occurs in North-Western Russia (Botsch, Smagin 1993). There are very poor data about subfossil communities that consisted of birch, pine and tussock cottongrass. For example Obidowicz (1990), who studied mires of the Podhale region, tried to relate them to the contemporary association *Betuletum pubescentis* Tüxen 1937, however it seems to be quite risky for the community recognized in the Knyszyńska Forest.

Concluding, we want to stress one more time the importance of palaeobotanical analyses in recognition of environmental changes in the past. This is the only way to reconstruct subfossil flora and vegetation of the territory studied, especially in mires. The occurrence and disappearance of species and plant communities was a result of climatic and habitat changes which happened in the Late Glacial and during the Holocene.

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Zmiany we florze i roślinności torfowisk Puszczy Knyszyńskiej od ostatniego zlodowacenia

Streszczenie

Torfowiska to jedne z najbardziej cennych elementów krajobrazu. Nierzadko są to obiekty zachowane w stanie niemal naturalnym, stanowiące refugia gatunków wrażliwych na antropopresję. Osady biogeniczne tworzące złoża torfowe, w tym przede wszystkim torf są jednocześnie jednymi z najważniejszych archiwów wiedzy o dziejach środowiska przyrodniczego. Zawarte w nich mikro- i makrofosylia stanowią bowiem, niezwykle cenne źródło wiedzy o minionym środowisku przyrodniczym. Jednym z najbardziej zatorfionych obszarów w Polsce jest Puszcza Knyszyńska.

Badania podjęte na tym obszarze miały na celu rozpoznanie subfosylnej flory i zbiorowisk roślinnych występujących na torfowiskach puszczańskich od późnego glacjału, poprzez holocen. W tym celu wykorzystano metodę analizy roślinnych szczątków makroskopowych. Wiek osadów oceniono za pomocą datowania radiowęglowego.

Szczątki roślinne zawarte w torfie i osadach jeziornych przypisano do 116 taksonów roślinnych różnej rangi. Z czego 16 stanowiły taksony obecnie niereprezentowane na terenie Puszczy Knyszyńskiej. Wśród nich znalazły się zarówno rośliny naczyniowe (np. *Betula nana*, *Cladium mariscus*, *Myriophyllum alternifolium*, *Potamogeton filiformis*, *P. friesii*, *P. paorminatus*), mchy brunatne (np. *Scorpidium scorpioides*, *Meesia triquetra*), jak i mchy torfowce (np. *Sphagnum centrale*). Opisano także kilka zbiorowisk subfosylnych znanych ze współczesnych stanowisk w Europie Zachodniej, jak i na Zachodniej Syberii (*Menyantho trifoliatae-Sphagnetum teretis*), oraz z obszaru Rosji Północno-Zachodniej (*Sphagnetum betulo-pinosum eriophoreto fruticuletosum*).

Zmiany klimatu, które nastąpiły po ostatnim zlodowaceniu, w holocenie sprawiały, że zasięgi pewnych zespołów przesuwały się z południa na północ i ze wschodu na zachód. Badania paleoekologiczne torfowisk stanowią zatem najwierniejsze źródło wiedzy na ten temat.

Range changes in Pleistocene as the source of intraspecific diversity of arctic-alpine plants in Europe

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Abstract

The present article reviews the relevant publications on the intraspecific diversity of arctic-alpine plants. The intraspecific diversity of plants is still insufficiently recognized because the methods of its study have been developing only since the end of the 20th century.

Plant protection requires the information on the gene pool of species, especially from the endangered areas, such as the arctic and alpine regions, which may suffer from climate warming. The arctic-alpine disjunction has developed as a consequence of plant migrations forced by climate fluctuations in the Pleistocene and the early Holocene. Different routes of migrations and the isolation of particular populations contributed to the development of interspecies variability. Studies of this variability conducted with molecular methods helped to trace the glacial history of many species, and to identify the phylogeographical patterns. The investigations of the morphological variability are less popular, although differences on this level do occur. Biogeographic structure based on the morphological characteristics of two dwarf *Salix* species showed some similarities to the structure obtained through molecular research. At the same time the dependence of some morphological features on the climate was proved. Factors influencing contemporary morphological variation of arctic-alpine plants are complex.

Key words: glacial refugia; molecular variability; morphological analyses; phylogeography;

1. Introduction – intraspecific diversity

The term ‘biodiversity’, introduced during the Convention on Biological Diversity in Rio de Janeiro 1992 (Andrzejewski, Weigle 2003) refers to a variety of organisms at all levels of the organization (Wilson 1992). In Europe the species diversity of vascular plants as well as the diversity of ecosystems and landscapes, is quite well recognized. The intra-species diversity, defined as the variety of the gene pool of plants, is obviously great and much poorer known, mainly because of the fact that the methods of its investigation have been developing only since the 1980s (Stace 1993). Increasing awareness of the importance of genetic diversity of species, and of the need of its protection, have led to rapidly growing number of studies and publications. Thanks to them the internal variation of several species has been revealed, and some results helped to solve taxonomic problems (Conti et al. 1999; Zhang et al. 2001; Hagen et al. 2002; Albach et al. 2004). In addition, the knowledge of intraspecific biodiversity is a prerequisite for modern plant protection (Taberlet 1998; Danielewicz 2003). An important reason for this research is also the concern for the preservation of biodiversity in the face of predicted climate change.

The present article reviews some relevant publications dealing with the intraspecific diversity of arctic-alpine plants. The purpose of the review was to compare the postglacial fate of several species and to compare their geographic structure obtained with molecular and morphological methods.

2. Arctic-alpine plants

The currently observed climate warming is a threat, particularly to plants growing in areas with cold climates, where changes in the variability of species and plant communities have already been noted (Gottfried, Grabherr 2003; Lesica, McCune 2004; Crawford 2008). That is why arctic-alpine plants inhabiting these areas are the subject of great concern.

The arctic-alpine disjunction was quite early recognized and its causes were described (Pawłowska 1972). In Europe, the species with this type of distribution occur in the north of the continent in the Arctic, and in the mountains of the Alpine system. The distribution developed as a consequence of plant migrations forced by climate fluctuations in the Pleistocene and the early Holocene (Hewitt 1999; Comes, Kadereit 1998; Birks,Willis 2008). It should be noted that in the Quaternary, Europe survived several drastic climate changes because in the Pleistocene its area experienced at least four glacial periods, separated by interglacials. In the northern and central parts of the continent the tertiary thermophilic flora was destroyed in most, and survived only in refugia. However, arctic plants for most of the Pleistocene could grow on the vast tundra in the foreland of the glacier, in the north-western part of the European Lowlands, and it is believed that their ranges were much larger than today (Birks, Willis 2008). Glacial conditions prevailed for about 80% of the duration of the Pleistocene, but they were interrupted by shorter warm periods. During warmer years thermophilic species entered the European Plains displacing arctic plants, whose ranges at that time moved to the areas liberated from the glaciers, that is to the north of the continent, as well as to the higher positions in the mountains. This resulted in the division of their ranges into various populations, isolated during interglacials. This process was repeated in the time of each climate change, until the modern warming (Marcysiak 2010).

3. Sources of diversity of arctic-alpine plants

It is believed that the rapidly changing environmental conditions, resulting in the adaptation processes in plants, can accelerate the rate of differentiation and speciation of organisms (Hewitt 2000; Willis, McElwain 2002; Kadereit et al. 2004; Willis, Niklas 2004).

The internal variability of arctic-alpine species during the ice ages may have been a resultant of the following processes: during cold periods – divergence at the edges of the wide species ranges, hybridization between closely related species, survival of population in the glacial refugia, and during warm periods – genetic processes occurring in isolated populations (Mitka 1997; Jones, Gliddon 1999; Abbott, Brochmann 2003; Kapralov et al., 2006; Birks 2008; Birks, Willis 2008). These phenomena were accompanied by the effect of the so-called migration of plants, that is the contractions and expansions of ranges, resulting from climate changes. During glacials the earlier isolated populations had a chance to re-connect and gene flow between them was possible (Marcysiak 2010). The extent of migrations is sometimes hard to assess, as the cases of the long distance dispersal (LDD) and trans-Atlantic dispersal were also reported (Abbott et al. 2000; Hagen et al. 2001; Kropf et al. 2006; Schönswetter et al. 2007).

Another factor influencing the variability of mountain and arctic plants is the possibility of survival of some populations in nunataks, which is a still controversial issue. However, their existence has been proved by biostratigraphical (Paus et al. 2006) or, for some species, molecular methods (Schönswetter et al. 2005).

The research, carried out with molecular methods, has allowed not only a recognition of this variability, but also tracing the likely routes of Pleistocene migrations of plants.

4. Genetic diversity and migration routes of arctic-alpine plants in the light of molecular studies

The analyses of genetic diversity and phylogenetic lineages, linked with the studies of their geographic distribution, have led to development of phylogeography as a new branch of biological science (Emerson, Hewitt 2005). First summaries of phylogeographic studies, tending to find some common features of fates of species affected by glaciations, did not bring clear results. The general trend found for thermophilic species, both plants and animals, was that the level of intraspecific polymorphism was lower at higher latitudes, that is in the areas glaciated during cold periods, while most of the variation was detected in possible refugia. The migration routes varied and were species-specific (Taberlet et al. 1998).

As for arctic-alpine plants the colder periods of Pleistocene were optimal, and warm conditions limited their ranges, all the contemporary ranges should be considered as refugia. Phylogeographic studies of these species allowed concluding that different species had different migration routes, which is a scheme found already for thermophillic species. For instance, the populations of *Dryas octopetala* L. in the Alps and Western Scandinavia originated from the glacier foreland on the European lowland, while the populations in the Carpathians and the Eastern Scandinavia came from the Northern Siberia (Skrede et al. 2006). *Dryas octopetala* is a dwarf, clonal shrub, preferring the limestone substratum and common throughout the contemporary range, abundant also in the glacial and post-glacial floras (Vasari 1999). *Salix herbacea* L. is another small shrub, often found in glacial sediments and also important nowadays in the arctic tundra as well as in the high mountains, but growing on the poor granite ground. Studies revealed that the European populations of this species, in Scandinavia (and further north), the Carpathians and the Alps had their source in the glacial tundra in the European Lowland (Alsos et al. 2009). Different preferences to the substrate may be partially responsible for the differences between the migration routes of the two species.

Glacial and postglacial fates of less common arctic-alpine species are also complex, because besides the migration ways listed above, the survival of small refugial populations played a role (Schönswetter et al. 2005; 2006).

Distribution of the genetic diversity of arctic-alpine plants usually reflects their migration routes. Some species show relatively great variation in Scandinavia. This may be an effect of either broad front of colonization after deglaciation (Alsos et al. 2009), or two or three sources of origination of contemporary populations (Nordal, Jonsell 1988). The area where populations originating from different sources meet is called suture zone and such a zone between the northern and southern (western) Scandinavia was reported for various organisms, both plants and animals (Taberlet 1998; Hewitt 2000). Besides, the *in situ* survival in some Arctic refugia was also proved (Alsos et al. 2005). Among the species showing a considerable molecular variability in Scandinavia are *Dryas octopetala* (Skrede et al. 2006), *Saxifraga paniculata* (Reisch 2008) and *Salix herbacea* (Alsos et al. 2009).

On the other hand, some species, usually forming small and scattered populations, show low genetic variability in Scandinavia. Often proposed explanations of this situation are the genetic bottleneck and founder effect. *Ranunculus glacialis* (Schönswetter et al. 2003) and *Veronica alpina* (Schönswetter et al. 2006) can be examples of these species.

Molecular variability patterns of the arctic-alpine species in mountains are also differentiated, being impacted by the species history, either glacial or reaching back to the Tertiary, and of course resulting from different genetic processes. In the Alps, two species of *Ranunculus* are good examples: *R. pygmaeus* Wahlenb. shows low diversity, while *R. glacialis* L. is quite variable there (Schönswetter et al. 2003, 2006). Populations of some species from the eastern and western Alps differ genetically (Schönswetter et al. 2003, Ronikier et al. 2008). The latter issue has been thoroughly investigated for more alpine species with the conclusion confirming the existence of two big break zones, generally consistent for inter- and intra-species variability. They are located on the Aosta valley and west to the Dolomites and have been reported previously from the middle of the twentieth century (Thiel-Egenter et al. 2011).

Molecular variation of the plant species of another huge mountain massif, the Carpathians has been much poorer recognised. Still, the division of the chain has been proposed and proved for several species, with the main border between northern (western) and south-eastern parts, the latter subsequently divided into southern and eastern regions. The Carpathians might be relevant for plants migrating from east to north, the species dispersal between them and the Alps has also been noted, but many populations remained isolated in particular regions for long periods of their Quaternary history (Ronikier 2011).

5. Studies of the morphological diversity

Analyses based on plants morphology are much less popular, and morphological features are often treated as unreliable, being dependent on environmental conditions (Panditharathna 2008). Thus, only a few studies have been made linking these features with molecular diversity of arctic-alpine plants, although Schönswetter et al. (2003) found out that the pattern of genetic diversity of *Ranunculus glacialis* reflects the morphological variability of the species described by Böcher (1972).

Investigations of biogeographical structure of morphological variability of arctic-alpine plants are generally lacking. Latest studies give some insights on *Salix reticulata* L. (Marcysiak 2012 a) and *S. herbacea* (Marcysiak 2012 b). Both willows are clonal and their ranges in Europe are similar. They differ in the substrate preferences: *S. reticulata* grows mostly on limestone rocks and *S. herbacea* on granite. *S. reticulata* is less frequent and common mainly in the Tatras (Pawłowski 1956; Rechinger 1964; De Bolòs, Vigo 1990; Castroviejo et al. 1993). The species remains poorly investigated. *S. herbacea* is quite common within its range, relevant also as a fossil material, and has been fairly well studied (Wijk 1986 a, b; Rundgren, Beerling 1999; Stamati et al. 2003; Reisch et al.2007; Alsos et al. 2009).

To avoid the use of characteristics whose variation may result from the influence of environment, the studies were based on the calculated characters, which describe the shape of the plant organs and are believed to be more reliable (Kremer et al. 2002). The studies, based on leaf characters, confirmed the lower variation in the shape characters than in the size characters (Marcysiak 2012 a, b, c.). However, greater stability of shape did not imply the complete independence of the external conditions, as a connection between climatic factors and shape characters for *S. herbacea* was proved. The dissimilarity of biogeographical structures found in the studies for two *Salix* species could be easily explained, for instance, by their substrate preferences. The structures seem to be consistent with the proposed ways of plants migrations, as they both split into two parts: western and eastern, indicating possible two ways of glacial migrations. For *S. reticulata,* the western part comprises the Western Alps, Western Carpathians and western Scandinavia, with Eastern Carpathians and eastern (northern) Scandinavia much distant from them and from each other (Marcysiak 2012 a). The pattern could confirm different migrations routes to western and eastern Scandinavia, proved earlier for another calcareous species, *Dryas octopetala* (Skrede et al. 2006) and also a separate source of the Eastern Carpathian populations. The structure for *S. herbacea* is also explicable, as it connected all Scandinavian and Pyrenean populations into one group, while

Carpathian with Alpine ones created the other. For this species, the leaf shape was different in different parts of the range: in Scandinavia and the Pyrenees leaves had a round shape, and in the Carpathians and the Alps they were more elongated. The subdivision neither for Scandinavia, the Alps, nor for Carpathians were found (Marcysiak 2012 b). But, this patterns is not fully congruent with previous molecular findings (Alsos et al. 2009) and probably it is not quite reliable in the light of the connection between the characters analyzed and the climate.

6. Summary

Despite the rapid development of phylogeography and many new species and areas under study, our knowledge of the intraspecific variability of arctic-alpine plants, and processes that had led to the emergence of contemporarily observed biogeographical structures, is still insufficient. The recognition of morphologic diversity is especially poor. It is difficult to draw conclusions about variability for regions, because plant reactions to the changing environment are complex and often species-specific. One of the conclusions is that climate change may cause some decrease in intra-species diversity of arctic-alpine plants.

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Zmiany zasięgów w plejstocenie jako źródło różnorodności wewnątrzgatunkowej roślin arktyczno-alpejskich w Europie

Streszczenie

W artykule dokonano przeglądu niektórych istotnych publikacji traktujących o wewnątrzgatunkowej różnorodności roślin arktyczno-alpejskich. Różnorodność biologiczna na poziomie wewnątrzgatunkowym jest nadal niedostatecznie poznana, jako że metody jej badania zaczęły się rozwijać dopiero w latach 80 XX wieku. Rozpoznanie zróżnicowania puli genetycznej dziko żyjących roślin i zwierząt jest niezbędne dla nowocześnie rozumianej ochrony przyrody. Dotyczy to szczególnie gatunków zamieszkujących obszary zagrożone, na przykład zmianami klimatu. Należą do nich rośliny arktyczno-alpejskie, których dysjunktywny zasięg jest efektem procesów wywołanych przez plejstoceńskie zlodowacenia. Różne drogi migracji tych gatunków, czyli zmniejszania i poszerzania ich zasięgów pod wpływem zlodowaceń, a także izolacja poszczególnych części zasięgów w okresach ciepłych, przyczyniły się do powstania ich wewnątrzgatunkowej zmienności.

Badania tej zmienności prowadzone metodami molekularnymi pozwoliły poznać glacjalną historię wielu gatunków oraz określić zagrożenia dla ich puli genowej, jakim może być obecne ocieplanie klimatu. Dotychczasowe wyniki wykazały wielką różnorodność zarówno dróg migracji jak i struktury biogeograficznej poszczególnych gatunków. Rozpoznano strefy podziału phylogeograficznego Skandynawii, Alp i Karpat. Opisano procesy które doprowadziły do współczesnego rozmieszczenia różnorodności wewnątrzgatunkowej, charakterystycznej dla poszczególnych gatunków i zależnej od wielu czynników.

Zmienność w obrębie poszczególnych gatunków arktyczno-alpejskich widoczna jest także na poziomie morfologicznym, ale jest znacznie słabiej udokumentowana. Biogeograficzna struktura analizowanych dotąd gatunków, oparta na badaniach morfologicznych cech syntetycznych, wykazuje pewne podobieństwa do struktury uzyskanej dzięki badaniom molekularnym. Jednocześnie udowodniono zależność niektórych cech morfologicznych od klimatu. Czynniki kształtujące współczesną zmienność morfologiczną roślin arktyczno-alpejskich są złożone i prawdopodobnie różne dla różnych gatunków. Natomiast efektem ocieplenia klimatu może być zanik części wewnątrzgatunkowego zróżnicowania roślin arktyczno-alpejskich.

The volume of dead wood in mixed coniferous forests of the Knyszyńska Forest versus nature conservation

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Abstract

Coniferous forests cover over 60% of the Knyszyńska Forest, however, only a few forest stands are protected. Felling economy is not conducive to keeping natural structure of forest communities. Impoverishment afflicts among others dead wood resources. The aim of this study was to establish a) the amount of downed woody material (DWM) and standing dead trees (SDT) in mixed coniferous forests, b) a dependence between the amount of dead wood and forest stand's origin/age, c) if reserve conservation guarantees maintenance of high resources of dead wood in coniferous forests. The study was carried out in managed/protected mature and young stands of *Serratulo-Pinetum*, *Querco-Pinetum* and *Vaccinio uliginosi-Pinetum*. The highest amount of coarse woody debris (CWD) was noted in old-growth forests in the nature reserve (58.13 m^3 ha^{-1}). Managed stands, both natural and of artificial origin, turned out to be poor in dead wood (8.33 m^3 ha^{-1} and 4.90 m^3 ha^{-1}, respectively), similarly to forest stands in boggy habitats (2.66 m^3 ha^{-1}). DWM was an essential part of dead woods' resources (51–58% in protected and 73–95% in managed stands). In managed mature stands, widely decomposed DWM prevailed (ca. 60%), whereas in younger stands it was mainly less decomposed DWM (ca. 70%). CWD with diameters smaller than 40 cm dominated in all types of coniferous forests. Large dead trees were noticed only in mature stands, in considerably higher amount in nature reserve. In managed mixed coniferous forests within the Knyszyńska Forest there is too little dead wood. It can result in a decrease in diversity of the species inhabiting dead wood. Only in protected areas the resources are bigger, thus reserve conservation has fulfilled its aim.

Key words: dead trees, coarse woody debris, downed woody material, standing dead trees, forest management, nature reserves

1. Introduction

Forest species or those connected with forest areas constitute over a half of all land-based organisms (Gutowski et al. 2004). Most of the forests in Poland is of secondary origin as they are mainly the ecosystems risen as a result of post-felling man-made afforestations as well as afforestations of formerly arable grounds. Ecosystems of this sort usually have simplified structure and low biodiversity level. What leads to such condition is, among others, intensive woodland maintenance, economic selection and clearing the forest of dead wood. Silviculture leads to acquisition of big-size trees and, at the same time, to removal of dead and withered ones. Introduction of the so-called "spatial and temporal order" in forest ecosystems (Rykowski 2003) has resulted in impoverishment of natural resources in timber forests.

One of more important ecological roles of dead wood in forest ecosystems is formation of biotops, which are a place of living for many organisms. Biocenotic role of dead wood has been emphasised in many studies (Piotrowski, Wołk 1975; Stevens 1997; Solon 2002; Wu et al. 2005; Holeksa, Maciejewski 2006; Jaroszewicz 2007; Zhou et al. 2007; Paletto et al. 2012). Dead wood, which is of great importance in terms of ecosystem's dynamics, can take different forms in forest environment: snags, stamps, logs, and fine woody debris (Harmon, Sexton 1996). Habitats of this kind can be colonised by numerous specialized species (stenobionts). It is assessed that over a half of organisms living in forests (starting with protozoans and fungus, through mosses, lichens, vascular plants and finishing with vertebrates) use dead wood as living environment to a lower or higher degree (Faliński, Mułenko 1997; Siitonen 2001; Gutowski et al.2004; Starzyk et al. 2008; Ciach 2011).

Ecological basis of forestry presume the protection of forests, also through the raise in biodiversity. In this respect the issue of dead wood in forest ecosystem gets the right perspective (Hagar 2007). As follows from numerous studies, increased amount of dead wood brings an increase in the species cover as well as their number (Müller, Bütler 2010). Permanently worsening forest conditions have changed the ways of dead wood use and initiated an ecological trend in this branch of economy. The intensification of methods of wood harvesting entails a definite decrease in dead wood of various types in forests. Introduction of new methods of cut has admittedly improved the situation, but has not solved the problem (Atlegrim, Sjöberg 2004). Protection of unmanaged forests from which the largest amount of wood is obtained, is therefore still essential. The problem is particularly important with reference to coniferous forest communities, the most often used for economy in Poland.

The aim of this study was to define the amount of dead wood in mixed coniferous forest in the Knyszyńska Forest and to search for dependences between the number of dead wood and origin/age of a given forest stand. Equally important was the question of reserve conservation and in particular if it guarantees sufficiently high resources of dead wood in coniferous forest communities.

2. Study site

The Knyszyńska Forest is one of the biggest forest complex in the Polish Lowland (ca. 105 ha) and one that has been the least changed due to forestry. Ecosystems only slightly modified by man can be found in large numbers. The Knyszyńska Forest is situated in north-east Poland within the boundaries of two mezoregions: Białostocka Plateau and Sokólskie Hills, which are a part of a macroregion called Północnopodlaska Lowland (Kondracki 2000). The territory mentioned is located within old glacial plains – its relief, however, is exceptionally diversified, especially in eastern part (Banaszuk 1995). The richness of relief features in the Knyszyńska Forest determines the appearance of varied habitats – 16 types of forest communities as well as numerous substitute plant communities have been identified (Czerwiński 1995). According to inventory data of National Forests, coniferous forest habitats dominate in the Knyszyńska Forest (66.82% of forest area). The majority of the forest complex is covered by mixed coniferous forest (43.37%) and fresh coniferous forest (19.2%) (Gątkiewicz, Tołwiński 1995). By far the predominant species is pine – its proportional share in forest stands exceeds 70%. Most of the forest stands in the Knyszyńska Forest are relatively young (up to 80 years). The old-growth forests cover merely a few percent of the area.

The Krzemienne Góry Reserve (area: 73.56 ha) was founded in 1987. It is situated in the central part of the Knyszyńska Forest, on Supraśl Division Forest's grounds. Its aim is to protect well preserved forest communities, mainly those in oligotrophic and mezotrophic coniferous forest habitats, overgrowing grand kame terrace. The land sculpture in the reserve is very diversified, with numerous steep elevations (the difference in altitude reaches 45 m). Southern and central parts of the reserve are overgrown with fresh coniferous forest communities and mixed coniferous forest communities, whereas its northern part is covered with mixed forests (Sokołowski 2006). Ninety percent of the area is covered with forest stands aged 120 or more. The reserve is a refuge of rare and protected plant species, e.g. *Lycopodium annotinum* L., *Diphasiastrum tristachyum* (Pursh)Holub, *Pulsatilla pratensis* (L.)Miller, *P. patens* (L.)Miller, *Goodyera repens* (L.)R.Br..

3. Methods

The studies were performed between 2007 and 2011 in mixed coniferous forest of natural origin (in managed forest stand as well as in nature reserve), mixed coniferous forest of artificial origin and bog-pine forest of natural origin. With regard to phytosociological classification, the mineral habitats were represented by communities of *Serratulo-Pinetum* (W.Mat. 1981) J.Mat. 1988 and *Querco-Pinetum* (W.Mat. 1981) J.Mat. 1988. On hydrogenic habitats, *Vaccinio uliginosi-Pinetum* Kleist 1929 in complex with *Ledo-Sphagnetum* Sukopp 1959 em. Neuchäusl 1969 was surveyed and assumed to be a specific control. Both the amount of downed woody material (DWM) and standing dead trees (SDT) were determined.

Forest stands of natural origin aged over 100 years were examined in the Krzemienne Góry Reserve in sections 181f, 191c and 204d as well as in the Żednia Forest Division (Michałowo District) in sections 7a, c and 8b, c. Those grown from planting (49–54 years) were studied in sections 100a, 80b and 81c. A well-preserved bog-pine forest with forest stand up to 60 years was studied in Waliły Forest Division (Waliły District) in sections 261a, 353h, 354h and 391d. The bog-pine forest and the forest stands within the conservation area were excluded from management.

In the field study measurements were made along line transects. The measurement lines were established in parallel, at every few dozen metres, within the boundaries of a chosen fragment of the forest. Total length of the measurement lines varied from 2950 to 3400 metres in particular locations. The length of downed dead trees and their diameters at the points of intersection of logs and measurement line were measured. The logs whose diameter did not exceed 5 cm were omitted. When found not farther than 5 metres from the transect, STD were taken into account. The diameter and height of snags were measured. The degree of decomposition of dead wood fragments was described according to the five-stage scale for DWM and a modified seven-stage scale for SDT (Pawlaczyk et al. 2002).

In order to estimate the amount of downed woody material in the forests studied, Van Wagner's formula was used (Bobiec 2002):

$$V = A\pi^2 \, \Sigma d^2 (8L)^{-1}$$

where V – DWM volume [$m^3 \cdot ha^{-1}$], A – the area under study [m^2], d – piece diameter [m], L – length of measurement line [m] .

The volume of SDT was calculated with the use of the cone formula (the majority of standing dead trees was cone-shaped).

4. Results

The largest amount of downed woody material, reaching 29.8 $m^3 ha^{-1}$ (Fig. 1, Table 1) was found in the phytocenoses of over hundred-year-old timber mixed coniferous forest, within the reserve area. Timber forest stands turned out to be poorer in DWM. In them the resources of woody debris were several times smaller and amounted to 6.09 $m^3 ha^{-1}$ in old-growth forests and 4.66 $m^3 ha^{-1}$ in the planted stands of middle age. The smallest amount of DWM (merely 1.55 $m^3 ha^{-1}$) lied in bog-pine forest. Downed woody material made nearly all dead wood resources in the managed stands, while the volume of standing dead trees per areal unit was inconsiderable and varied from 0.24 $m^3 ha^{-1}$ to 2.24 $m^3 ha^{-1}$ (Tab. 1). The only exception was the fragment of forest situated in the nature reserve. There, the amount of SDT per one hectare reached 28.33 m^3 (which makes 49 % of total resources).

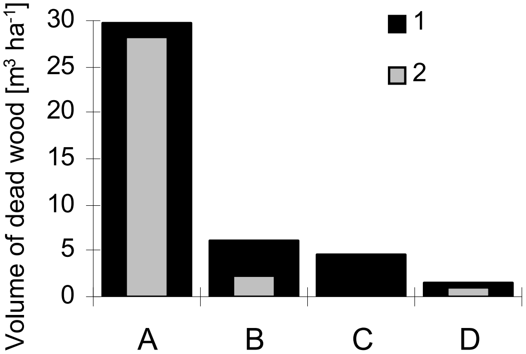

Figure 1. Amount of DWM (1) and SDT (2) in $m^3 ha^{-1}$ in selected types of coniferous forests in the Knyszyńska Forest. Measurements were made in mixed coniferous forest: A – unmanaged mature stands (120–130 years) in the Krzemienne Góry nature reserve, B – managed mature stands (94–104 years), C – managed young stands (49–54 years) and in unmanaged stands (25–60 years) of bog-pine forest (D)

Rycina 1. Zasoby ($m^3 ha^{-1}$) martwego drewna leżącego (1) i stojącego (2) w wybranych typach borów w Puszczy Knyszyńskiej. Pomiary były wykonywane w borach mieszanych: A – nieużytkowanych starodrzewach (120–130 lat) w rezerwacie przyrody Krzemienne Góry, B – dojrzałych drzewostanach gospodarczych (94–104 lata), C – młodych drzewostanach gospodarczych (49–54 lat), a także nieużytkowanych drzewostanach (25–60 lat) boru bagiennego (D)

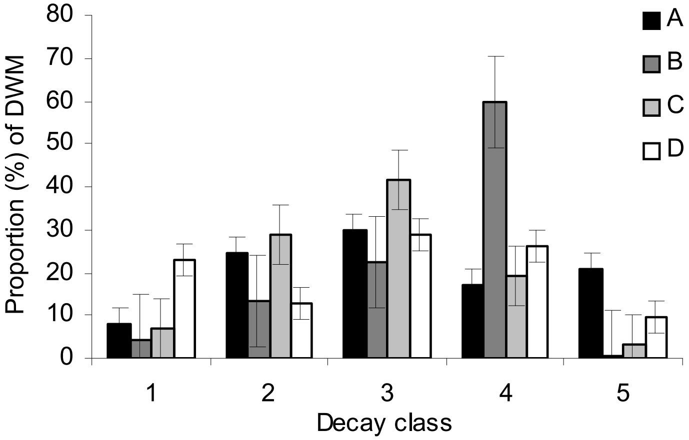

Figure 2. Percentage share of DWM volume in decay classes in selected types of coniferous forests in the Knyszyńska Forest. Measurements were made in mixed coniferous forest: A – unmanaged mature stands (120–130 years) in the Krzemienne Góry nature reserve, B – managed mature stands (94–104 years), C – managed young stands (49–54 years) and in unmanaged stands (25–60 years) of bog-pine forest (D)

Rycina 2. Procentowy udział leżaniny w klasach rozkładu w wybranych typach borów Puszczy Knyszyńskiej. Pomiary były wykonywane w borach mieszanych: A – nieużytkowanych starodrzewach (120–130 lat) w rezerwacie przyrody Krzemienne Góry, B – dojrzałych drzewostanach gospodarczych (94–104 lata), C – młodych drzewostanach gospodarczych (49–54 lat), a także nieużytkowanych drzewostanach (25–60 lat) boru bagiennego (D)

The volume of dead wood representing particular decay classes was different in different types of coniferous forests (Fig. 2). In mature stands located in nature reserve the distribution of DWM resources in all classes of decay was quite uniform, but among SDT the trees in class 3 strongly prevailed (65%). In managed old-forest stands, considerably decomposed DWM (class 4) had the biggest proportional share (almost 60% of resources), while in SDT the largest share was taken by class 1 and 2 DWM (almost 80% of resources). In fifty-year-old mixed planted coniferous forest, the highest amount of weakly decomposed DWM (class 2 and 3) was found making together ca. 70% of resources. STD in the same forest represented mainly medium classes of decomposition, i.e. class 2, 4 and 5. On the other hand, pretty regular share of DWM in successive decay classes was marked in bog-pine forest. The amount of SDT was concentrated in utmost ranges, both in low (class 1 and 2) and in high classes of decomposition (class 5 and 6).

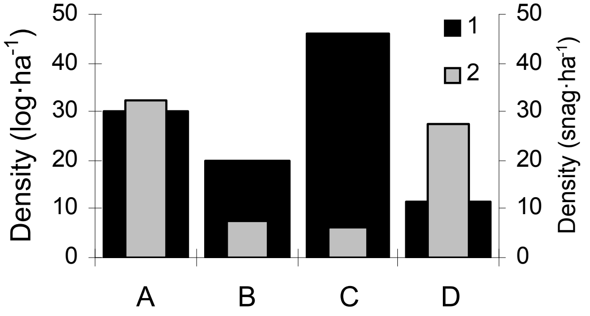

Figure 3. Density of logs (1) and snags (2) per hectare in selected types of coniferous forests in the Knyszyńska Forest. Research was made in mixed coniferous forest: A – unmanaged mature stands (120–130 years) in the Krzemienne Góry nature reserve, B – managed mature stands (94–104 years), C – managed young stands (49–54 years) and in unmanaged stands (25–60 years) of bog-pine forest (D)

Rycina 3. Zagęszczenie kłód (1) i stojących martwych pni (2) (sztuk· ha^{-1}) w wybranych typach borów Puszczy Knyszyńskiej. Badania prowadzono w borach mieszanych: A – nieużytkowanych starodrzewach (120–130 lat) w rezerwacie przyrody Krzemienne Góry, B – dojrzałych drzewostanach gospodarczych (94–104 lata), C – młodych drzewostanach gospodarczych (49–54 lat), a także nieużytkowanych drzewostanach (25–60 lat) boru bagiennego (D)

Considering the number of logs lying on the forests floor, the most distinctive was the coniferous forest arisen as a result of man-made plantings (Fig. 3, Tab. 2). Along the sampling line there were 137 fragments counted (average of 4.5 log per hectare). The smallest amount of dawned trees pieces was noted in the bog-pine forest (merely 35, average of 1.09 log per hectare). Different relations were found for SDT: those in the reserve and in the bog-pine forest were numerous (respectively 103 and 84 trees counted along sampling line) in the presence of barely 18 to 26 trees in other types of forest. Over ¾ of standing and downed trees had the diameter lesser than 40 cm (Tab. 2). Large dead wood could be found only in forest stands aged over 100: in the reserve (14 snags and 9 logs along sampling line) as well as in the managed mature stands (7 snags and 1 log along sampling line). Within the protected area, the large dead wood composed 37% of DWM total amount and 53% of SDT total amount. Significant relationship between the number of logs and the amount of downed dead wood in particular decay classes was stated. This connection is most emphasised for fifty-year-old forest stands of mixed coniferous forest (Fig. 4).

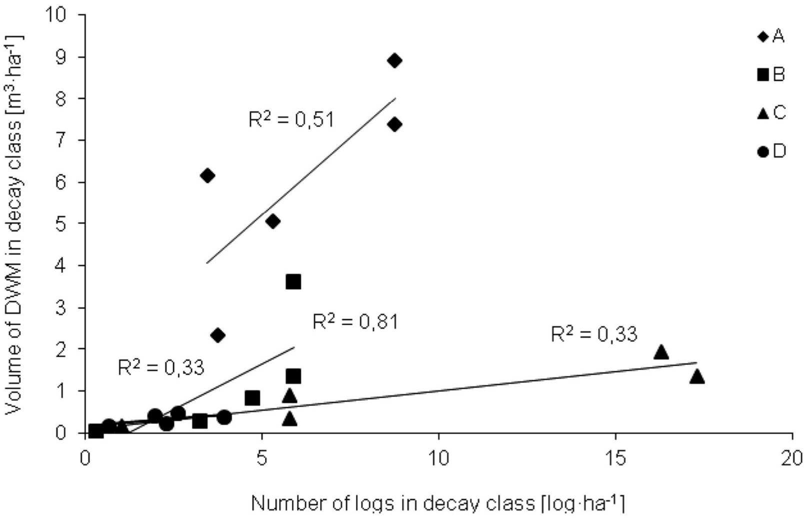

Figure 4. Relationship between volume of DWM ($m^3 \cdot ha^{-1}$) and number of logs ($log \cdot ha^{-1}$) in decay classes in selected types of coniferous forests in the Knyszyńska Forest. Research was made in mixed coniferous forest: A – unmanaged mature stands (120–130 years) in the Krzemienne Góry nature reserve, B – managed mature stands (94–104 years), C – managed young stands (49–54 years) and in unmanaged stands (25–60 years) of bog-pine forest (D)

Rycina 4. Zależność między zasobami leżaniny ($m^3 \cdot ha^{-1}$) i liczbą kłód (sztuk· ha^{-1}) w klasach rozkładu w wybranych typach borów Puszczy Knyszyńskiej. Badania były wykonywane w borach mieszanych: A – nieużytkowanych starodrzewach (120–130 lat) w rezerwacie przyrody Krzemienne Góry, B – dojrzałych drzewostanach gospodarczych (94–104 lata), C – młodych drzewostanach gospodarczych (49–54 lat), a także nieużytkowanych drzewostanach (25–60 lat) boru bagiennego (D)

5. Discussion

Dead wood contributes to a high level of biodiversity within the forest ecosystems (Müller, Bütler 2010). Woody debris make a biotope for numerous species of animals, plants and fungi – beyond this environment they could not survive. Because of that dead wood is one of the most important factors that shape the forest environment.

It was shown in our study that on 1 hectare of managed stands of mixed coniferous forests in the Knyszyńska Forest (both of artificial and natural origin) the amount of dead wood was small (from 4.90 to 8.33 m^3). In the reserve excluded from the forest use, however, the amount of woody debris was higher (58.13 $m^3 \cdot ha^{-1}$), though it does not mean that it was sufficient. Good benchmarks are natural forests situated in the margins of national parks. In natural forests the volume of dead wood is 100–200 $m^3 \cdot ha^{-1}$ (Pawlaczyk 2002). Reduction of this amount could lead to loss of many species. In some types of forests in the Białowieża National Park, 130 to 140 m^3 of dead wood occurs per one hectare, which is 1/5 of the total overground biomass (Gutowski i in. 2004). Likewise, a fair amount of dead wood was noted in upper mountain spruce coniferous forest in the Babiogórski National Park. Nearly 160 logs (95 m^3) and 82 snags (77 m^3) were catalogued on one hectare (Holeksa, Maciejewski 2006). Considerable amounts of dead wood were also noticed in spruce-fir-beech forests in the Babiogórski National Park (218 logs per hectare/164 m^3 and 63 snags per hectare/86 m^3), as well as in the forests of the Roztoczański National Park (170 snags with 200 m^3 per hectare). In managed forests in the Knyszyńska Forest the amount of dead wood significantly differs from the natural pattern. The amount of woody debris which guarantees a proper level of biodiversity should be between 10 and 150 $m^3 \cdot ha^{-1}$ – depending on timber forest type (Müller, Bütler 2010). Unfortunately, this amount decreases at times to merely 1 $m^3 \cdot ha^{-1}$. It is not only the problem of Polish forests – in managed forest complexes of Western Europe and Scandinavia only from a few to over a dozen cubic metres of dead wood has been noted (Green, Peterken 1997; Dudley et al. 2004). It has been estimated that in the forests of such type the woody debris should make 5 to 20 percent of stand's thickness, and the number of standing large dead trees (up to 40 cm diameter) should vary between 7 and 10 per one hectare. For instance, to guarantee the continuity of populations of birds connected with such environment, the minimal density of standing hollow trees should reach 2.4 item per one hectare (the distribution pattern of most species is not relevant) (Bunnell et al. 2002). In the area we studied along 12.6 kilometres of trial line, there were only 21 such trees.

Table 1. Amount and density of downed woody material (DWM) and standing dead trees (SDT) in decay classes in selected types of coniferous forests in the Knyszyńska Forest

Tabela 1. Objętość i zagęszczenie leżących oraz stojących martwych pni w klasach rozkładu w wybranych typach borów Puszczy Knyszyńskiej

		Mixed coniferous forest			Mixed coniferous forest			Mixed coniferous forest			Bog-pine forest		
Stand age		120-130			94-104			49-54			25-60		
Management		Unmanaged			Managed			Managed			Unmanaged		
	Decay class	Density [log·ha^{-1}] [snag·ha^{-1}]	Volume [m^3·ha^{-1}]	Percentage share [%]	Density [log·ha^{-1}] [snag·ha^{-1}]	Volume [m^3·ha^{-1}]	Percentage share [%]	Density [log·ha^{-1}] [snag·ha^{-1}]	Volume [m^3·ha^{-1}]	Percentage share [%]	Density [log·ha^{-1}] [snag·ha^{-1}]	Volume [m^3·ha^{-1}]	Percentage share [%]
	1	3.75	2.34	7.8	3.24	0.27	4.4	5.76	0.33	7.1	3.93	0.35	22.8
	2	8.75	7.37	24.7	4.71	0.81	13.4	17.29	1.34	28.8	2.3	0.2	12.9
	3	8.75	8.9	29.9	5.88	1.36	22.3	16.27	1.94	41.6	2.62	0.44	28.6
	4	5.31	5.05	17	5.88	3.63	59.5	6.1	0.9	19.3	1.97	0.41	26.1
	5	3.44	6.14	20.6	0.29	0.02	0.4	1.02	0.15	3.2	0.66	0.15	9.6
Total DWM		30	29.8	100	20	6.09	100	46.44	4.66	100	11.48	1.55	100
	1	3.13	4.89	17.3	1.18	1.26	56.1	-	-	-	3.93	0.32	28.4
	2	5	1.42	5	1.47	0.53	23.6	2.71	0.06	26.6	3.93	0.17	15.6
	3	18.13	18.41	65	-	-	-	0.34	0.004	1.8	0.33	0.07	5.9
	4	2.5	2.43	8.6	-	-	-	0.34	0.07	28	0.66	0.04	3.6
	5	1.25	0.72	2.5	1.18	0.1	4.5	1.36	0.06	24.6	8.2	0.22	20.3
	6	1.88	0.43	1.5	0.59	0.01	0.7	0.34	0.01	2.1	7.54	0.23	20.9
	7	0.31	0.03	0.1	3.23	0.34	15.1	1.01	0.04	16.9	2.95	0.06	5.3
Total SDT		32.2	28.33	100	7.65	2.24	100	6.1	0.24	100	27.54	1.11	100

Table 2. Volume and density of downed woody material (DWM) and standing dead trees (SDT) in size classes in selected types of coniferous forests in the Knyszyńska Forest

Tabela 2. Objętość i zagęszczenie leżących oraz stojących martwych pni w klasach wielkości w wybranych typach borów Puszczy Knyszyńskiej

		Mixed coniferous forest			Mixed coniferous forest			Mixed coniferous forest			Bog-pine forest		
Stand age		120-130			94-104			49-54			25-60		
Management		Unmanaged			Managed			Managed			Unmanaged		
	Diameter [cm]	Density $[log \cdot ha^{-1}]$ $[snag \cdot ha^{-1}]$	Volume $[m^3 \cdot ha^{-1}]$	Percentage share [%]	Density $[log \cdot ha^{-1}]$ $[snag \cdot ha^{-1}]$	Volume $[m^3 \cdot ha^{-1}]$	Percentage share [%]	Density $[log \cdot ha^{-1}]$ $[snag \cdot ha^{-1}]$	Volume $[m^3 \cdot ha^{-1}]$	Percentage share [%]	Density $[log \cdot ha^{-1}]$ $[snag \cdot ha^{-1}]$	Volume $[m^3 \cdot ha^{-1}]$	Percentage share [%]
	5-20	10.63	3.6	12.1	17.35	2.94	48.3	45.76	3.97	85.2	11.15	1.37	88.4
	21-40	16.56	15.16	50.9	2.35	2.02	33.2	0.68	0.69	14.8	0.33	0.18	11.6
	>40	2.81	11.04	37	0.3	1.13	18.5	-	-	-	-	-	-
Total DWM		30	29.8	100	20	6.09	100	46.44	4.66	100	11.48	1.55	100
	5-20	18.44	2.63	9.3	4.41	0.23	10.3	5.42	0.21	87.5	24.59	0.73	65.8
	21-40	9.38	10.82	38.2	1.18	0.69	30.8	0.68	0.03	12.5	2.95	0.38	34.2
	>40	4.38	14.88	52.5	2.06	1.32	58.9	-	-	-	-	-	-
Total SDT		32.2	28.33	100	7.65	2.24	100	6.1	0.24	100	27.54	1.11	100

Many researches emphasise that not only the total amount of dead wood has important meaning for species occurrence but also its quality (degree of decomposition) and thickness (Lassauce et al. 2011). The amount of dead wood in investigated managed forests is generally reliant on the number of logs and standing dead trees, regardless of the age of the forest stand. It means that in these types of forests, the basic part of resources originates from withering of young trees, whereas the fraction of large wood (the most precious material from the viewpoint of biodiversity) is insignificant. In reality this type of wood is first and foremost harvested and reluctantly left in forest because of its economic value. Admittedly, the presence of dead wood is more and more noticeable in managed forests, but most often it is fine woody debris (or at most decaying so-called "ecological trees" left on felling site on purpose). Production forests are poor in large trees in different phases of decomposition (Marage, Lemperiere 2005). Large trees are of great importance, as the slow process of their decomposition ensures continuity of the specific type of biotopes in forest ecosystems.

The reasons for such a small amount of dead wood in coniferous forests in the Knyszyńska Forest are quite a few. One of the most important is non-observance of ecological forest economy rules (including the order of leaving significant amounts of dead wood in the forest) and excessive exploitation of resources. In young forest stands of artificial origin the amount of dead wood is smaller, because they are meticulously maintained. In this connection, there is relatively more wood harvested in such areas than in mature forest stands. Small amount of woody debris is linked also with the age of a forest stand. In a hundred-year-old managed stand there was 40% more dead wood than in fifty-year-old forest in the same habitat. Relatively many trees would have to wither in young forest stands to increase the woody debris resources. In old forest stands, the level of resources rises after withering of merely a few large trees. The amount of dead wood is also influenced by habitat features. First of all it is a resultant of biomass growth rate and the rate of tree fragments decomposition (Holeksa, Maciejewski 2006). In less favourable habitat conditions (i.e. in bog-pine forest) the biomass of trees which build a forest stand is lower. Thus, the volume of decomposed trees also shrinks. Simultaneously, their decomposition is slower in such conditions. In mixed coniferous forest, where the habitat conditions are more favourable, the biomass of dead trees is much higher. Mainly Scandinavian researchers have pointed out the importance of the above-mentioned factors in shaping the resources of dead wood in forests (Fridman, Walheim 2000). In respect to the abundance of dead wood, both young and old managed forest stands of the Knyszyńska Forest are similar to bog-pine forests, that is poor with woody debris as a rule (2.66 m^3 ha^{-1}).

The resources of dead wood in coniferous forest communities of the Knyszyńska Forest, which are protected in nature reserves, are considerably greater than in managed forest stands. This is probably a general rule, also including other types of forests (Maślak, Orczewska 2010). Whether the resources are sufficient is, however, disputable (Pasierbek et al. 2007). There is still no doubt that the number of reserves protecting mature stands of coniferous forests in the Knyszyńska Forest is inadequate. Up to date, 21 reserves have been established there, from among which only in 6 coniferous forest communities are the main object of protection. Mature stands of mixed coniferous forests and fresh coniferous forests appear on 500 hectares which is 30% of all protected mature stands within the reserves in the Knyszyńska Forest. Considering the size of the Knyszyńska Forest the area is definitely too small. Newly established reserves surely will not deplete wood resources. They do not have to be large objects, on the contrary, they should cover relatively small areas of old-growth forests which can be perfect reservoirs of dead wood. According to the study carried out in Scandinavia, both the density of large trees and the density of dead wood (snags, logs) decreased with increasing reserve size (Götmark, Thorell 2003).

The state of knowledge about dead wood importance in forest ecosystems is still insufficient. Repeating after Holeksa and Maciejewski (2006), dead wood is still removed from protected areas (both reserves and national parks), although there is no rational explanation for such actions. It is hard to expect this field of knowledge will by fully put into practice in forestry and nature conservation in the near future. Every effort to change it should be made though.

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Zasoby martwego drewna w zbiorowiskach borów mieszanych Puszczy Knyszyńskiej a ochrona rezerwatowa

Streszczenie

Lasy iglaste zajmują ponad 60% powierzchni Puszczy Knyszyńskiej jednak tylko nieliczne drzewostany są objęte ochroną. Prowadzona od dziesięcioleci gospodarka zrębowa nie sprzyja zachowaniu naturalnej struktury zbiorowisk leśnych. Zubożeniu ulegają m.in. zapasy martwego drewna, w związku z czym radykalnie obniża się różnorodność biotyczna takich ekosystemów. Celem badań było określenie: a) wielkości zasobów leżącego i stojącego martwego drewna w najczęściej występujących w Puszczy Knyszyńskiej borach mieszanych, b) zależności między ilością martwego drewna i pochodzeniem/wiekiem drzewostanu, c) czy ochrona rezerwatowa gwarantuje zachowanie odpowiednio wysokich zasobów martwego drewna w zbiorowiskach borowych. Badania wykonano w naturalnych starodrzewach (użytkowanych/objętych ochroną) oraz sztucznie nasadzonych młodszych drzewostanach *Serratulo-Pinetum* i *Querco-Pinetum*. Zbiorowisko *Vaccinio uliginosi-Pinetum* w kompleksie z *Ledo-Sphagnetum* potraktowano jako swoistą próbę kontrolną. Najwięcej martwego drewna stwierdzono w starodrzewach położonych w rezerwacie przyrody − 58.13 m^3 ha^{-1}.

Mało zasobne okazały się drzewostany użytkowane gospodarczo, zarówno naturalne jak i sztucznego pochodzenia (odpowiednio 8.33 m^3 ha^{-1} i 4.90 m^3 ha^{-1}); pod tym względem nawiązywały one do z natury ubogich w martwe drewno drzewostanów na siedliskach bagiennych (2.66 m^3 ha^{-1}). Drewno leżące stanowiło zasadniczą część zasobów w drzewostanach gospodarczych (73–95%) oraz nieco ponad połowę zasobów w lasach nieużytkowanych (51–58%). W dojrzałych drzewostanach gospodarczych przeważała leżanina znacznie rozłożona (ok. 60%), natomiast w młodszych − słabo rozłożona (ok. 70%). W drzewostanach na terenie rezerwatu odnotowano dość równomierny udział leżaniny w kolejnych klasach

rozkładu. We wszystkich typach borów dominowało drewno leżące o średnicy mniejszej niż 40 cm. Grubowymiarowe martwe drewno występowało jedynie w starych drzewostanach, przy czym znacznie liczniej i obficiej na terenie rezerwatu przyrody. W użytkowanych gospodarczo borach mieszanych w Puszczy Knyszyńskiej zalega zbyt mało martwego drewna. Jedynie na terenach chronionych zasoby te są wyższe. Ochrona konserwatorska spełnia zatem swoje zadanie w tym zakresie. Są to jednak ciągle zasoby znacząco odbiegające od wielkości charakterystycznych dla lasów naturalnych, co może oznaczać wielokrotnie niższą od oczekiwanej różnorodność wśród roślin i zwierząt, dla których biotopem jest murszejące drewno.

The Romincka Forest – arguments for and against establishment of a national park

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Abstract

North-eastern Poland is not represented in the network of National Parks. This paper attempts to determine whether the natural features of the Romincka Forest justify establishment of a national park on its territory. The presented arguments for and against are based on a review of published and unpublished sources as well as own research, including a map of potential natural vegetation.

The proposed territory covers 85% of the Romincka Forest Landscape Park. It is characterized by resources of considerable scenic and natural value:

- The local flora has a high proportion of forest communities representing a wide range of habitats and phytosociological characteristics, which are found nearly exclusively in the northern regions of Poland;
- The Romincka Forest is a an abundant source of flora characteristic of boreal regions. The majority of taxa in hydrogenic forests are listed in the Red Book of Endangered Species and the Red List of Threatened Species;
- Most sources indicate that the Romincka Forest, in particular its forest, marsh, meadow and rush ecosystems, are undergoing natural regeneration;
- The Romincka Forest provides habitat for wolves, lynxes, otters, beavers, 10 bat species, bird species of special concern and many invertebrate species;
- The Romincka Forest is not a mass tourism site, and it is subject to low anthropogenic pressure;
- The Romincka Forest has a small percentage share of farmland and potential sites for industrial, tourist and recreational development which are generally a source of social conflict.

1. Introduction

This study compiles information from various sources dedicated to the natural resources of the Romincka Forest, with main focus on floristic and phytosociological data and the age structure of tree stands. An evaluation of natural resources, including the local fauna, will contribute to the debate regarding the pros and cons of establishing a national park in the territory of the Romincka Forest.

2. General characteristics of the area analyzed

A botanical evaluation was carried out in the territory of the Romincka Forest Landscape Park which was created pursuant to the Regulation of Suwałki Province Governor No. 6/98 of 14 January 1998 (Journal of Laws of Suwałki Province Governor No. 2/98). The Landscape Park spans an area of 14 820 ha. The proposed National Park would cover 85% of that territory, i.e. 12 588 ha (Table 1). Non-forested land in the vicinity of a local village and Lake Gołdap will be excluded from the territory of the park proposed. The above areas and the buffer zone of the Landscape Park would constitute a buffer zone of the new national park.

Table 1. Area of the Romincka Forest Landscape Park and the proposed Romincka Forest National Park according to administrative boundaries

Tabela 1. Powierzchnia PK Puszczy Rominckiej i projektowanego PN Puszcza Romincka wg podziału administracyjnego

Municipality	Area [ha]		Area [ha]	
	Landscape Park	Buffer zone of Landscape Park	Proposed National Park	Buffer zone ofNational Park
Dubeninki	10 507	6 720	8 824	8 403
Gołdap – rural municipality	4 154	978	3 764	1 368
Gołdap – urban municipality	159	6	0	165
Total	14 820	7 704	12 588	9 936

The Romincka Forest occupies an area of 36,000 ha in north-eastern Poland between Gołdap and Żytkiejmy near the Polish border. A larger section of Romincka Forest (21,000 ha) lies in the Kaliningrad Oblast of Russia, whereas its southern part with an area of around 15,000 ha is situated in the Polish Region of Warmia and Mazury, Gołdap county, municipalities of Gołdap and Dubeninki. This paper will discuss only the Polish section of the Romincka Forest.

The Romincka Forest is part of the Regional Directorate of State Forests in Białystok, and it is administered by the Gołdap Forest District with its seat in Gołdap. The Gołdap Forest District manages State-owned land with an area of 13 731 ha, including 11 112 ha on the territory of the proposed National Park (81% of its area).

According to the geobotanical regionalization system proposed by Matuszkiewicz (1993), the Romincka Forest Landscape Park is situated in the Central European province, Proper Central European subprovince, North Masurian and Belarusian divide (F), Augustów and Suwałki syntaxonomic region (F.2), Suwałki Lakeland landscape region (F.2.1), Romincka Forest (F.2.1.a) and Wiżajny (F.2.1.b) landscape subregions. A part of the Romincka Forest Landscape Park occupies the Masurian syntaxonomic region (F.1), East Masurian syntaxonomic subregion (F.1.b), Węgorapy landscape region (F.1.b.6) and Gołdap landscape subregion (F.1.b.6.c).

In the geobotanical classification system proposed by Szafer (1972), the Northern Division roughly corresponds to the Giżycko and Suwałki landscape region in the Masurian Lakeland syntaxonomic region of East Pomerania, as identified by Polakowski (1963). Boreal forms of plant communities characteristic of the Northern Divide have been identified by Szafer and Polakowski, and forms characteristic of the Giżycko and Suwałki landscape region – by Polakowski. The most characteristic feature of the Northern Divide is the boreal spruce zone. The spruce entered Polish territory from the north-east, and it formed dense communities in the analyzed area (Boratyński, Boratyńska, Hanz 1980).

The contemporary landscape of the Romincka Forest and the surrounding areas was shaped during the youngest geologic epochs of the Pleistocene and Holocene (Krzywicki 2000).

3. Materials and Methods

This paper is based on reviews of available literature, manuscripts released by the Regional Directorate for Environmental Protection in Olsztyn and the Management Board of the Romincka Forest Landscape Park with its seat in Żytkiejmy, as well as the results of own floristic and phytosociological research. It contains a description of the local flora whose distribution is shown on the map of potential natural vegetation (presented for the discussed segment of the forest). The map has been developed on the basis of the following materials and resources:

- detailed maps and descriptions of plant communities in reserve protection schemes (manuscripts of the Regional Directorate for Environmental Protection in Olsztyn),
- phytosociological studies of the Romincka Forest published after World War II (Czerwiński 1965, 1970, 1973, 1978, 1986, 1995; Endler 1987; Polakowski 1962, 1963; Sokołowski 1971,1980; Zaręba 1979),
- indirect information obtained from Forest Management Plans of the Gołdap Forest District for 2005–2014 (Bureau for Forest Management and Geodesy (BFMG), Warsaw Division, 2005, manuscript of the Regional Directorate for State Forests (RDSF) in Białystok),
- maps of soil types and forest habitats in the Gołdap Forest District (BFMG, Warsaw Division, 2003, manuscript of RDSF in Białystok),
- Plan for the Protection of the Romincka Forest Landscape Park (Białystok 2005, manuscript of the Management Board of the Landscape Park in Żytkiejmy),
- own topographic survey performed in 2011 by surface levelling of scattered points.

The above resources supported the identification and delimitation of plant communities which were confronted with soil and habitat maps to verify typological units and develop a vegetation map. Detailed cartography was obtained for forest communities in the rank of plant associations and for oak-linden-hornbeam forests – in the rank of three sub-associations. Cartographic data were used only to describe the surface structure of forest communities in the discussed area.

The applied classification system of identified plant communities is based on *Przewodnik do oznaczania zbiorowisk roślinnych Polski* (Guide for identification of plant communities of Poland, Matuszkiewicz 2008), and selected forest communities were described on the basis of the work of Czerwiński (1995). The study relies on the plant nomenclature proposed in the above monographs, and synonymous names describing the communities discussed (or the names of communities which – according to the author – are similar to the described ones) are given in parentheses.

This study does not cover aqueous and rush plants in rivers and lakes, rush communities, plant communities in meadows, pastures and psammophilous grasslands, segetal and ruderal vegetation in farmland, which occupy an area of 1476 ha, i.e. 11.7% of the total area of the national park proposed.

4. Evaluation of natural resources

4.1. Syntaxonomic classification

The following vegetation units were identified in the Rominncka Forest on the basis of the syntaxonomic classification system proposed by Matuszkiewicz (2008) and Czerwiński (1985):

Cl. *Stellarietea mediae* R.Tx., Lohm. et Prsg 1950
        Anthropogenic communities in farmland with Cl. *Stellarietea mediae* R.Tx., Lohm. et Prsg 1950
Cl. *Artemisietea vulgaris* Lohm., Prsg et R.Tx in R.Tx. 1950
        Communities of perennial ruderal plants and shrubs in anthropogenic habitats with Cl. *Artemisietea vulgaris* Lohm., Prsg et R.T in R.Tx. 1950
Cl. (Subcl.) *Galio-Urticenea* (Pass. 1967)
        Communities of nitrophilous herbaceous plants with Subcl. *Galio-Urticenea* (Pass. 1967)
Cl. *Montio-Cardaminetea* Br.Bl. et R.Tx. 1943
    Order *Montio-Cardaminetalia* Pawł.1928
        All. *Cardamino-Montion* Br.-Bl. 1925
            Community *Cardamine amara-Chrysosplenium alternifolium* Oberd. 1977 (= *Cardamino-Alnetum glutinosae* (Meijer-Dress 1936) Pass. 1968 – spring alder forest
Cl. *Phragmitetea* R.Tx. et Prsg 1942
    Order *Phragmitetalia* Koch 1926
        All. *Phragmition* Koch 1926 – rush communities
        All. *Magnocaricion* Koch 1926 – sedge communities
        Communities with plants of All. *Magnocaricion* Koch 1926 and *Phragmition* Koch 1926 – mosaic of rush and sedge communities
Cl. *Molinio-Arrhenetheretea*
    Order *Molinietalia caeruleae* W. Koch 1926
        All. *Filipendulion ulmariae* Segal 1966 – communities of herbaceous plants
        All. *Molinion caeruleae* W.Koch 1926 – communities of moor-grass meadows
        All. *Calthion palustris* R.Tx. 1936 em. Oberd. 1957 – communities of marshy and wet meadows

- Order *Arrhenatheretalia* Pawł. 1928
    - All. *Arrhenatherion elatioris* (Br.-Bl. 1925) Koch 1926
    - Lowland hay meadows of All. *Arrhenatherion elatioris* (Br.-Bl. 1925) Koch 1926 and hay meadows on mineral soil

Cl. *Scheuchzerio-Caricetea* (Nordh. 1937) R.Tx 1937 – small-sedge mires in fens and raised bogs

Cl. *Oxycocco-Sphagnetea* Br.-Bl. et R.Tx. 1943
- Order *Sphagnetalia magellanici* (Pawł. 1928) Moore (1964) 1968
    - All. *Sphagnion magellanici* Kästner et Flössner 1933 em. Dierss. 1975
        - Ass. *Sphagnetum magellanici* (Malc. 1929) Kästner et Flössner 1933 – Sphagnum peat bog
        - Ass. *Ledo-Sphagnetum magellanici* Sukopp 1959 em. Neuhäusl 1969 –peat bog pine forest

Cl. *Alnetea glutinosae* Br.-Bl. et R.Tx. 1943
- Order *Alnetalia glutinosae* R.Tx. 1937
    - All. *Alnion glutinosae* (Malc. 1929) Meijer Drees 1936
        - Ass. *Salicetum pentandro-cinereae* (Almq. 1929) Pass. 1961 – willow shrubs
        - Ass. *Ribeso nigri-Alnetum* Sol.-Górn. (1975) 1987 – alder carr
        - Ass. *Sphagno squarosi-Alnetum* Sol.-Gór. (1975) 1987 – alder peat forest
        - Ass. *Thelypteridi-Betuletum* Czerw. 1972 – marshy forest with pine and birch (= Community *Betula pubescens-Thelypteris palustris*)

Cl. *Querco-Fagetea* Br.-Bl. et Vlieg. 1937
- Order *Fagetalia sylvaticae* Pawł. in Pawł., Sokoł. et Wall. 1928
    - All. *Alno-Ulmion* Br.-Bl. et R.Tx. 1943 – riparian forests
        - Ass. *Fraxino-Alnetum* W. Mat. 1952 – alder-ash riparian forest
        - Ass. *Stellario nemorum-Alnetum glutinosae* Lohm. 1957 – alder-ash riparian forest with wood stichwort
        - Ass. *Ficario-Ulmetum minoris* Knapp 1943 em. J.Mat.1976 – elm-ash riparian forest
        - Ass. *Piceo-Alnetum* Sokoł.1980 – spruce-alder riparian forest
    - All. *Carpinion betuli* Issl. 1931 em. Oberd. 1953
        - Ass. *Tilio cordatae-Carpinetum betuli* Tracz. 1962
        - Sub-ass. *Tilio cordatae-Carpinetum betuli calamagrostietosum* – sub-continental oak-linden-hornbeam forest with reed grass (poorly developed oak-linden-hornbeam forest)

Sub-ass. *Tilio cordatae-Carpinetum betuli typicum* – typical sub-continental oak-linden-hornbeam forest (typical oak-linden-hornbeam forest)

Sub-ass. *Tilio cordatae-Carpinetum betuli stachyetosum* – fertile sub-continental oak-linden-hornbeam forest (fertile oak-linden-hornbeam forest)

Ass. *Tilio-Piceetum* Czerw. 1978 boreal spruce forest (= *Corylo-Piceetum* Sokoł. 1980)

Cl. *Vaccinio-Piceetea* Br.-Bl. 1939

Order *Cladonio-Vaccinietalia* Kiell.-Lund 1967

All. *Dicrano-Pinion* Libb. 1933

Sub-all. *Dicrano-Pinenion* Seibert in Oberd. (ed.) 1992

Ass. *Peucedano-Pinetum* W.Mat. (1962)1973 – sub-continental fresh coniferous forest

Ass. *Molinio* (*caeruleae*)*-Pinetum* W.Mat et J.Mat 1973 – moist coniferous forest

Ass. *Vaccinio uliginosi-Pinetum* Kleist 1929 – marshy pine forest

Order *Vaccinio-Piceetalia* Br.-Bl. 1939

All. *Vaccinio-Piceenion* Oberd. 1957

Ass. *Vaccinio myrtilli-Piceetum* Sokoł. 1980 moist spruce forest, (= *Vaccinio myrtilli-Pinetum* Kob.(1930) em. Czerw. 1978 mixed coniferous forest (= *Carici digitatae-Piceetum* Czerw.1978)

Ass. *Serratulo-Piceetum* Sokoł 1968 – subboreal mixed coniferous forest (= *Calamagrostio arundinaceae-Piceetum* Sokoł. 1968 mixed coniferous forest with reed grass and spruce)

Ass. *Querco-Piceetum* (W.Mat. 1952) W.Mat. et Poak. 1955 – moist mixed coniferous forest with spruce and oak (Jegiel)

Ass. *Myceli-Piceetum* Czerw. 1978 marshy coniferous forest

Ass. *Sphagno girgensohnii-Piceetum* Pol. 1962 – boreal peatland spruce forest

The local flora has a high proportion of forest communities representing a wide range of habitats and phytosociological characteristics, which are found nearly exclusively in the northern regions of Poland. This includes bog woodlands, extensive riparian forests with a high degree of phytoceonotic variation, boreal forms of oak-linden-hornbeam forests, including the boreal spruce forest identified by Czerwiński (1973), the moist mixed coniferous forest (Jagiel) and the boreal peatland spruce forest.

Non-forest communities have not been inventoried in detail to date, therefore taxonomic units in higher ranks are given.

4.2. Area of the identified forest communities in the Romincka Forest

Forest communities were classified in line with the described methodology, and their area was estimated on the basis of GIS data (Table 2).

The existing habitat conditions support the development of oak-linden-hornbeam forests in 60% of the area analyzed and riparian forests in an estimated area of 1000 ha (10%). Owing to an optimal climate and supportive habitat conditions, boreal peatland spruce forests occupy a significant part of the Romincka Forest.

Table 2. Area of potential natural vegetation in the proposed National Park

Tabela 2. Struktura powierzchniowa dzisiejszej potencjalnej roślinności naturalnej w granicach projektowanego Parku Narodowego

No.	Syntaxonomic unit	area [ha]	% share of forest area
1.	Ass. *Ledo-Sphagnetum magellanici* Sukopp 1959 em. Neuhäusl 1969 – forest peat bog	39.5	0.41
2.	Ass. *Ribeso nigri-Alnetum* Sol.-Górn. (1975) 1987 – alder carr	26.0	0.27
3.	Ass. *Sphagno squarosi-Alnetum* Sol.-Gór. (1975) 1987 – alder peat forest	73.6	0.76
4.	Ass. *Thelypteridi-Betuletum* Czerw. 1972 – marshy forest with pine and birch (= Community *Betula pubescens-Thelypteris palustris*)	93.4	0.96
5.	All. *Alno-Ulmion* Br.-Bl. et R.Tx. 1943 – riparian forests	990.2	10.16
6.	Sub-all. *Tilio cordatae-Carpinetum betuli calamagrostietosum* – sub-continental oak-linden-hornbeam forest with reed grass (poorly developed oak-linden-hornbeam forest)	1 342.2	13.78
7.	Sub-all. *Tilio cordatae-Carpinetum betuli typicum* typical sub-continental oak-linden-hornbeam forest (typical oak-linden-hornbeam forest)	3 286.1	33.73
8.	Sub-all. *Tilio cordatae-Carpinetum betuli stachyetosum* – fertile sub-continental oak-linden-hornbeam forest (fertile oak-linden-hornbeam forest)	859.0	8.82

No.	Syntaxonomic unit	area [ha]	% share of forest area
9.	Ass. *Tilio-Piceetum* Czerw. 1978 boreal spruce forest (= *Corylo-Piceetum* Sokoł. 1980)	373.1	3.83
10.	Ass. *Peucedano-Pinetum* W.Mat. (1962) 1973 – sub-continental fresh coniferous forest	14.5	0.15
11.	Ass. *Molinio* (*caeruleae*)-*Pinetum* W.Mat et J.Mat 1973 – moist coniferous forest	12.7	0.13
12.	Ass. *Vaccinio uliginosi-Pinetum* Kleist 1929 – marshy pine forest	94.8	0.97
13.	Ass. *Vaccinio myrtilli-Piceetum* Sokoł. 1980 moist spruce forest	258.2	2.65
14.	Ass. *Serratulo-Piceetum* Sokoł 1968 – subboreal mixed coniferous forest (= *Calamagrostio arundinaceae-Piceetum* Sokoł. 1968 mixed coniferous forest with reed grass and spruce)	945.4	9.70
15.	Ass. *Querco-Piceetum* (W.Mat. 1952) W.Mat. et Poak. 1955 – moist mixed coniferous forest with spruce and oak (Jegiel)	529.9	5.44
16.	Ass. *Sphagno girgensohnii-Piceetum* Pol. 1962 – boreal peatland spruce forest	803.6	8.25
	Total	**9 742.2**	**100.0%**
	Non-forest communities within the boundaries of the Landscape Park	1 362.5	

4.3. Vascular plants in the Landscape Park

Data regarding the flora of the Romincka Forest and the surrounding areas (Table 8) have been compiled by Wołkowycki (2004) in the Protection Plan for the Romincka Forest Landscape Park. Historical data were verified and updated on the basis of own research and publications available before 2005 (Łachacz 2002; Olesiński 1962; Pawlikowski 2000a, b, 2001; Polakowski 1962, 1963; Zaręba 1975; Zając A., Zając M. 2001). For the needs of this study, the above database was expanded to account for the resources published after 2005 (Pawlikowski 2011), inventory data from the Gołdap Forest District and own research performed in 2011. The above resources were used to develop a list of special concern species (Table 3).

Table 3. Protected species in the Romincka Forest, listed in the Red Book of Endangered Species and the Red List of Threatened Species

Tabela 3. Gatunki chronione, z Czerwonej Księgi i Czerwonej Listy podawane z Puszczy Rominckiej

Botanical name	Common name	Number of identified localities or frequency of occurrence
Aconitum x cammarum L. emend. FR.	Monkshood	probably of anthropogenic origin, dispersed in cemeteries
Agrimonia pilosa LEDEB.	Hairy agrimony	dispersed
Allium ursinum L.	Ramsons	5
Anemone sylvestris L.	Snowdrop anemone	locality not confirmed
Aquilegia vulgaris L.	European columbine	dispersed
Arnica montana L.	Leopard's bane	locality not confirmed
Aruncus sylvestris Kostel.	Goat's beard	very rare
Batrachium fluitans (LAM.) WIMM.	River crowfoot	dispersed
Betula humilis SCHRANK	Shrub birch	3
Blechnum spicant (L.) ROTH	Deer fern	locality not confirmed
Botrychium lunaria (L.) SW.	Common moonwort	locality not confirmed
Campanula latifolia L.	Wide-leaved bellflower	very rare
Carex atherodes SPRENG.	Wheat sedge	13
Carex chordorrhiza L. F.	String sedge	2
Carex disperma DEWEY	Softleaf sedge	7
Carex globularis L.	Globular sedge	locality not confirmed
Carex limosa L.	Mud sedge	rare
Carex loliacea L.	Ryegrass sedge	6
Centaurium erythraea RAFN	Common centaury	dispersed
Chimaphila umbellata (L.) W. P. C. BARTON	Umbellate wintergreen	very rare
Convallaria majalis L.	Lily of the valley	frequent
Corallorhiza trifida CHÂTEL.	Early coralroot	3
Dactylorhiza baltica (KLINGE) N. I. ORLOVA	Baltic orchid	1
Dactylorhiza fuchsii (DRUCE) SOÓ	Common spotted orchid	12
Dactylorhiza incarnata (L.) SOÓ	Early marsh orchid	16
Dactylorhiza x *kernerorum* (SOÓ) SOÓ	Early marsh orchid x Common spotted orchid	9
Dactylorhiza maculata (L.) SOÓ	Heath spotted orchid	very rare

Botanical name	Common name	Number of identified localities or frequency of occurrence
Dactylorhiza majalis (RCHB.) P. F. HUNT & SUMMERH.	Western marsh orchid	rare
Dactylorhiza ruthei (R. RUTHE ET M. SCHULZE IN R. RUTHE) SOÓ	Ruthe's orchid	2
Daphne mezereum L.	Mezereon	dispersed
Digitalis grandiflora MILL.	Big-flowered foxglove	dispersed
Dracocephalum ruyschiana L.	Northern dragonhead	locality not confirmed
Drosera anglica HUDS.	Great sundew	locality not confirmed
Drosera intermedia HUDS.	Spoonleaf sundew	1
Drosera rotundifolia L.	Common sundew	quite frequent in peatlands
Epipactis atrorubens (HOFFM.) BESSER	Dark red helleborine	1
Epipactis helleborine (L.) CRANTZ	Broad-leaved helleborine	4
Epipactis palustris (L.) CRANTZ	Marsh helleborine	rare
Frangula alnus MILL.	Alder buckthorn	common
Galanthus nivalis L.	Common snowdrop	rare, anthropogenic origin
Galium odoratum (L.) SCOP.	Woodruff	frequent
Gentianella uliginosa (WILLD.) BÖRNER	Dune gentian	locality not confirmed
Goodyera repens (L.) R. BR.	Creeping lady's tresses	5
Gymnadenia odoratissima (L.) RICH.	Short-spurred fragrant orchid	locality not confirmed
Hammarbya paludosa (L.) KUNTZE	Bog orchid	very rare
Hedera helix L.	Common ivy	rare, anthropogenic origin
Helichrysum arenarium (L.) MOENCH	Dwarf everlast	rare
Hepatica nobilis SCHREB.	Kidneywort	frequent
Hierochloë odorata (L.) P. BEAUV.	Sweet grass (Buffalo grass)	very rare
Huperzia selago (L.) BERNH. EX SCHRANK & MART.	Northern firmoss	11
Jovibarba sobolifera (SIMS) OPIZ	Houseleek	rare, anthropogenic origin
Lathyrus palustris L.	Marsh pea	rare
Ledum palustre L.	Marsh Labrador tea	quite frequent

Botanical name	Common name	Number of identified localities or frequency of occurrence
Lilium bulbiferum L.	Orange lily	rare, anthropogenic origin
Lilium martagon L.	Turk's cap lily	dispersed
Linnaea borealis L.	Twinflower	locality not confirmed
Liparis loeselii (L). RICH.	Fen orchid	2
Listera cordata (L.) R. BR.	Lesser twayblade	3
Listera ovata (L.) R. BR.	Common twayblade	6
Lycopodium annotinum L.	Stiff clubmoss	frequent
Lycopodium clavatum L.	Wolf's foot-clubmoss	rare
Malaxis monophyllos (L.) SW.	White adder's mouth	1
Matteucia struthiopteris (L.) TOD.	Ostrich fern	3
Menyanthes trifoliata L.	Bog-bean	dispersed
Neottia nidus-avis (L.) RICH.	Bird's-nest orchid	dispersed
Nuphar lutea (L.) SIBTH. & SM.	Yellow water lily	rare
Nuphar pumila (TIMM) DC.	Dwarf water lily	probably not encountered
Nymphaea alba L.	European white waterlily	very rare
Nymphaea candida C. PRESL	Hard waterlily	locality not confirmed
Ononis arvensis L.	Field restharrow	rare
Ophioglossum vulgatum L.	Southern adderstongue	2
Orchis mascula (L.) L. *subsp. signifera* (VEST) SOÓ	Early purple orchid	locality not confirmed
Orobanche pallidiflora WIMM. & GRAB.	Pale thistle broomrape	1
Pedicularis palustris L.	Marsh lousewort	very rare
Platanthera bifolia (L.) RICH.	Lesser-butterfly orchid	rare
Platanthera chlorantha (CUSTER) RCHB.	Greater-butterfly orchid	dispersed
Polemonium coeruleum L.	Jacob's ladder	88
Polypodium vulgare L.	Common polyplody	rare
Primula veris L.	Cowslip	dispersed
Ranunculus lingua L.	Greater spearwort	very rare
Ribes nigrum L.	Black currant	frequent
Rubus chamaemorus L.	Cloudberry	2
Salix myrtilloides L.	Swamp willow	locality not confirmed
Saxifraga hirculus L.	Marsh saxifrage	1
Scheuchzeria palustris L.	Rannoch-rush	rare

Botanical name	Common name	Number of identified localities or frequency of occurrence
Stellaria crassifolia EHRH.	Fleshy starwort	2
Swertia perennis L.	Felwort	locality not confirmed
Taxus baccata L.	Yew	very rare
Tofieldia calyculata (L.) WAHLENB.	German asphodel	locality not confirmed
Trisetum sibiricum RUPR.	Siberian oatgrass	7
Trollius europaeus L.	Globe-flower	5
Utricularia australis R. BR.	Yellow bladderwort	rare
Utricularia intermedia HAYNE	Flatleaf bladderwort	rare
Utricularia minor L.	Lesser bladderwort	rare
Utricularia vulgaris L.	Common bladderwort	rare
Viburnum opulus L.	Guelder rose	frequent
Vinca minor L.	Myrtle	dispersed in anthropogenic localities
Viola epipsila LEDEB.	Dwarf marsh violet	12

The list contains 98 species, some of which are frequently or even commonly found in the Romincka Forest, including mezereon *Daphne mezereum,* big-flowered foxglove *Digitalis grandiflora*, kidneywort *Hepatica nobilis*, northern firmoss *Huperzia selago*, Turk's cap lily *Lilium martagon*, Marsh Labrador tea *Ledum palustre*, stiff clubmoss *Lycopodium annotinum*, wolf's-foot clubmoss *Lycopodium clavatum*, bird's-nest orchid *Neottia nidus-avis* and greater butterfly orchid *Platanthera chlorantha*. Around 16 taxa are cited by Polakowski (1963) after Abromeit (1898) and are listed in the Atlas of Poland (ATPOL), but their presence has not been confirmed by contemporary sources. The list also features protected species of anthropogenic origin. Most of them colonize cemeteries, gardens and former settlements (villages) in the Romincka Forest.

4.4. The age and species structure of tree stands, the degree of transformation and growth trends

The present age and species structure of tree stands in the Romincka Forest is a reflection on former forest management practices and natural disasters in the past 150 years. In the mid 1840s, a rapid expansion of the black arches population led to a nearly complete destruction of the forest's resources. The forest was largely recreated by planting the European spruce as well as Scots pine in less fertile habitats. After World War II, coniferous trees aged 90 to 100 years had a predominant share in the forest's resources. In recent years (1997 and 1999), strong winds caused significant damage to tree stands, and a total of 122,000 m^3 of large timber was obtained in the process of waste removal. Between the 19th century and World War II, the main objective of forest management practices was to keep red deer populations high. Extensive grassland reclamation efforts led to radical changes in water relations in the forest.

The current age structure of tree stands within the boundaries of the proposed National Park is presented in Table 4.

Table 4. Age structure of tree stands

Tabela 4. Struktura wiekowa drzewostanów

Age classes	Area [ha]	Share (%) of total forest area
Age class I (thickets up to 20 years)	793.47	8.08
Age class II (21–40 years)	2 824.39	28.78
Age class III (41–60 years)	3 237.74	33.00
Age classes IV and V (61–100 years)	1 693.75	17.26
Stands aged 101–140 years	1 054.07	10.74
Stands older than 141 years	210.18	2.14
Total	**9 813.60**	**100.00**

Around 70% of tree stands are aged up to 60 years. Old stands occupy only 210 ha, mostly on the territory of protected timber reserves. The average age of tree stands in the Romincka Forest is 53 years, and the predominant spruce stands are relatively dispersed (236 m^3/ha) with the average age of 54 years.

Changes in the age structure of tree stands observed over the last 50 years point to a significant decrease in the proportion of older trees. The above is a natural consequence of timber production and harvesting of mature trees.

5. Conclusions – arguments for and against the creation of the Romincka Forest National Park

Arguments for the creation of the Romincka Forest National Park:

1. The local flora has a high proportion of forest communities representing a wide range of habitats and phytosociological characteristics, which are found nearly exclusively in the northern regions of Poland.
2. Local resources significantly contribute to the conservation of boreal forest communities in Poland and Europe. They are of great scientific and practical value, they provide valuable insights into resource management methods, including in marshy habitats or degraded oak-linden-hornbeam forests, by promoting spruce communities.
3. The Romincka Forest is a an abundant source of flora characteristic of boreal regions. The majority of taxa in hydrogenic forests are listed in the Red Book of Endangered Species and the Red List of Threatened Species. The most valuable species include Lithuanian mannagrass *Glyceria lithuanica*, softleaf sedge *Carex disperma*, fewflower sedge *C. pauciflora*, ryegrass sedge C. *loliacea*, string sedge *C. chordorrhiza*, mud sedge *C. limosa*, pale sedge *C. pallescens* and shrub birch *Betula humilis.*
4. Most sources indicate that the Romincka Forest, in particular its forest, marsh, meadow and rush ecosystems, are undergoing natural regeneration. The abandonment of drainage systems in extensive meadows and forest marshes, water retention resulting from beaver activity, the implementation of small water retention reservoirs, the introduction of new methods for forest resource management and the implementation of programs for the reconstruction of tree stands support the above goal.
5. The Romincka Forest provides habitat for wolves, lynxes, otters, beavers, 10 bat species, bird species of special concern and many invertebrate species which remain poorly known in their respective taxonomic groups.
6. The Romincka Forest is not a mass tourism site, and it is subject to low anthropogenic pressure. Ventures in professional and educational tourism can be developed on a broader scale without generating adverse consequences for the local habitat.
7. The Romincka Forest has a small percentage share of farmland and potential sites for industrial, tourist and recreational development which are generally a source of social conflict.
8. The National Park scheme is likely to be approved by the local community.

Arguments against the creation of the Romincka Forest National Park:

1. Non-forest ecosystems are relatively poorly diversified. There are no lakes or communities of aquatic fauna and flora.
2. In the 19th century and the first half of the 20th century, vast efforts were made to reclaim non-forest areas (conversion of peatlands into grasslands) and forests (promoting spruce stands, hunting priorities). The consequences of those anthropogenic transformations are still visible today.
3. Nearly 70% of tree stands are aged up to 60 years, and trees older than 120 years have a negligent share in the forest. For the National Park scheme to take effect, tree stands should be reconstructed and adapted to local habitat requirements. The present map of potential natural vegetation indicates that the existing habitat conditions are conducive to the growth of boreal oak-linden-hornbeam forests in 60% of the analyzed area, fertile riparian forests in 10%, boreal peatland spruce forests – in 9–10% and moist spruce and oak forests – in 5% of the analyzed territory.

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Puszcza Romincka – za i przeciw powołaniu Parku Narodowego

Streszczenie

W sieci Parków Narodowych w Polsce, jej część północno-wschodnia nie jest reprezentowana. Artykuł stara się odpowiedzieć na pytanie czy walory przyrodnicze Puszczy Rominckiej są na tyle istotne, że zasługują na tą formę ochrony wielkoobszarowej. W znacznej części opiera się on na kwerendzie dostępnej literatury i materiałów niepublikowanych jak też badań własnych szczególnie w zakresie prezentowanej mapy dzisiejszej potencjalnej roślinności naturalnej.

Proponowany obszar obejmuje 85% powierzchni dotychczasowego Parku Krajobrazowego Puszcza Romincka.

Istotne walory przyrodnicze tego terenu są następujące:

- Szata roślinna omawianego obszaru wyróżnia się przede wszystkim dużym udziałem zbiorowisk leśnych charakterystycznych niemal wyłącznie dla działu północnego lub (oraz) występujących tutaj w pełnej skali zmienności siedliskowej i fitosocjologicznej.
- Obszar Puszczy Rominckiej charakteryzuje się wyjątkowo bogatą florą o charakterze borealnym. Większość gatunków występujących w zbiorowiskach lasów hydrogenicznych to gatunki zagrożone z Czerwonej księgi lub Czerwonej listy. Do najcenniejszych należą: manna litewska Glyceria lithuanica, turzyca szczupła Carex disperma, turzyca skąpokwiatowa C. pauciflora, turzyca życicowa C. loliacea, turzyca strunowa C. chordorrhiza, turzyca bagienna C. limosa, turzyca blada C. palescens, brzoza niska Betula humilis.
- Większość danych przyrodniczych wskazuje, że obszar Puszczy Rominckej, a szczególnie ekosystemy leśne, bagienne i łąkowo-szuwarowe są w stadium regeneracji w kierunku zbiorowisk naturalnych. Sprzyjają temu: zaniechanie melioracji odwadniających

rozległych łąk i bagien puszczańskich, retencja wody wywołana przez bobry, wdrożenie niewielkich, ale znaczących programów małej retencji, zmian metod gospodarowania w lesie oraz wdrażanie programów przebudowy drzewostanów.

- Puszcza jest ważną ostoją wilka, rysia, wydry, bobra, 10 gatunków nietoperzy, gatunków ptaków „specjalnej troski” oraz wielu gatunków słabo rozpoznanych w niektórych grupach systematycznych bezkręgowców.
- Stosunkowo mała presja antropogeniczna o charakterze masowej turystyki. Turystyka kwalifikowana i o charakterze edukacyjnym może być realizowana w szerszym niż dotychczas zakresie, bez ujemnych skutków dla walorów przyrodniczych obszaru.
- Mały udział powierzchniowy obszarów rolniczych i potencjalnych terenów do zainwestowania o charakterze przemysłowym, turystycznym, rekreacyjnym, które z reguły są źródłem konfliktów społecznych.

Fungi and fungus-like organisms from the lower course of the Horodnianka river, Podlasie Province

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Abstract

Studies concerning the occurrence of some fungi and fungus-like organisms, in the lower course of the Horodnianka river in Podlasie Province, were made in the years 2011–2012. Bait method was used to isolate the fungi from water. Aquatic fungi found in Horodnianka river included 19 fungus-like organisms from the kingdom Chromista, class Oomycetes and 11 fungi from the kingdom Fungi, anamorphic fungi (5), Chytridiomycetes (5) and Zygomycetes (1). The fungi identified in the water samples represented 30 species, 20 genera: Achlya – 5 species, Saprolegnia – 4 species, Aphanomyces, Catenaria and Pythium, 2 species each and 15 single species of Apodachlya, Alternaria, Aspergillus, Catenophlyctis, Cladosporium, Dictyuchus, Fusarium, Lagenidium, Leptolegnia, Leptomitus, Nowakowskiella, Penicillium, Phlyctochytrium, Thraustotheca and Zoophagus. The most common species were Achlya polyandra, Aphanomyces laevis, Apodachlya pyrifera, Catenophlyctis variabilis, Fusarium aqueductum, Pythium rostratum, Saprolegnia diclina, S. ferax and S. parasitica. Several aquatic fungi and fungus-like organisms species found in the river were either new to Polish fungal biota – Leptolegnia caudata and Zoophagus insidians or rare – Lagenidium giganteum.

Key words: fungi, fungus-like organisms, Poland, river.

1. Introduction

Fungi can be found in all kinds of aquatic habitats both in freshwater and marine waters. They occur as saprophytes or parasites of plants and animals. Aquatic fungi play an important role among reducers, taking active part in the mineralization of the organic matter of plant origin, including branches, fruits and tree leaves that fell to water (Alexopoulos et al. 1996; Dick 2001). Some of them act as parasites of plants, animals and humans (Dick 2001; Massoud 2012). A separate group of aquatic fungi is that of predatory species which grow on dead fragments of plants and animals and sometimes catch as prey some invertebrate animals (Kiziewicz, Czeczuga 2003). About 200 predatory species have been identified so far (Czygier, Boguś 2001; Barron 2003). The main aim of the present study was to establish the diversity of fungi and fungus-like organisms found in the Hordnianka river from the lower course of the river in sites: Choroszcz and Żółtki located in Podlasie Province.

2. Study area

Water samples for the experiments were collected from the Horodnianka river flowing through Choroszcz and agricultural areas. It is a right-bank tributary of the Narew river, of the length of 27.4 km, catchment area of 76 km^2, depth ranging from 0.3 to 0.5 m depending on the season. Horodnianka river joins the Narew river near the bridge in the village Żółtki. The area is located in south western part of Białystok (53^0 05$'$ N, 23^0 10$'$ E).

3. Methods

The research material were water samples collected in 2011 and 2012 from the lower course of the Horodnianka river in sites: Choroszcz and Żółtki. At each site of sample collection, water was scooped directly into sterile 500 ml polyethylene bottles from the surface layer of water.

Microbial analysis for baiting and recovery of fungi and fungus-like organisms. Water samples collected from the Horodnianka river were brought directly to the laboratory for the recovery of aquatic fungi and fungus – like organisms. Baiting technique with the use of onion skin (*Allium cepa*), hemp-seeds (*Cannabis sativa*), cellophane, crucian carp eggs (*Carassius carassius*), and snake skin (*Natrix natrix*), was applied to isolate fungi from water. The baits were transferred to one-litre vessels and poured with water from the respective site of the river and placed in the laboratory at a room temperature (15–20^{0}C). After 3–4 days, the baits (onion skin, seeds, cellophane, eggs, fragments of snake skin) were colonized, a single hypha or sporangium was isolated under microscope and were placed in sterilized Petri dishes containing sterile distilled water and hemp seeds halves in order to obtain new colonies. Microscopic examinations of the mycelia were repeated after a few days. Several microscopic preparations were made each time. The duration of experiments was four weeks. The identification of the fungi and fungus-like organisms was based on morphological and biometric data of the vegetative organs – shape and size of the hyphae, asexual reproductive organs – shape of sporangium and spores, and generative organs – the structure of the oogonium, oospores and antheridium and conidiophores and conidia of the anamorphic fungi. Measurements and observations were made on those colonies growing in water cultures using Nicon Eklipse 50*i* microscope. The macro- and microscopic observations were photographically documented. The fungi were identified using the works by Dudka (1974), Batko (1975), Fassatiová (1983), Seymour, Fuller (1987) and Dick (1990a, 2001).

Statistical analysis. The results were subjected to statistical analysis using t-test to determine the significance of differences ($p \leq 0.05$) between sampling sites (river) in total number of species (richness) and total frequency.

4. Results

The fungi and fungus-like organisms identified in the water samples studied are listed in Table 1 and drawn in Figs 1–3. Aquatic fungi identified in the Horodnianka river included 19 fungus-like organisms from the kingdom Chromista, class Oomycetes and 11 fungi from the kingdom Fungi, anamorphic fungi (5), Chytridiomycetes (5) and Zygomycetes (1). The fungi represented 30 species, 20 genera: *Achlya* – 5 species, *Saprolegnia* – 4 species, *Aphanomyces, Catenaria* and *Pythium,* 2 species and 15 other single species 15: *Apodachlya*, *Alternaria, Aspergillus, Catenophlyctis, Cladosporium, Dictyuchus, Fusarium, Lagenidium, Leptolegnia, Leptomitus, Nowakowskiella, Penicillium, Phlyctochytrium, Thraustotheca* and *Zoophagus.*

The highest number of species and the highest total frequency were obtained at the Horodnianka river site in Żółtki while the lowest number of species and the lowest total frequency were recorded from the Horodnianka river site Choroszcz.

Differences in the total number of species and in total frequency between the site Żółtki and Choroszcz were statistically significant (Tab. 1; p≤00.5). The most common species included *Achlya polyandra, Aphanomyces laevis, Apodachlya pyrifera, Catenophlyctis variabilis, Fusarium aqueductum, Pythium rostratum, Saprolegnia diclina, S. ferax* and *S. parasitica*. Several aquatic fungi and fungus-like organisms species found in the river were either new to Polish fungal biota – like *Leptolegnia caudata* and *Zoophagus insidians* or rare – like *Lagenidium giganteum*.

Table 1. Diversity of fungi identified in water samples from each site of the Horodnianka river

Tabela 1. Różnorodność gatunkowa grzybów izolowanych z każdego stanowiska na rzece Horodnianka

Fungal taxa (Kingdom, class, order, species)	Choroszcz	Żółtki	Total
Chromista = Stramenopila			
Oomycetes = Oomycota = Peronosporomycetes			
Leptomitales			
Apodachlya pyrifera Zopf.	+	+	+
Leptomitus lacteus Agardh	+	-	+
Pythiales			
Lagenidium giganteum Couch	+	-	+
Pythium debaryanum R.Hesse	-	+	+
Py. rostratum Butler	+	+	+
Saprolegniales			
Achlya americana Humphrey	-	+	+
Ac. colorata Pringhs	-	+	+
Ac. debaryana Humphrey	+	-	+
Ac. dubia Coker	+	-	+
Ac. polyandra Hildebrand	+	+	+
Aphanomyces laevis de Bary	+	+	+
A. irregularis W.W. Scott	-	+	+
Dictyuchus monosporus Leitg	-	+	+
Leptolegnia caudata de Bary	-	+	+

Fungal taxa (Kingdom, class, order, species)	Choroszcz	Żółtki	Total
Saprolegnia diclina Humhrey	+	+	+
S. ferax (Gruith.) Thur.	+	+	+
S. parasitica Coker	+	+	+
S. unispora (Coker & Couch) R.L. Seym.	-	+	+
Thraustotheca clavata de (Bary) Humphrey	-	+	+
Zygomycetes			
Zoopagales			
Zoophagus insidians Sommerst.	-	+	+
Fungi			
Chytridiomycetes			
Blastocladiales			
Catenaria anguillulae Sorokin	-	+	+
Catenaria verrucosa Karling	-	+	+
Catenophlyctis variabilis (Karling) Karling	+	+	+
Chytridiales			
Nowakowskiella elegans (Nowakowski) Schroeter	-	+	+
Phlyctochytrium aureliae Ajello	-	+	+
Anamorphic fungi			
Alternaria alternata Fries	-	+	+
Aspergillus niger var *niger* Tiegh.	-	+	+
Cladosporium herbarum (Pers.) Link	-	+	+
Fusarium aqueductum Rabenh. and Radlk.	+	+	+
Penicillium notatum Westling	-	+	+
S	13	26	30
TF(%)	43.00*	87.00	100

Legend:

S – total number of species; TF(%) – total frequency.

* – significant differences ($p \leq 0.05$).

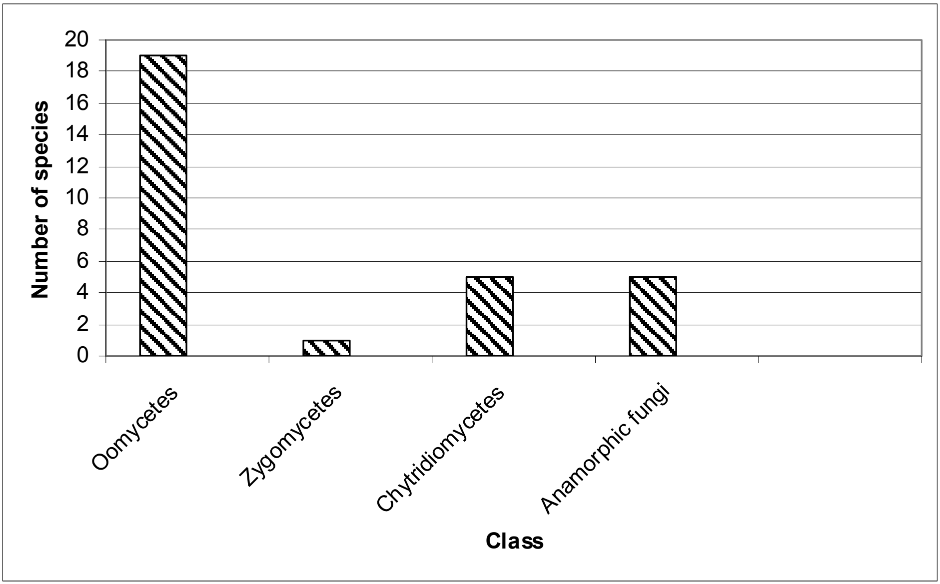

Figure 1. Number of fungal species in Class recovered from Horodnianka River

Rycina 1. Liczba gatunków grzybów w klasach znalezionych w rzece Horodnianka

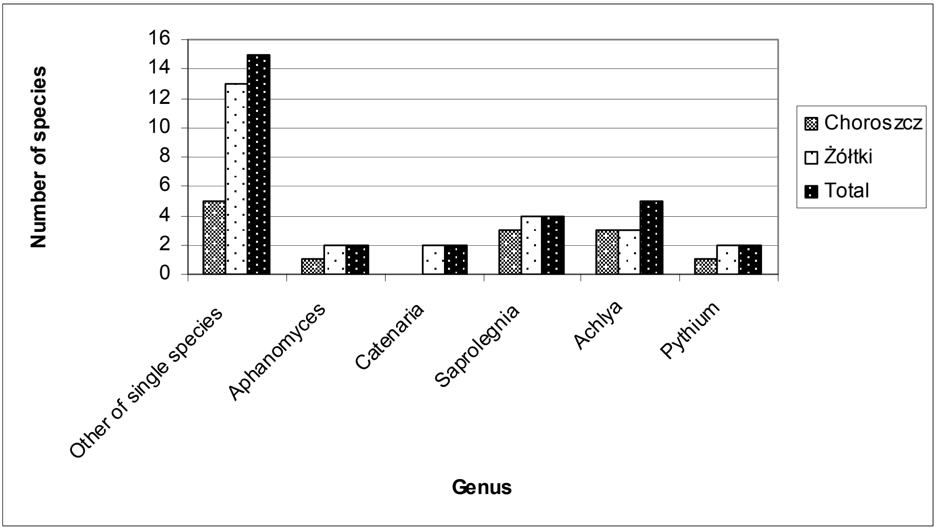

Figure 2. Number of species in the different of fungal general recovered from Horodnianka River

Rycina 2. Liczba gatunków w poszczególnych rodzajach grzybów oznaczonych w rzece Horodnianka

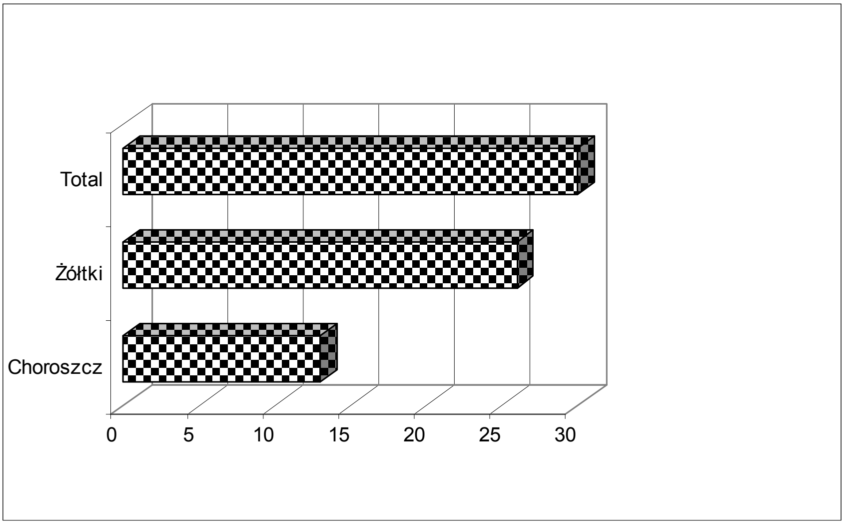

Figure 3. Number of fungal species recovered in each site of Horodnianka River

Rycina 3. Liczba gatunków grzybów oznaczonych na każdym stanowisku na rzece Horodnianka

5. Discussion

The present study the fungi and fungus-like organisms originating from water samples of the Horodnianka river were identified on the onion, hemp-seeds, crucian carp eggs, and snake skin which were used as baits. In total, 30 species of fungi were identified in the water of the river studied. The fungi linked with water environment were represented by over one thousand species (Batko 1975; Dick 2001). Aquatic fungi mainly belong to Mastigomycotina (the zoosporic fungi) and to the Deuteromycotina. However, some Ascomycota and Basidiomycota are known to have aquatic forms. In rivers and streams a lot of representatives of the Class Oomycetes are met. The phylogenetic relationship of Oomycetes (watermolds) to fungi has been debated for many years. Oomycota (oomycota means egg fungus) or Oomycetes have been for a long time recognized as significantly different from the organisms classified as the Phylum Oomycetes in the Kingdom Fungi (True fungi) (Alexopoulos et al. 1996). Scientific studies have shown that some organisms may look like fungi yet are not really members of the Kingdom Fungi. A cladistic classification based on modern insights supports a relatively close relationship between Oomycetes with photosynthetic organisms such as brown algae and diatoms, within the heterokonts. The Oomycetes have been differently classified by numerous taxonomists, for instance they have been classified as heterokont organisms in the

Kingdom Stamenopila, Phylum Heterokonta, Class Peronosporomycetes (Alexopoulos et al. 1996; Dick 2001). Presently, Oomycetes are referred to as fungus-like eukaryotic microorganisms and they are grouped in the Chromalveolata Super-Kingdom/Supgroup, Chromista/Stramenopila Kingdom, Heterokontophyta Phylum, Oomycota = Oomyctes Class (Adl et al. 2005; Kirk et al. 2008). Members of the family of Saprolegniaceae from the Class Oomycota are cosmopolitan and yet very little known; they are difficult to key out and their identification is mainly based on features of the reproductive structures, that is, the size and shape of oogonia, antheridia and sporangia. All members of Oomycetes have motile zoospores. Fungus-like organisms from the Class Oomycetes were the most frequent microorganisms in the running waters studied. There are typical species like *Achlya americana, A. debaryana, A. polyandra, Aphanomyces laevis, Dictyuchus monosporus, Pythium debaryanum, Saprolegnia ferax* and *Saprolegnia parasitica* (Kiziewicz 2012). *Achlya americana* has been known as fungus-like organism mostly saprotrophic which occurred in soil land and surface waters. Khulbe and Sati (1981) have studied the composition of fungus species in the Asian mountain rivers and have isolated *Achlya americana* together with the other microorganisms from the bodies of fish living in these rivers. They have shown that this saprotrophic species can also be fungal pathogen and may cause fungal infection in fish. In Poland this species as a saprotroph was detected in waters of the Bug River by Czeczuga et al. (2002c).

Common taxons like *Saprolegnia diclina*, *S. ferax* and *S. parasitica* have been found in all studies. The genus *Saprolegnia* is cosmopolitan, which means that it occurs everywhere in freshwater in the world. *Saprolegnia parasitica* is the best known out of all the known parasites of the genus. It causes saprolegniosis of fish and their eggs and broodstocks. Saprolegniosis is the most trouble making infection of salmonids, common carp in fresh water farms in the world (Hatai, Hoshiai 1992; Hussein et al. 2001; Johnson et al 2002; Czeczuga et al. 2004, 2005; Steciov et al. 2007; Fadaeifard et al. 2011). The most common strain in this study was *Penicillium notatum* which is a common species in nature, however it has not been identified from fish as a pathogenic agent. However some species of the genus *Penicillium* can cause fungal diseases of fish. Mycosis infections associated with the family Saprolegniaceae have been widely reported in freshwater fish (Hussein, Hatai 2002; Czeczuga et al. 2002a, b; Fadaeifard et al. 2011).

The remaining lower incidence fungus species isolated in the river Horodnianka, such as *Catenaria anguillulae, C. verrucosa, Nowakowskiella elegans, Pythium debaryanum,* and *Py. rostratum*, belong to phytosaprobionts. These fungi contain numerous pectin and cellulose enzymes that decompose pectin and cellulose found in seeds, fruits, flower petals, leaves, stems and other parts of plants submerged in water. Thanks to the enzymatic capabilities they mineralize the plant organic matter (Batko 1975; Chandrashekar, Kaveriappa 1988).

During the studies in Horodnianka river, some fungus-like organisms from the genus *Pythium* were detected. At present about one hundred species from this genus are known and half of them live in water (Dick 1990a). These species are considered as soil saprotroph or parasites of plants (Kiziewicz 2005; Czeczuga, Snarska 2001). An interesting finding was that of the two species of fungus-like organisms that induce plant diseases *Pythium debaryanum* and *Py. rostratum* (Batko 1975).

Some fungi could change saprophytisms to other kinds of ecological interactions e.g. parasitism or predation. Sometimes they catch living aquatic animals, and use them as a source of food containing nitrogen (Dick 2001; Barron 2003). Representatives of fungi have been relatively often found at various latitudes (Kiziewicz, Czeczuga 2003; Kiziewicz 2004a, b). For example *Zoophagus insidians* which was detected in the Horodnianka river, has been described to catch small water animals such as rotifers by Dick (1990b), Powell et al. (1990) and Kiziewicz (2004b). The river fungus *Catenaria anguillulae*, identified in the water samples of Horodnianka, was known as a phyto- and zoosaprotroph, however later it was discovered to colonize living aquatic animals, similarly as *Zoophagus insidians* (Czeczuga, Godlewska 1998; Czeczuga et al. 2002b).

An important *Lagenidium giganteum* fungus was detected in the Horodnianka river. The genus *Lagenidium* includes more than 50 species, the majority of which are natural parasites of algae, fungi, rotifers, nematodes, crustaceans and mosquito larvae. *Lagenidium* sp. was found in lakes and ponds in the southeastern United States (Grooters 2003). Only one species of the genus *Lagenidium,* namely *Lagenidium giganteum* is known as a facultative parasite of mosquito larvae. It has recently been registered by U.S. Environmental Protection Agency for operational mosquito control (Singh, Prakash 2010). *Lagenidium giganteum* causes a disease named lagenidiosis in animals especially in dogs (Grooters 2003).

Another species found in the samples of the Horodnianka river water was a nitrophilic fungus *Leptomitus lacteus*. This fungus is commonly known as a sewage fungus and a typical representative of microorganism living in waters strongly polluted with municipal wastes (Dick 2001). The Voivodeship Inspectorate Environmental Protection in Białystok (WIOŚ Białystok, 2012) classified the water of the Horodnianka river below Choroszcz in 2011 as of poor ecological status. The water samples studied in this work were also quite polluted. The sewage fungus *Leptomitus lacteus* can also be a parasite and necrotroph of fish (Willoughby, Roberts 1991; Riethmüller et al. 2006).

Rare species of fungi found in Horodnianka river include *Alternaria alternata, Aspergillus niger* and *Cladosporium herbarum. Alternaria alternata* and *Cladosporium herbarum* are responsible for several diseases affecting plants, animals and humans (Breitenbach, Simon-Nobbe 2002).

Some of these species such as *Aspergillus niger, Penicillium notatum* are common saprotrophs but can be potentially pathogenic to humans, inhabiting the human skin, alimentary tract, and other tissues and organs (Ulfig 1996).

Catenophlyctis variabilis was frequently recovered in the Horodnianka river. This species of fungus has been described in literature as a widely spread saprotroph found on keratin substrates such as human skin and hair and has been frequently found in various aquatic bodies (Batko 1975; Godlewska et al. 2012).

These species of fungi and fungus-like organisms supplemented the list of fungi already found in the rivers of north-east part of Poland. The Horodnianka river was quite polluted, however despite that a large diversity of fungi species were detected in its water. This feature is advantageous form the point of view of the natural purification of water bodies.

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Grzyby i organizmy grzybopodobne w dolnym biegu rzeki Horodnianka na terenie województwa podlaskiego

Streszczenie

Badania dotyczące występowania niektórych grzybów i organizmów grzybopodobnych, w rzece Horodnianka w dolnym biegu w województwie podlaskim, zostały wykonane w latach 2011–2012. Do izolowania grzybów z wody zastosowano metodę przynęt. Grzyby wodne stwierdzone w wodzie rzeki Horodnianka, reprezentowane są przez 19 organizmów grzybopodobnych z królestwa Chromista, klasy Oomycetes oraz 11 grzybów z królestwa Fungi, w tym grzyby anamorficzne (5) oraz klasy Chytridiomycetes (5) i Zygomycetes (1). Ze wszystkich badanych stanowisk oznaczono 30 gatunków grzybów, 20 rodzajów: z rodzaju *Achlya* – 5 gatunków, z rodzaju *Saprolegnia* – 4 gatunki, z rodzaju *Aphanomyces, Catenaria* i *Pythium* po 2 gatunki oraz 15 pojedynczych gatunków z rodzaju *Apodachlya*, *Alternaria, Aspergillus, Catenophlyctis, Cladosporium, Dictyuchus, Fusarium, Lagenidium, Leptolegnia, Leptomitus, Nowakowskiella, Penicillium, Phlyctochytrium, Thraustotheca* i *Zoophagus.* Do pospolitych gatunków należały: *Achlya polyandra, Aphanomyces laevis, Apodachlya pyrifera, Catenophlyctis variabilis, Fusarium aqueductum, Pythium rostratum, Saprolegnia diclina, S. ferax* i *S. parasitica*. Kilka gatunków grzybów i organizmów grzybopodobnych występujących w rzece zanotowano jako nowe dla Polski: *Leptolegnia caudata* i *Zoophagus insidians* lub rzadko występujące: *Lagenidium giganteum*.

Species diversity of fungi in communities in selected types of post-bog soil

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Abstract

The qualitative and quantitative compositions of communities of fungi living at particular genetic levels of the soil studied were determined and the species dominant in individual communities were identified. Species diversity of fungi in communities occurring at different genetic levels of post-bog soil differing in the degree of dehydration was studied. Analyses of the fungi communities were made at five sites in peat-muck soil (weakly, medium and strongly mucked). Two sites were established in the Narew river valley and the other three in the Biebrza river valley. The total number of 1853 fungi isolates represented 53 species. The peat-muck soils of medium and strong degree of transformation revealed much greater number of fungi communities and their higher species diversity than the weakly transformed soil, indicating that increasing intensity of soil transformation favours greater diversity of fungi species in the communities growing in dehydrated peatland. The muck layers, especially the turf layer, were occupied by fungi communities of much stronger diversified qualitative and quantitative structures than those found in deeper layers of peat. The fungi species most frequently met in the soil analyses included *Penicillium simplicissimum, P. janczewskii* and *P. waksmanii* along with *Helicosporium vegetum*.

Key words: peat-muck soils, soil fungi species

1. Introduction

The hitherto recognised species diversity of fungi makes only a small part of the expected and potentially present number of species (Kirk et al. 2001). From the area of Poland about 6.5 thousand fungi species have been described, which are estimated to make about 52% of expected and potentially present species. Unfortunately, no region of Poland has been sufficiently well examined as to the presence of fungi species. The same is true about the most valuable natural objects such as national parks, landscape parks or nature reserves (Grzywacz 2003).

It should be noted that determination of the species richness in a given area is not sufficient for evaluation of their biodiversity as besides the diversity of taxons their abundance, spatial distribution, their interrelations, intraspecies variation and many other factors hitherto neglected or unrecognised should be taken into regard. These factors contribute to the relations in the communities of organisms that have developed in response to the environmental conditions and depend on them (Mańka 2000).

The soil environment poses particular problems in assessment of diversity of microorganisms and hence fungi as well. High heterogeneity of texture, structure and chemical conditions hinder the analyses so that the degree of recognition of the real composition of soil mycoorganisms, which is their biodiversity in the sense of richness of species and their distribution in the community, is poor (Kennedy 1999; Torsvik et al. 2002; Badura 2003). Moreover, the quantitative and qualitative compositions of the communities of microorganisms occurring in a given soil undergo continuous changes in response to disturbances in physical and chemical conditions as well as those caused by physiological and metabolic activity of individual populations or even species (Liesack et al. 1997; Kozdrój 2004).

For these reasons a study was undertaken to identify the qualitative and quantitative structures of the fungi living in selected post-bog soils differing in the degree of dehydration and thus density and muck character.

2. Materials and methods

The study was performed in July 2007 in the soil types classified by the Polish soil classification, division hydrogenic soils, order post-bog soil. The peat-muck soils studied differed in the degree of dehydration. Classification of peat-muck soils was taken after Okruszko (1988): weakly mucked – profiles 1, 2 and 3, medium mucked – profile 4 and strongly mucked – profile 5. The weakly transformed peat-muck soil samples from profiles 1 and 2 were from the south part of the Narwiański National Park. These two sites were at a distance of about 2.7 km from Suraż and about 0.9 km from the Narew river. The site of profile 1 was on the east, while that of profile 2 on the west side of the river. The profiles were in the habitat of flooded overdried peatlands. The two profiles were periodically flooded by the river water with a similar intensity. The other 3 sites of profile 3 (weakly transformed peat-muck soil), 4 (medium transformed) and 5 (strongly transformed) were in drained meadows in different parts of the Biebrza river valley. The profiles were in the peat bogs in the decession phase with no periodical floodings. The weakly transformed peat-muck soil (profile 3) was in the Lower Biebrza river valley, at about 1 km SE of Uścianek village, the medium transformed peat-muck soil (profile 4) was in the Upper Biebrza river valley at about 1.8 km S of Jaminy, while the strongly transformed peat-muck soil was in the Middle Biebrza river valley, near Modzelówka village.

The weakly transformed peat-muck soils from the Narew river valley (profile 1 and 2) were formed by mucking of sedge peat and had very similar soil profile. The turf layer of these soils was 20 cm thick, filled with a network of plant roots and made of fine-aggregate fresh humus peat, almost black in colour. Below the turf layer, down to about 80 cm, there was the layer of medium-decomposed, silted, breaking to pieces sedge peat. The peat was moist towards wet. Below the peat there was a 25 cm thick layer of black, wet peat-silt formation set upon wet loose sand. The ground water table was at a depth of 35 cm.

The peat-muck soils from the sites studied in the Biebrza river valley were much different. The main factor determining the differences was different depth of drainage of the relevant peatlands. Because of the differences in the depth of drainage the muck layer at each site had different thickness and character. The soil profiles of the soils at particular sites are characterised below.

Profile 3 – weakly transformed peat-muck soil from the Lower Biebrza river valley:

0–20 cm – turf horizon interwoven with plant roots, finely aggregated humic muck, breaking up under pressure, dark-grey, fresh,

20–70 cm – medium – decomposed sedge peat of fibre structure; against a background of black humic fragments of peat-making sedges, with domination of roots and sporadic larger parts of plants; down to the water table (at 37 cm) the formation was moist, below the water table – wet,

70–130 cm – loose, light-grey sand, wet.

The ground water table was at the depth of 37 cm.

Profile 4 – medium-transformed peat-muck soil from the Upper Biebrza river valley:

0–15 cm – turf horizon interwoven with plant roots, finely aggregated humic muck, breaking up, dry,
15–26cm – subturf horizon, made of coarse-grained muck, easily breaking up under pressure, fresh
26–70 cm – medium-decomposed sedge peat of poorly developed fibre structure, fragments of sedges are seen among the peat-producing mass, fresh,
70–130 cm – strongly-decomposed alder peat, making a uniform mass of a mixture of amorphous humus with fragments of wood, breaking up under pressure, work silted of small stratification of mineral formation, black, fresh.

No ground water table was established in the soil profile.

Profile 5 – peat-muck soil, strongly transformed, from the Middle Biebrza river valley:

0–6 cm – turf horizon interwoven with plant roots, humus muck of fine-grain structure, fresh,
6–17 cm – subturf horizon of coarse-grain structure, breaking up under pressure, fresh,
17–36 cm – transitory horizon of coarse-aggregate lumpy structure, fresh,
36–79 cm – sedge peat, medium-decomposed with fragments of peat producing plants, fresh,
79–130 cm – loose sand, light-grey in colour, wet.

The ground water table established at 80 cm.

Soil samples for mycological studies were collected from each horizon of muck and from the peat layer at all sites, i.e. the samples were collected from the turf horizons in all soils studied, from the subturf horizons in medium and strongly transformed peat-muck soils, from transitory horizon developed only in strongly transformed peat-muck soli and from the peat layer in all soils studied.

For isolation of fungi *micromycetes* the Warcup (1950) method of soil cells was chosen in modification of Johnson and Mańka (1961) and Mańka and Salmanowicz (1987).

3. Results

In total 1853 of fungi isolates making 13 communities were identified (Tab. 1). They lived in the turf horizons in all soils studied (5 communities), subturf horizons of medium and strongly transformed peat-muck soil (2 communities) and transitory horizon of strongly transformed peat-muck soil (1 community). The other 5 communities were obtained from the peat relining muck in all soils studied. The greatest number of isolates were obtained from strongly transformed peat-muck soil (1241). This statement refers to the sum of abundance of fungi communities in particular genetic horizons and the communities from each horizon. A considerable number of isolates – 504 – was also found in medium transformed peat-muck soil. The lowest number of isolates, of just 32, was found in weakly transformed peat-muck soil from the site on the east bank of the Narew river (profile 1). It should be noted that the number of isolates in weakly transformed peat-muck soil was similarly low, irrespective of whether it came from the Narew river valley or Biebrza river valley, although the number of isolates from the turf layer in the Lower Biebrza valley (profile 3) was a bit higher than that from the surface muck layer in the soil from the Narwiański National Park (profile 1, 2, Tab. 1).

It should be noted that the differences in the number of fungi isolates living in the mock from the turf horizon in medium and strongly transformed soil and those living in the mock from weakly transformed soil are evident. The number of isolates obtained from profile 5 (strongly transformed peat-muck soil) was 303, from profile 4 (medium transformed peat-muck soil) – 268, while from the mock of weakly transformed peat-mock soil – only from 18 (profile 2) to 29 (profile 3, Tab. 1).

Significant numbers of isolates were found in the deeper muck layers in the soil from much dehydrated peatland in the Biebrza river valley, in the subturf horizon of medium transformed peat-muck soil – 207 isolates, while in strongly transformed peat-muck soil – 354 ones. In the transitory horizon mock of strongly transformed peat-muck soil as many as 454 isolates were identified. The number of fungi isolates from the peat layer of the same soil type was the highest than in all other types of soil, irrespectively of the degree of transformation, and medium transformed peat-muck soil (Tab. 1).

Table 1. The number of fungi isolates (frequency) in the soils studied

Tabela 1. Liczba izolatów (frekwencja) grzybów otrzymanych z badanych gleb

Genetic horizon	Soil profiles				
	Profile 1	Profile 2	Profile 3	Profile 4	Profile 5
Turf horizon	23	18	29	268	303
Subturf horizon	-	-	-	207	354
Transitory horizon	-	-	-	-	454
Peat layer	9	15	14	29	130
Summary of individual profiles	32	33	43	504	1241
Total number	1853				

Explanations
profiles 1 and 2 – peat-muck soil weakly transformed from the Narwiański National Park
profile 3 – peat-muck soil weakly transformed from the Lower Biebrza Valley
profile 4 – peat-muck soil medium transformed from the Upper Biebrza Valley
profile 5 – peat-muck soil strongly transformed from the Middle Biebrza Valley

Table 2. The number of fungi species in the soils studied

Tabela 2. Liczba gatunków grzybów otrzymanych z badanych gleb

Genetic horizon	Soil profiles				
	Profile 1	Profile 2	Profile 3	Profile 4	Profile 5
Turf horizon	5	6	4	14	27
Subturf horizon	-	-	-	9	12
Transitory horizon	-	-	-	-	15
Peat layer	3	1	3	6	6
Summary of individual profiles	7	7	5	18	32
Total number	53				

Explanations as in Table 1

Analysis of the Table 1 data shows that in general the number of fungi isolates decreases with increasing depth. The exception was strongly transformed peat-muck soil in which the number of isolates in the subsequent deeper muck horizons increased. The number of isolates in the pear layer was always much smaller than that of the isolates in the higher layer of muck. The fungi communities living in the soils studied differed not only in quantitative but also in qualitative composition. The total number of fungi species represented was 53 (Tab. 2). It should be mentioned that the numerical data given in Table 2 do not add up. The number of species identified in particular genetic horizons of a given soil profile do not add up as the species living in subsequent genetic horizons often repeat. Moreover, in different soils the same species can occur.

The highest number of 32 species was obtained from the strongly transformed peat-muck soil. Also the diversity of species in the surface muck layer was the greatest (27 ones) in the turf horizon of this soil. The fungi community in the turf horizon in medium transformed peat-muck soil is made by 14 species. The lowest species diversity (4–6 species) was noted in the communities in turf muck layer in weakly transformed peat-muck soil, both from the sites in the Narew and Biebrza river valleys (Tab. 2).

It should be emphasised that the highest qualitative and quantitative diversity was established for the fungi communities living in strongly transformed peat-muck soil (Tabl. 1, 2).

As follows from the above data, with increasing depth in the soil profile the number of fungi species decreases. The lowest qualitative diversity was found in the peat layers of the soils studied (Tab. 2).

Table 3 presents the fungi species most abundant in a given community in particular soil types and their genetic horizons. Highly abundant in all horizons of strongly transformed peat-muck soil was *Penicillium simplicissimum* (Oudem.) Thom. This fungus species was also isolated from the other types of soil studied. The other species represented by a large number of isolates were *Penicillium janczewskii* Zaleski, *P. waksmanii* Zaleski and *Helicosporium vegetum* Nees. Besides the above mentioned species, also other ones representing the genus *Penicillium* were abundant in the communities observed (Tab. 3).

Table 3. Fungi species of the highest abundances in the soils studied

Tabela 3. Gatunki grzybów najliczniej występujące w omawianych glebach

Species of fungi	Genetic horizon			
	M1	M2	M3	Ot
	Frequency			
Profile 1 – peat-muck soil weakly transformed from the Narwiański National Park				
Mucor racemosus Fresen.	-	-	-	4
Penicillium simplicissimum (Oudem.) Thom	10	-	-	4
Trichoderma koningii Oudem.	6	-	-	-

Species of fungi	Genetic horizon			
	M1	M2	M3	Ot
	Frequency			
Profile 2 – peat-muck soil weakly transformed from the Narwiański National Park				
Gonatobotryum sp.	5	-	-	-
Pseudeurotium ovale Stolk	4	-	-	-
Penicillium simplicissimum (Oudem.) Thom	-	-	-	15
Profile 3 – peat-muck soil weakly transformed from the Lower Biebrza Valley				
Helicosporium vegetum Nees	24	-	-	11
Profile 4 – peat-muck soil medium transformed from the Upper Biebrza Valley				
Helicosporium vegetum Nees	49	145	-	18
No spores 1	17	8	-	3
Penicillium janczewskii Zaleski	30	4	-	-
Penicillium implicatum Biourge	28	7	-	3
Penicillium simplicissimum (Oudem.) Thom	28	4	-	-
Penicillium waksmanii Zaleski	116	35	-	3
Trichoderma harzianum Rifai	8	-	-	-
Profile 5 – peat-muck soil strongly transformed from the Middle Biebrza Valley				
Absidia spinosa Lendn.	17	-	-	-
Chaetomium homopilatum Omvik	15	3	-	-
Fusarium oxysporum E.F. Sm. & Swingle	15	-	-	-
Gliomastix murorum (Corda) S. Hughes	5	-	-	-
Mucor hiemalis Wehmer	3	-	-	-
Paecilomyces marquandii (Massee) S. Hughes	5	-	-	-
Penicillium brevicompactum Dierckx	8	-	-	-
Penicillium implicatum Biourge	12	-	-	-
Penicillium janczewskii Zaleski	80	3	3	5
Penicillium janthinellum Biourge	8	-	-	-
Penicillium simplicissimum (Oudem.) Thom	49	310	407	115
Penicillium waksmanii Zaleski	49	18	11	6
Pseudogymnoascus roseus Raillo	4	9	8	-
Trichoderma koningii Oudem.	6	-	-	-
Verticillium bulbillosum W. Gams & Malla	3	-	4	-
Zygorrhynchus moelleri Vuill.	8	-	4	-

Explanations
M1 – turf horizon
M2 – subturf horizon
M3 – transitory horizon
Ot – peat layer

4. Discussion

In the second half of the 20th century large boggy river valleys in Poland have undergone significant habitat transformations. These transformations have been taking place till today with different intensity in the valleys of Narew and Biebrza rivers. As can be inferred from the directions of these transformations, they are consequences of adverse hydrological changes caused by man as well as nature (Banaszuk 1999). Development of the network of drainage channels, considerable lowering of ground water table and utilisation of peat soils have initiated a fast process of degradation. The degradation led to far-reaching and often irreversible changes in the environment. The aeration of the upper surface of peat has hindered the process of peat-production and initiated intense mucking of organic substance and diversification of soil properties (Kajak, Okruszko 1992; Okruszko 2000). The process of mineralisation of organic compounds is realised with the involvement of different groups of microorganisms which undergo dynamic qualitative and quantitative transformations (Gonet, Markiewicz 2007). In other words, the mocking process leads not only to changes in the physical and chemical conditions of the soil substrate but also in the character of the microorganisms (including fungi) living in this substrate (Kaczmarek 1991). The reduced moisture content in dehydrated peatlands is accompanied by an increase in the organic substance mineralisation indices and an increase in the abundance of fungi (Kajak 1985; Kajak et al. 1985). The physicochemical properties of soil have definite effect on the group of microorganisms living in this soil and on their activity (Barabasz, Vořišek 2002). Each change in the substrate affects the biotic relations between microorganisms and the quantitative and qualitative structures of their communities (Paul, Clark 2000). It has been proved that the direction and dynamics of changes in the organic substance making peat soil depend also on the activity of microorganisms (Andrzejewska et al., 1983). The number and types of these organisms can be treated as a sensitive indicator of the condition of the soil and the whole ecosystem, moreover informing about the direction of the soil-producing processes (Barabasz, Vořišek 2002).

As follows from the data collected, the mucking process and mainly its intensity, has considerable effect on the qualitative and quantitative composition of the fungi communities living in peat-muck soils. The quantitative and qualitative diversity was directly proportional to the intensity of dehydration of hydrogenic soils and the highest in strongly transformed peat-muck soil. This greatest diversity should not be linked only to the number of samples of this soil, the large number of samples was related to the greatest diversity of the morphological features of this soil profile when compared to the other types of soil. It has been found that irrespective of the number of soil samples studied, the higher the intensity of the mocking process the greater the quantitative and qualitative diversity of the fungi communities living in the mucking soil. A similar relation has been noted by other authors (Andrzejewska et al. 1983; Bogacz et al. 2004) and interpreted as a consequence of the fact that increased mineralisation of organic substance favours the development of fungi (Bogacz et al. 2004). Aeration of near surface muck (turf layer) is favourable for intense development and diversification of fungi communities thus also for the increase in mineralisation and peat mucking in which the fungi are involved (Ławrynowicz, Mułenko 2008). The fungi are also involved in soil-producing processes and plant nourishment (Bis 2002; Badura 2003, 2004). Taking into account all the above, the greater number and diversity of fungi communities in turf horizons than in the lower peat are directly related to the role of fungi in the environment.

It should also be mentioned that according to the results of mycological analysis of strongly transformed peat-muck soil, the frequency of communities living in the turf and subturf horizons was higher than in the transitory horizon, so the number of communities increased with increasing depth in the muck horizon. The peat layer in this type of soil, similarly as in the other soil types, was occupied by much less abundant and much poorer diversified fungi community than the communities living in the muck. The structures of fungi communities occupying weakly transformed peat-muck soil from the flooded overdried peatlands in the Narwiański National Park did not differ significantly from those of the communities present in weakly transformed peat-muck soil from the Biebrza river valley. It was however, mainly true about the abundance of isolates and the number of species as the species were different. Nevertheless, it can be concluded that the fungi communities forming and undergoing transformations in dehydrated soils in peatlands are mainly influenced by the changes taking place in the mucking process. this means that the character of fungi communities in post-bog soils is determined by the process of soil formation. However, the role of the type of muck and vegetation should also be regarded (Badura 2003; Barabasz, Vořišek 2002) as there is a close connection between the general habitat conditions and structures of fungi communities living in the soils of hydrogenic habitats (Wielgosz 2001).

One of the most abundant species in the soils studied was *Penicillium simplicissimum*. It reached a particularly high frequency in all genetic horizons of strongly transformed peat-muck soil. It was also noted in the communities in the other types of soil but in lower abundance. The species of high frequency in strongly and medium transformed peat-muck soils were *P. waksmanii* and *P. janczewskii*. In the weakly transformed peat-muck soil from the Biebrza river valley the most abundant was *Helicosporium vegetum*. It was also obtained from the medium transformed peat-muck soil. Because of their abundance the above-mentioned species have a significant effect on transformation of the organic substance in the soils studied. Analysis of the abundance of fungi species in the communities in weakly transformed peat-muck soil from the Narew river valley has not permitted identification of one clearly dominant species.

5. Conclusions

1. Quantitative and qualitative diversity of fungi communities living in strongly and medium transformed peat-muck soils is much greater than that in the weakly transformed peat-muck soil. Thus, increased intensity of the mucking process favours the diversity of fungi communities in the soil of dehydrated peatland.
2. The fungi communities living in the muck layer, in particular in the turf horizon, were characterised by much stronger diversified qualitative and quantitative structures than those living in the lower layer of peat.
3. The species most abundantly represented in the soil studied were *Penicillium simplicissimum, P. janczewskii, P. waksmanii* and *Helicosporium vegetum*, which means that these species determined to the highest degree the transformation of organic substance in post-bog soils.

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Różnorodność gatunkowa zbiorowisk grzybów występujących w wybranych glebach pobagiennych

Streszczenie

Analizie poddano pięć gleb torfowo-murszowych (słabo, średnio i silnie zmurszała). Dwa z wybranych punktów badawczych pochodziły z doliny Narwi, kolejne trzy znajdowały się w Kotlinie Biebrzy. W efekcie przeprowadzonych prac uzyskano 1853 izolatów grzybów, które były reprezentowane przez 53 różne gatunki. Gleby torfowo-murszowe silnie i średnio zmurszałe charakteryzowały się zdecydowanie większym zróżnicowaniem gatunkowym i ilościowym zasiedlających je zbiorowisk grzybów w porównaniu z glebami torfowo-murszowymi słabo zmurszałymi. Wynika stąd, że wzrost intensywności procesu murszenia przyczynia się do zwiększenia różnorodności zbiorowisk grzybów zasiedlających gleby odwodnionych torfowisk. Warstwy murszu, szczególnie poziomy darniowe, były zasiedlane przez zbiorowiska grzybów o zdecydowanie silniej zróżnicowanych strukturach jakościowo-ilościowych w porównaniu ze strukturami zbiorowisk występującymi w niżej zalegającym torfie. Do najliczniej występujących grzybów w analizowanych glebach należały *Penicillium simplicissimum, P. janczewskii* i *P. waksmanii* oraz *Helicosporium vegetum*.

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Differentiation and dynamic tendencies of epiphytic lichen associations of birch (*Betula* sp.) in the Biebrza National Park

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Abstract

Birch in the Biebrza National Park is the habitat of 6 epiphytic lichen associations: Calicietum viridis, Chaenothecetum ferrugineae, Hypocenomycetum scalaris, Lecanoretum conizaeoidis, Parmeliopsidetum ambiguae and Pseudevernietum furfuraceae. They have been recorded in forest communities, mainly coniferous forests, as well as in the open area. Exclusive forest associations, characteristic of natural forests are Calicietum viridis, Chaenothecetum ferrugineae and Parmeliopsidetum ambiguae. The remaining associations occur both in forest and non-forest areas. Hypocenomycetum scalaris is highly xerothermic and heliophytic, which is reflected both in the composition of species forming it as well as in its preference for the most illuminated side of the trunk. The smallest and most species – impoverished patches are formed by toxitolerant association Lecanoretum conizaeoidis. The most frequently recorded in the area under study, both in forest communities and open areas, is Pseudevernietum furfuraceae. It constitutes an association most abundant in species and occupies the phorophyte from the base of the trunk up to the tree crown. The ecological factors which influence the formation and differentiation of epiphytic lichen associations include the phorophyte properties, forest community, abiotic and anthropogenic factors.

Key words: phytosociology, lichens, protected areas

1. Introduction

With the development of research into phytosociology of vascular plants investigation on lichen communities began. Epiphytic lichen associations in Poland have been studied, among others, by Glanc (1978, 1981), Zielińska (1967), Fabiszewski (1968), Cieśliński, Halicz (1971), Bystrek, Anisimowicz (1981), Bielczyk (1986), Kolanko (2001). Their investigation concerned mainly large forest complexes and less frequently concentrated on isolated trees growing outside forest areas.

Birch is one of the most instantly recognizable trees in Poland. Its lichenbiota is rich and taxonomically diversified. In north-eastern Poland 151 species have been recorded from its bark (Cieśliński 2003), including 74 in the Białowieska Forest (Cieśliński, Tobolewski 1988), 95 in the Knyszyńska Forest (Bystrek, Kolanko 2000). Lichen species on birch are can grow individually or can form populations and associations of different sizes.

The aim of the study presented in this paper is phytosociological characterisation of epiphytic associations of birch and their dynamic tendencies in the Biebrzański National Park.

2. Study area and methods

The Biebrzański National Park located in the Biebrza Basin in the Podlaskie Voivodeship covers an area of 59 223 ha. Its plant cover is distinguished by a great diversity, high degree of naturalness and presence of many rare species. Environmental factors, including diversified sculpture and abundance of substrata provide perfect living conditions for lichens. However, the lichenbiota of this territory has not been studied thoroughly yet. Among all groups of ecological lichens, the dominant are epiphytes (Kolanko 2005), even though forest communities of the park cover as little as 26 % of its surface area (Bartoszuk 2005). Birch can be found in dry and fresh pinewood , mixed coniferous forests, less frequently in deciduous forests. It also grows in open areas subject to anthropopressure. This enables a comparison of requirements of particular lichen species and their associations in different environmental conditions and evaluation of their development dynamics.

Field studies into epiphytic lichen associations of birch were conducted in the years 2003–2009 over the whole territory of the park. Trees of different ages, growing in forest communities and in open country were examined. The observations were carried out according to the principles of the Braun – Blanquet method, modified and adapted for lichen examination by Klement (1955) and Barkman (1958). Phytosociological records were taken from the tree base up to the height of about 2.5 m. The size of the coverage area of the associations ranged from 2 to 24 dcm^2. For each record the diameter of birch trunk at the height of approximately 1 m, the height of the association position on the trunk, its exposure and the character of forest community were noted. The associations were differentiated with regard to characteristic species, based on literature data and local faithfulness and species combination. Constancy coefficients for particular species were calculated following to the 5-degree scale of Braun-Blanquet.

From among different phytosociological systems, the classification of Barkman (1958) was adopted, which treats epiphytic lichen associations as independent units, in accordance with the assumptions of the French-Swiss school. This enabled the phytosociological studies on lichens on trees growing individually in open areas and allowed a comparison with associations from other territories of Poland.

The nomenclature of lichen species have been accepted after Fałtynowicz (2003) and Santesson et al. (2004).

3. Results

In the Biebrzański National Park 6 associations of epiphytic lichens growing on bark of birch were found. The classification of these lichen communities is as follows.

I. Order: *Leprarietalia* Barkman 1958 emend. Wirth 1972
Alliance: *Calicion viridis* (Čern et Hadač 1944) emend. Barkman 1958
Association: *Calicietum viridis* Hilitzer 1925
Association: *Chaenothecetum ferrugineae* Barkman 1958

II. Order: *Lecanoretalia variae* Barkman 1958
Alliance: *Lecanorion variae* Barkman 1958
Association: *Lecanoretum conizaeoidis* Barkman 1958
Association: *Hypocenomycetum scalaris* Hilitzer 1925

III. Order: *Parmelietalia physodo-tubulosae* Barkman 1958
Alliance: *Parmelion saxatilis* Barkman 1958
Association: *Pseudevernietum furfuraceae* (Hilitzer 1925) Ochsner 1928
Association: *Parmeliopsidetum ambiguae* Hilitzer 1925

3.1. Characterisation of epiphytic lichen associations on birch

Calicietum viridis Hilitzer 1925 very seldom occupies birchbark in the area studied. It has been noted only on 4 stands in the mixed coniferous forest in the central Biebrza basin. It colonizes the lowest, basal parts of birch with intensively fissured bark forming small , elongated patches located along the cracks of bark, whose area does not exceed 6 dcm². It shows preference for shaded habitats, so is found mainly on the northern side of the trunk and it is poor in species. The highest degrees of constancy and coverage are attained by the species characteristic of the association (Tab.1). Its physiognomy is determined by *Chaenotheca chrysocephala*, *C. ferruginea* and *Calicium viridis*, which give it a characteristic yellow-green colour. The association is ombrophobous and occupies sites on trunk which are sheltered from direct atmospheric precipitation.

Chaenothecetum ferrugineae Barkman 1958. This association has been recorded on 9 stands, mainly in the southern basin of the Biebrzański National Park. It can be found only in forest communities. It is an impoverished community built up by 3 to 8 species, among which the representatives of *Caliciaceae* dominate. Thalli of lepropose type determine the physiognomic spectrum of the association, giving it an olive green colour. *Chaenotheca ferruginea*, a species characteristic of this association and *C. chrysocephala* attain high degrees of constancy and coverage (Tab. 1). Among the accompanying species, *Hypocenomyce scalaris* occurs at high constancy, but it attains low coverage degree. The patches of this association are not large and usually cover an area up to 9 dcm². They grow in the bottom, basal parts of trunks on the northern side.

Lecanoretum conizaeoidis Barkman 1958 similarly to the above mentioned association was recorded on 9 stands. It colonizes the middle parts of birch trunks in mixed coniferous forest, in dry pinewood and on trees growing individually in non-forest areas. It was noted near roads with high traffic density. It is composed of a small number of species with a wide ecological amplitude, exhibiting great tolerance to atmospheric pollution and to changing conditions of the natural environment. The highest constancy and coverage degree is shown by the species characteristic of association *Lecanora conizaeoides* and locally distinctive, *L. expallens* (Tab.1). Crustose and leprolose thalli dominate. This association forms relatively small patches of areas not exceeding 8 dcm². It prefers the northern and eastern side of the trunk.

Table 1. Composition and structure of epiphytic lichen associations of birch

Tabela 1. Skład i struktura zespołów porostów epifitycznych brzozy

	A	B	C	D	E	F
Characteristic species (gatunki charakterystyczne) *Leprarietalia* and *Calicion viride*						
Lepraria incana sensu lato	1.II	+. I	1.II	+.II	+.I	1.II
Chaenotheca phaeocephala	+.I	1.II	.	.	.	.
Characteristic species (gatunki charakterystyczne) *Calicietum viridis*						
Calicium viride	3.V	+.I	.	.	.	.
Chaenotheca chrysocephala	2.IV	3.IV	.	.	.	.
Characteristic species (gatunki charakterystyczne) *Chenothecetum ferrugineae*						
Chaenotheca ferruginea	2.III	4.V	.	.	.	.
Characteristic species (gatunki charakterystyczne) *Lecanoretum conizaeoidis*						
Lecanora conizaeoides	.	.	4.V	+.III	1.I	+.I
Lecanora varia	.	.	3.V	.	.	.
Lecanora expallens	.	.	4.IV	.	+.I	.
Characteristic species (gatunki charakterystyczne) *Hypocenomycetum scalaris*						
Hypocenomyce scalaris	.	+.III	.	5.V	.	+.I
Characteristic species (gatunki charakterystyczne) *Hypogymnietalia physodo-tubulosae*						
Hypogymnia physodes	+.I	1.I	1.II	1.I	5.V	4.V
Hypogymnia tubulosa	.	.	.	+.I	2.III	.
Platismatia glauca	.	.	.	.	2.V	+.I
Usnea filipendula	.	.	.	.	1.IV	+.I
Characteristic species (gatunki charakterystyczne) *Parmelion saxatilis*						
Cetraria chlorophylla	.	.	.	+.I	1.II	+.I
Vulpicida pinastri	.	.	.	+.I	+.I	+.II
Usnea hirta	.	.	.	.	1.II	+.I
Characteristic species (gatunki charakterystyczne) *Pseudevernietum furfuraceae*						
Pseudevernia furfuracea	.	.	.	+.I	3.V	+.I
Characteristic species (gatunki charakterystyczne) *Parmeliopsidetum ambiguae*						
Imshaugia aleurites	.	.	.	+.II	1.I	2.IV

	A	B	C	D	E	F
Parmeliopsis ambigua	.	.	.	+.I	+.I	2.IV
Others species (inne gatunki)						
Cladonia coniocraea	+.I	+.I	.	1.II	1.I	1.I
Cladonia chlorophaea	.	+.I	.	+.I	.	+.I
Cetraria sepincola	.	.	.	+.I	1.II	.
Lecanora pulicaris	.	.	+.I	.	+.I	.
Bryoria fuscescens	.	.	.	.	1.I	.
Scoliciosporum chlorococcum	.	.	+.I	.	.	.
Evernia prunastri	.	.	.	+.I	1.II	.
Parmelia sulcata	.	.	+.I	.	+.I	+.I
Ramalina farinacea	.	.	.	.	1.II	.

Explanations:
Associations of epiphytic lichens: A – *Calicietum viridis*; B – *Chaenothecetum ferrugineae*; C – *Lecanoretum conizaeoidis*; D – *Hypocenomycetum scalaris*; E – *Pseudevernietum furfuraceae*; F – *Parmeliopsidetum ambiguae*
+–5 – degree of coverage; I-V – constancy

Hypocenomycetum scalaris Hilitzer 1925 frequently colonizes birchbark. It was noted on 23 stands. It was found in most forest communities, mainly in illuminated places, on the edge of forest and in open country. It is composed of a small number of species, most of which are photophilous and xerophilous lichens, often encountered in open areas. The physiognomy of this community is determined by its xerophilous character. At the sites of its occurrence there is dry, hot microclimate, in which only this type of epiphytic association is able to develop. The characteristic species of *Hypocenomyce scalaris* attains here high constancy and the highest degree of coverage. *Lecanora conizaeoides*, the characteristic species of association *Lecanorion variae*, similarly to *Imshaugia aleurites*, a characteristic species of order *Hypogymnietalia physodo-tubulosae*, also exhibit high constancy but with evidently lower coverage (Tab.1). This association colonizes lower and middle parts of trunks and definitely prefers the southern side of trees. On the edge of forest it often forms large patches of areas reaching even up to 20 dcm^2.

Pseudevernietum furfuraceae (Hilitzer 1925) Ochsner 1928 is the most common on birch, and at the same time it is the only association dominated by macrolichens. It was noted on 29 stands, mainly in pine coniferous forests, mixed coniferous forests and on trees growing in open country. The initial stages of *Pseudevernietum furfuraceae* are already formed on trees aged between ten and twenty years old but in very small patches. The process of building this association begins with the appearance of *Pseudevernia furfuracea* and *Platismatia glauca* between the thalli of *Hypogymnia physodes*. In older treestands the structure of the association and the occupied areas depend on microhabitat conditions, the species and age of tree as well as the height on trunk. Due to the occurrence of three morphological thallus types in patches a layered structure appears. Patches of the association with a big participation of *Evernia prunastri* and *Ramalina farinacea* differ. Species of *Bryoria,* rare in Poland, mark their appearance on birch. It appears in the best developed form on bark of the oldest birch trees. Foliose and fruticose species dominate in its patches. *Usneaceae* and *Parmeliaceae* attain large sizes here, and their thalli are well developed. The highest constancy and coverage degree are attained by *Pseudevernia furfuracea* , characteristic of this association and species characteristic of order *Hypogymnietalia physodo-tubulosae* (Tab.1). The association is found along all the height of the phorophyte. Its patches cover areas up to 24 dcm^2.

Parmeliopsidetum ambiguae Hilitzer 1925 was noted on 7 stands, exclusively in forests. It forms on birchbark mainly in dry and fresh coniferous forests and in mixed coniferous forests. It is composed of 5 to 13 species, among which the representatives of *Parmeliaceae* dominate. The physiognomy of *Parmeliopsidetum ambiguae* is determined by placodial and foliose thalli. The contribution of crustose and lepropose lichens is also considerable. In the lowest parts of the patches there grow *Cladonia chlorophaea* and *C. coniocraea*. Among the species characteristic of this association only *Parmeliopsis ambigua* and *Imshaugia aleurites* reach high constancy. *Vulpicida pinastri* does not frequently appear in the studied area , hence its participation in the community was definitely the lowest (Tab. 1). The association colonizes the lowest parts of trunk, most often on the northern side. The area of its patches ranges from 4–9 dcm^2.

3.2. Distribution of epiphytic lichen associations on birch in forest communities and in open area

On birchbark the epiphytic lichen associations occur both in forest communities and on isolated trees growing in open country (meadows, pastures, and along roads). Two groups of associations can be distinguished: those found exclusively in forests and the ones recorded both in forest communities and in the open.

The first group comprises *Calicietum viridis*, *Chaenothecetum ferrugineae* and *Parmeliopsidetum ambiguae*. These associations are built up of species reaching the optimum stage of development inside large forest communities, which provide them with most favourable living conditions – an appropriate degree of air humidity and illumination, as well as numerous microhabitats available for colonization.

The second group comprises *Pseudevernietum furfuraceae*, *Hypocenomycetum scalaris* and *Lecanoretum conizaeoidis*, which grow in forests and open areas. Unlike the above mentioned associations, they are distinguished for their wider ecological spectrum.

Associations which show distinct preference for coniferous forests include *Hypocenomycetum scalaris* and *Lecanoretum conizaeoidis*. It is there on birchbark that they form the largest and best developed patches. By contrast, *Calicietum viridis*, *Chaenothecetum ferrugineae*, *Parmeliopsidetum ambiguae* and *Pseudevernietum furfuraceae* are also found on birch in deciduous forests.

3.3. Vertical distribution of epiphytic lichen associations on birchbark

A tree (phorophyte) is a specific substratum for epiphytes. As a living organism, it undergoes various changes with time. This leads to change in the conditions on its surface. A number of zones can be delimited on a phorophyte: the base of the trunk, the middle part of the trunk and the part near the crown. Each of them offers different conditions for the epiphytes colonizing the birch trees.

Associations of tree lichens of birch in the area studied populate all habitats available on a phorophyte, from the base of the trunk up to the crown. *Calicietum viridis*, *Chaenothecetum ferrugineae* and *Parmeliopsidetum ambiguae* form at the base of the trunk. These are ombrophobous communities, they grow mainly in fissures and deep cracks of bark, exclusively in the lowest parts of trunks with relatively high humidity but sheltered from atmospheric precipitation. Both in the lowest and middle part of the trunk of birch, *Hypocenomycetum scalaris* forms patches of different sizes. *Lecanoretum conizaeoidis* was noted only in the middle part of the trunk. The only association which forms and colonizes birch all over its height is *Pseudevernietum furfuraceae*, however, the biggest and best developed patches cover the middle part of the trunk.

3.4. Epiphytic lichen associations of birch versus the age of the phorophyte

Many lichen species, mainly those with crustose thalli, colonize birchbark of the youngest age brackets, in thicket and brushwood. However, associations of tree lichens develop later. Associations which appear relatively early, on trees aged ten to twenty are *Lecanoretum conizaeoidis* and *Pseudevernietum furfuraceae*. The former community is relatively short-lived in forest areas and as the phorophyte develops and external conditions change it retreats and is replaced by other, more competitive lichen species or other more persistent associations. In the open, in favourable lighting conditions it lives longer.

Calicietum viridis, *Chaenothecetum ferrugineae*, *Hypocenomycetum scalaris* and *Parmeliopsidetum ambiguae* form only on trees of older age brackets, which provide them with appropriate illumination and humidity conditions. Only on older trees they form patches of bigger sizes and richer in species composition. Similarly, *Pseudevernietum furfuraceae* reaches its optimal occurrence on oldest trees which provide it with constant, stable conditions of substratum and surroundings.

3.5. Illumination requirements of epiphytic lichen associations

An important ecological factor influencing the development and distribution of epiphytic lichen communities is light. In the studied area 3 groups of associations with different light preferences can be distinguished.

Hypocenomycetum scalaris – a highly photophilous association. Its composition includes heliophytic, highly xerophilous species, hence the occurrence of the association is restricted to the most illuminated side of trunks, and the best developed patches are formed on the southern, south-eastern, eastern and south-western exposition. In the area under study it populates the base and middle part of trunks on forest edges and in the open.

Photophilous associations, showing tolerance to shade include *Lecanoretum conizaeoidis* and *Pseudevernietum furfuraceae*. They are made up of species with lower requirements for illumination, they grow on every exposition, but the best formed patches develop on the north-eastern, south-eastern, eastern and southern side. These associations have been noted on the middle part of trunks and in crowns in the open as well as in forest communities on trees growing in loose clusters on the edges of glades.

Strongly skiophilous communities include *Calicietum viridis*, *Chaenothecetum ferrugineae* and *Parmeliopsidetum ambiguae*. They are often composed of ombrophobous species with extremely high requirements regarding humidity, most often skiophilous, evidently avoiding southern and south-eastern exposition, the best developed patches have been noted on the northern and north-western side, they grow at the base of deeply cracked trunks , exclusively in forest communities.

4. Discussion and summary

Epiphytic lichen associations recorded on the bark of birch in the Biebrzański National Park have been reported from several other, mainly mountainous regions of Poland. The majority of them (*Chaenothecetum ferrugineae*, *Lecanoretum conizaeoidis*, *Hypocenomycetum scalaris*, *Parmeliopsidetum ambiguae* and *Pseudevernietum furfuraceae*) are common communities, frequently found on different tree species. Only *Calicietum viridis* is a rare association. The patches of this association observed in this study follow the description given by Bielczyk (1986), however, here it lacks *Lecanactis abietina*, which was not found in the studied area. This association has been evidently more frequently noted on the bark of spruce, fir and sycamore than on birch (Fabiszewski 1968; Glanc 1978; Bielczyk 1986). The composition of *Calicietum viridis* in the Sudety Mts includes *Candelaria concolor* (Fabiszewski 1968), which both in the Beskidy Mts (Bielczyk 1986) and in the studied area forms only on bark of trees growing in the open.

Chaenothecetum ferrugineae is an association widely distributed, common in European lowlands (Barkman 1958). It is ombrophobous, aerohygrophilous and acidophilous. It finds favourable growth conditions on trees living in large forest complexes. Apart from birch, it forms on other species of trees with thick, fissured bark, mainly on basal parts of trunks (Barkman 1958). This association observed in this study does not exhibit relevant differences in composition and ecological requirements in comparison to data from other regions of Poland (Zielińska 1967; Bielczyk 1986; Bystrek, Kolanko 2000; Kolanko 2001).

Lecanoretum conizaeoidis has been also reported from spruce, alder and beech. As a toxitolerant association (Barkman 1958) in the area studied it was formed on birch trees growing near roads with higher traffic volume. The species composition of *Lecanoretum conizaeoidis* from the area studied corresponded to the descriptions by Glanc (1978) and Bielczyk (1986) but had greater participation of *Lecanora expallens.*

Hypocenomycetum scalaris growing on birchbark in the Biebrzański National Park does not differ considerably from the communities described from the other territories in Poland. It is found in similar habitats and possesses identical physiognomy. The associations observed in this mostly resemble *Hypocenomycetum scalaris* described from Kampinoska Forest (Zielińska 1967). This association described by Cieśliński and Halicz (1971) from the Świętokrzyskie Mountains is richer in species. Of special interest is the presence of the following species in patches of this association: *Bryoria crispa*, *Platismatia glauca*, *Usnea hirta*, from order *Parmelietalia physodo-tubulosae*.

Parmeliopsidetum ambiguae, according to Klement (1955) and Barkman (1958), primarily forms in mountainous and foothill areas, in lowlands it is replaced by *Hypocenomycetum scalaris*. The patches of this association in the studied area lack the characteristic species, such as *Cetraria sepincola* and *Parmeliopsis hyperopta*, reported by Fabiszewski (1968) and Bielczyk (1986) from mountainous areas. However, we noted *Imshaugia aleurites*, a species which best characterises the association in lowlands (Zielińska 1967) and in the Świętokrzyskie Mountains (Cieśliński, Halicz 1971). The occurrence of *Hypogymnia physodes* is clearly marked, and in some patches also *Cetraria chlorophylla*. The participation of lichens from the association *Pseudevernietum furfuraceae* might reflect a possibility of transforming *Parmeliopsidetum ambiguae* into *Pseudevernietum furfuraceae*.

The last of the associations – *Pseudevernietum furfuraceae,* noted on birchbark is a widely distributed, familiar within the range of occurrence of *Hypogymnia physodes*, *H. tubulosa*, *Pseudevernia furfuracea* and *Platismatia glauca* and repeatedly reported from various tree species in literature (Barkman 1958; Zielińska 1967; Fabiszewski 1968; Cieśliński, Halicz 1971; Bystrek, Anisimowicz 1981; Glanc 1981; Bielczyk 1986; Kolanko 2001). Macrolichens dominate in it and the patches of this association from lowland areas are poorer with regard to species composition. In comparison with *Pseudevernietum furfuraceae* reported earlier from other regions of Poland, its evident impoverishment can be noted. It is most likely caused by the disappearance of characteristic species: *Bryoria fuscescens*, *B. implexa*, *B. subcana*, *Evernia mesomorpha* and also some taxa from genus *Usnea*. They belong to a group of lichens threatened with extinction, in categories with the highest risk level (Cieśliński et al. 2006).

The formation and further development of epiphytic lichen associations of birch, as well as other trees is influenced by a complex of ecological factors. They include both biological properties of phorophytes (pH of bark, its texture and rate of exfoliation), the closest vicinity (forest community, open area), abiotic factors, and in recent years also the impact of human activity.

A tree offers a great diversity of microhabitats for lichen colonization and formation of associations with various ecological requirements. Hence the possibility of different epiphytic lichen communities to grow next to one another, and even the transformation of some into others (Barkman 1958; Bielczyk 1986). An important role for epiphytes is played by physical and chemical properties of bark, which was emphasised, among others, by Barkman (1958), Fabiszewski (1968), Cieśliński and Halicz (1971). Birch is characterized by low bark pH (Barkman 1958), that is why all the associations populating it are acidophilous. Its cracked bark in basal parts of trunks facilitates the development of lichens and formation of associations.

On birchbark in the Biebrza National Park, 2 epiphytic lichen associations were identified whose occurrence is connected with natural forest communities. These are *Calicietum viridis* and *Chaenothecetum ferrugineae*. Their composition includes rare lichen species, recorded in well preserved forests of natural character. Disturbances in the balance of phytocenosis cause the death of thalli which build up associations, impoverishing their patches or leading to their elimination. *Pseudevernietum furfuraceae* is also exposed to great danger, its characteristic and distinctive species from families *Parmeliaceae* and *Usneaceae* are threatened on the territory of Poland (Cieśliński et al. 2006).

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Zróżnicowanie i tendencje dynamiczne zespołów porostów epifitycznych brzozy (*Betula* sp.) w Biebrzańskim Parku Narodowym

Streszczenie

Na korze brzozy w Biebrzańskim Parku Narodowym odnotowano 6 zespołów porostów epifitycznych: *Calicietum viridis*, *Chaenothecetum ferrugineae*, *Hypocenomycetum scalaris*, *Lecanoretum conizaeoidis*, *Parmeliopsidetum ambiguae* i *Pseudevernietum furfuraceae*. Występują one na brzozach rosnących w zbiorowiskach leśnych, głównie borowych, jak i na terenach otwartych. Zespołami wyłącznie leśnymi są *Calicietum viridis*, *Chaenothecetum ferrugineae* i *Parmeliopsidetum ambiguae*. Rozwijają się w lasach o charakterze naturalnym, ale ograniczają się tylko do nasadowych i dolnych części pni. Są cieniolubne i ombrofobowe. Pozostałe

zespoły występują zarówno w lasach, jak i na terenach nieleśnych. *Hypocenomycetum scalaris* jest wybitnie kserotermiczny i światłożądny, co znajduje odzwierciedlenie zarówno w składzie budujących go gatunków i preferencji do południowej, najsilniej oświetlonej strony pni. Jego płaty zajmują duże powierzchnie w nasadowej i środkowej części pnia. Najmniejsze i najuboższe gatunkowo płaty tworzy *Lecanoretum conizaeoidis*. Wchodzące w jego skład gatunki odznaczają się znacznym stopniem toksytolerancji, dlatego zespół ten rozwija się w pobliżu większych szlaków komunikacyjnych. Najczęściej na badanym terenie, zarówno w zbiorowiskach leśnych jak i na terenach otwartych, występuje *Pseudevernietum furfuraceae*. Jest zespołem najbogatszym w gatunki. Dominują wśród nich przedstawiciele rodziny *Parmeliaceae*, *Hypogymniaceae* i *Usneaceae*.

Pionowe rozmieszczenie na pniu poszczególnych zespołów jest zróżnicowane. Ugrupowania porostów epifitycznych kolonizują najczęściej dolną i środkową część pnia, rzadziej koronę. Zdecydowana większość zespołów porostów epifitycznych wykształca się dopiero na drzewach w średniej klasie wiekowej, powyżej 60 lat. W młodszych klasach wiekowych, rozwijają się niewielkie płaty zespołów *Lecanoretum conizaeoidis* i *Pseudevernietum furfuraceae*.

Do czynników ekologicznych, które wpływają na powstawanie i różnicowanie zespołów porostów epifitycznych należą właściwości forofitu, zbiorowisko leśne, czynniki abiotyczne i antropogeniczne.

Lichens of birch *(Betula* sp.*)* on area with differentiated anthropopressure within city limits of Białystok – floristic-ecological study

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Abstract

The paper presents a list of 46 taxa of lichens living on the bark of birch *Betula* sp. growing in Bialystok. Around 80% of the species occurred with phytosociological constancy of degree I and the lowest number with degrees IV and V, 4% and 2%, respectively. The largest number of species has been recorded in green areas, mainly in the forests and the lowest number in the city centre and along the exit roadsides. For many lichen species significant differences in frequency and abundance in individual areas have been observed. In the forests of Białystok clear vertical structures have been found in the distribution of lichens on birchbark.

Key words: lichens epiphytic, birch, Białystok, NE Poland

1. Introduction

Cities are specific types of ecological systems, urbicenoses, whose existence is determined by anthropogenic factors, as well as natural physiographic conditions resulting from their location. These are structural-functional systems consisting of biotic and abiotic elements of the environment in which fundamental processes of matter circulation and energy flow take place. The creator of these systems is a man, who is affected back by previously made changes in the environment and lifestyle (Jackowiak 1998). The development of civilisation, progressive increase in population, raising standards of living make living organisms face the alternative – die or adapt to extremely unfavourable urban environmental conditions. The extinction of lichens is the first and most clearly legible signal of the appearance of danger threatening other organisms and as a consequence the entire biocenosis (Lipnicki, Wójciak 1995). Cities should be subjected to monitoring, ecological and floristic research, because this is exactly where all the changes, those negative and positive, proceed at enhanced magnitude and are easier to be observed.

In Poland studies on lichens have been carried out in many large cities and small towns, often those of health resort status, located in lowlands, foothills and mountains. Lichenbiota has been developed for larger cities, such as Lublin (Rydzak 1953), Poznań (Dziabaszewski 1962), Toruń (Wilkoń-Michalska et al. 1968), Radom (Cieśliński 1974), Kielce (Toborowicz 1976), Kraków (Kiszka 1977), Szczecin (Marska 1979), Słupsk (Śpiewakowski, Izydorek 1981), Gdańsk, Sopot, Gdynia (Fałtynowicz et al. 1991), Rzeszów (Pustelniak 1991), Przemyśl (Kiszka 1999), Olsztyn (Kubiak 2005) and Białystok (Matwiejuk 2007).

Some authors present only lists of lichen species found, some others besides the floristic lists give maps with zones of atmospheric contamination made on the basis of lichen observations. Not many papers have been devoted to lichens in urban forest communities (Matwiejuk 2000, 2003; Matwiejuk, Kolanko 2001; Adamska 2004; Kubiak 2005), which is partly a consequence of the fact that only a few cities in Poland have forest communities within their administrative borders. To them belong Bialystok and Olsztyn in the north-eastern Poland.

Białystok, the capital of the Podlaskie Voivodeship, located in the centre of Green Lungs of Poland, is a perfect place to carry out the floristic-ecological analysis of lichens on birch in the areas with varying degrees of anthropopressure (centre, outskirts, green areas, exit roads). It is generally considered that cities are lichen deserts, in which adverse conditions for their growth prevail. This work does not confirm such a view. Białystok, outside the built-up area in the city centre, is characterized by abundance and diversity of lichenbiota. Ecological studies of lichens growing on bark of different tree species have been carried out, among others, in large forest complexes – Knyszyńska Forest (Bystrek, Kolanko 2000), Tuchola Forest (Kowalewska 2004), Biebrza Valley (Kolanko 2003) and Northern Poland (Rutkowski, Kukwa 2000), the Olsztyn Lake District (Kubiak 2006), Western Pomerania (Kowalewska 2007) and Western Wielkopolska (Zarabska 2009). The research subject were lichens on birch, poplar, oak and beech trees.

The aim of the study was the floristic-ecological analysis of birch lichens in the area subjected to different anthropopressure, within the city limits of Białystok, with a special focus on examination of the impact of different habitat conditions on areas with different degrees of anthropopressure on the species composition of lichens, abundance and distribution of lichens on the trunk of birch up to 2.5 m in height.

2. Study area

Białystok is the biggest city of north-eastern Poland, the capital of Podlaskie Voivodeship. It is at 53° 20' north latitude and 23° 10' east longitude. The city area comprises 102.12 km^2. According to the physico-geographical division of Poland (Kondracki 1994, 1998) Białystok is located in the Podlaska Lowland, in the western part of the Białostocka Upland. The climate of Białystok has typical features for the area of north-eastern Poland (Górniak 2000).

The location and nature of urban green is one of the main factors affecting the distribution of the particular lichen species in the area of Białystok. Green areas occupy around 32% of the city area (25.9 ha). Within the administrative boundaries of the city there are extensive forest complexes (total forest area within the city is 17,8 ha): Pietrasze Forest, Zwierzyniecki Forest, municipal forests to the west of 11 Listopada Street and at the junction of Kawaleryjska and Ciołkowskiego Street, Solnicki Forest, Bagno Forest, municipal forest at the Dojlidzkie Ponds and Bacieczki Forest. Also within the city there are eight parks, covering a total area of 80 ha. The city green area also includes squares (15), greenery, allotment complexes, green areas alongside sports facilities and high greenery of streets.

3. Study species. Birch as a phorophyte

Birch *Betula* sp. is a pioneer species, growing on dry and barren soils, on dunes, wasteland, but always in well-lit places. It endures atmospheric pollution, but impoverishes soil and prevents growth of many other plants in its immediate vicinity. It reaches the age of 120 years. Birch has acidic bark (under natural conditions the pH of bark varies from 3.5 to 5), poor in nutritional compounds and of small water capacity. The bark of birch is firm and rough. Birch has an unevenly developed bark surface and in places it is not suitable for settling lichens. White bark on the tree peels off making thin patches and as a result fresh layers of cork become continuously exposed.

Trees with acidic bark, such as birch or pine usually have a set of epiphytic lichens poorer in species in comparison with those on trees with neutral or slightly alkaline bark. This poverty of lichens is aggravated by other factors, such as low water capacity of the birch bark, low concentration of elements, and moreover, bark peels off intensively, which is not conducive to lichens. The trunk of birch frequently becomes colonized by demonstrably acidophilous and fast-growing lichens, e.g. *Hypogymnia physodes* and *Lecanora conizaeoides*.

4. Methods

The field investigation was carried out in 2007–2010, on 81 stands. At each stand, observations were carried out on the trunks of birch up to 2.5 m. Floristic index of species was compiled taking into account lichen species growing at the base of the stem (0–0.5 m), in the middle of the stem (0.5–2.5 m) and in the tree crown (branches), the degree of thallus cover was also determined. In order to evaluate the modifying impact of anthropogenic factors on epiphytic lichens of birch, the city was divided into three main sectors. One of them included exit roads outside the city buildings planted with trees. The second one included built-up areas and open city. The third one was green areas, large complexes of trees: parks, cemeteries and forests. In the areas taken by housing estates (built-up land) city centre and peripheral areas were distinguished, including estates with detached houses, residential areas, open spaces outside the built-up areas.

Table 1. Lichens on the bark of birch *Betula* sp.

Tabela 1. Porosty rosnące na korze brzozy *Betula* sp.

Species	C	Distribution in the city											
		Built-up area				Green areas						Roads outlet	
		Centre		Periphery		Park		Cemetery		Forest			
		A	B	A	B	A	B	A	B	A	B	A	B
Lecanora conizaeoides Nyl. ex Cromb.	V	+	5	+–1	18	+	1	+	7	+	14	+	1
Hypocenomyce scalaris (Ach.) M. Choisy	IV	+	3	+–2	20	2	2	+–2	9	+–4	29	+	1
Hypogymnia physodes (L.) Nyl.	IV	+	3	+–2	26	+	1	+–4	7	+–4	32	+	1
Lepraria sp.	III	1	1	+–1	17	1	1	+–2	9	+–3	23		
Scoliciosporum chlorococcum (Graewe ex Stenh.) Vězda	III	1	3	+–1	17	+	3	+	5	+	17		
Physcia dubia (Hoffm.) Lettau	II	+	2	+–1	12	+	1	+	3	+	2		
Cladonia coniocraea (Flörke) Spreng.	II	+	1	+–1	7			1	3	+–3	24		
Parmelia sulcata Taylor	I	+	2	+	4	+	1	+	1	+	8		
Phaeophyscia orbicularis (Neck.) Moberg	I	+	3	+	6	6	1	+	2				
Xanthoria parietina (L.) Th. Fr.	I	+	2	+	9	+	1			+	6	+	1
Cladonia fimbriata (L.) Fr.	II			+	3			+	1	+–2	18		
Xanthoria polycarpa (Hoffm.) Th. Fr. ex Rieber	II			+	16	+	1			+	3	+	1
Hypogymnia tubulosa (Schaer.) Hav.	I			+	1			+	2	+	5		
Lecanora allophana Nyl.	I			+	1			+	1	+	2		
Melanelixia fuliginosa (Fr. ex Duby) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	I			+	2			+	1				
Amandinea punctata (Hoffm.) Coppins & Scheid.	I			+	2			+	1				

Species	C	Distribution in the city											
		Built-up area				Green areas						Roads outlet	
		Centre		Periphery		Park		Cemetery		Forest			
		A	B	A	B	A	B	A	B	A	B	A	B
Candelariella xanthostigma (Ach.) Lettau	I	+	1	+	2								
Physcia adscendens H. Olivier nom. cons.	I	+	1	+	5								
Physcia stellaris (L.) Nyl.	I			+	5					+	2		
Lecanora pulicaris (Pers.) Ach.	I			+	4					+	1		
Lecanora carpinea (L.) Vain.	I			+	1					+	2		
Physcia tenella (Scop.) DC.	I			+	1					+	1	+	1
Trapeliopsis flexuosa (Fr.) Coppins & P. James	I			+	1					+	1		
Pseudevernia furfuracea (L.) Zopf.	I			+	1			+	1				
Vulpicida pinastri (Scop.) J.-E. Mattsson & M.J. Lai	I							+	1	+–1	4		
Platismatia glauca (L.) W.L. Culb. & C.F. Culb.	I							+	1	+	3		
Candelaria concolor (Dicks.) Stein	I			+	1								
Phaeophyscia nigricans (Flörke) Moberg	I			+	1								
Phlyctis argena (Spreng.) Flot.	I							+	1				
Tuckermanopsis chlorophylla (Willd.) Hale	I									+	7		
Cladonia digitata (L.) Hoffm.	I									+	4		
Cladonia glauca Flörke	I									+	2		
Cladonia chlorophaea (Flörke ex Sommerf.) Spreng.	I									+	2		
Placynthiella uliginosa (Schrad.) Coppins & P. James	I									+	2		
Trapeliopsis granulosa (Hoffm.) Lumbsch	I									+	2		

Species	C	Distribution in the city											
		Built-up area				Green areas						Roads outlet	
		Centre		Periphery		Park		Cemetery		Forest			
		A	B	A	B	A	B	A	B	A	B	A	B
Cladonia macilenta Hoffm.	I									+	2		
Bryoria crispa (Mot.) Bystr.	I									+	1		
Bryoria vrangiana (Gyeln.) Brodo & D. Hawksw.	I									+	1		
Caloplaca holocarpa (Hoffm. ex Ach.) A.E. Wade	I									+	1		
Chaenotheca chrysocephala (Turner ex Ach.) Th. Fr.	I									+	1		
Lecanora hagenii (Ach.) Ach.	I									+	1		
Lecanora varia (Hoffm.) Ach.	I									+	1		
Lepraria elobata Tønsberg	I									+	1		
Lepraria incana (L.) Ach.	I									+	1		
Melanohalea exasperatula (Nyl.) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch	I									+	1		
Usnea subfloridana Stirt.	I									+	1		

Explanatory notes: A – coverage degree: + – rare or very rare species of very small coverage degree,1 – species covering less than 1/20 (5%) of the area, 2 – species covering 1/20–1/4 (5–25%) of the area; 3 – species covering 1/4–1/2 (25–50%) of the area, 4 – species covering 1/2–3/4 (50–75%) of the area, 5 – species covering over 3/4 (over 75%) of the area; B – number of sites studied; C – degree of constancy, V – species present at 80.1–100% of sites studied, IV – species present at 60.1–80.0% of sites studied, III – species present at 40.1%–60.0% of sites studied, II – species present at 20.1–40.0% of sites studied, I – species present at 0.1–20.0% sites studied.

In the floristic table (Tab. 1) the cover degree is given in the 6-degree progressive scale of Braun-Blanquet and the degrees of constancy are calculated within the 5-degree scale of Braun-Blanquet (Barkmann 1958). Some specimens of genus *Lepraria* have been marked by thin layer chromatography (TLC) (Orange et al. 2001). The species have been named according to Santesson et al. (2004) and genus *Bryoria* and *Usnea* to Bystrek (1986, 1994), *Melanohalea exasperatula* to Blanco et al. (2004), *Melanelixia fuliginosa* to Arup, Sandler Berlin (2011), *Cladonia coniocraea* to Pino-Bodas et al. (2011). The lichen material has been deposited at the Herbarium of the Institute of Biology, University of Białystok.

5. Results

5.1. Lichen species differentiation on the bark of birch *Betula* sp.

In the area of Białystok, birch is not distributed evenly. In the natural landscape of the city it prevails in the woods and on the outskirts. Lichens on the bark of birch have been documented on 81 trees (11 in the city centre, 47 on the periphery, 23 in parks and forests). They have not been reported on 3 trees studied that grew in the city centre. The bark of birch was found to be colonized by 46 taxa of lichens belonging to 13 families and 26 genera (Tab. 1). The most frequently represented families are: *Lecanoraceae*, *Parmeliaceae*, *Physciaceae* and *Lecideaceae*. Lichens with crustose (37%) and foliose (35%) thallus dominate. The share of other forms is less marked. On the trunks and branches of birch, common species have been most frequently found, such as *Hypogymnia physodes* (70 stands), *Hypocenomyce scalaris* (64), *Lepraria* sp. (51), *Lecanora conizaeoides* (46), *Scoliciosporum chlorococcum* (45), *Cladonia coniocraea* (35).

The participation of lichens in colonization of the bark of trunks and branches of birch is the largest in the woods (Fig 1) where 37 species have been recorded, and on individual trees the number of species ranged from five to ten. Fifteen species have been found only in the woods, for example, *Bryoria crispa, B. vrangiana, Tuckermanopsis chlorophylla* and *Usnea subfloridana,* under full legal protection and included in the national Red List (Cieśliński et al. 2006). On the bark of birch trees growing on the fringes of forests and along roads, where their bark is often enriched as a result of dust and where there are trees whose cuts exude juice, the lichen species included *Phaeophyscia orbicularis*, *Physcia tenella, Xanthoria polycarpa* and sometimes also *Lecanora carpinea* and *L. pulicaris*. In the heart of the tree stands the bark of birch was colonized mainly by *Hypocenomyce scalaris,* *Hypogymnia physodes, Pseudevernia furfuracea, Lepraria* sp., less often by *Chaenotheca chrysocephala, Hypogymnia tubulosa, Platismatia glauca, Vulpicida pinastri* and the base of trunks by the lichens from the genus *Cladonia*.

On birch trees in parks and cemeteries, lichens grew only on certain trees, but mostly in the form of a single thallus or in populations of several individuals. These are species common throughout the area or rare species of a characteristic association *Pseudevernietum furfuraceae*: *Pseudevernia furfuracea, Platismatia glauca* and *Hypogymnia tubulosa.*

A diverse composition of lichen species on birchbark was found on the outskirts of the city, on the premises of residential estates and on the newly built housing estates and those under construction. Nitrophilous species of the genus *Phaeophyscia, Physcia* and *Xanthoria* predominated. Among them *Candelaria concolor* and *Phaeophyscia nigricans* were the lichen species not found in the other parts of the city.

On the trees growing in the centre and on exit roads, there were lichen species identified also in the other parts of the city. On the trees in the city centre 12 taxa were found on 11 stands (Table 1). Lichens with crustose and leprorose thalli prevailed: *Lecanora conizaeoides, Scoliciosporum chlorococcum* and *Lepraria* sp. On a few trees, lichens with foliose thalli were found: *Hypogymnia physodes, Phaeophyscia orbicularis, Physcia adscendens, P. dubia, P. stellaris, Xanthoria parietina* and *X. polycarpa.* The presence of *Lepraria* sp., *Phaeophyscia orbicularis* and *Scoliciosporum chlorococcum* was most often established at the bases of tree trunks. The frequency of prevalence and trunk cover was low (Table 1). The thalli growing on tree trunks were small. The dominant epiphyte was an aerial alga *Pleurococcus vulgaris*.

Around 80% of the taxa of lichens identified on the premises of Białystok were recorded with degree I of phytosociological constancy, while the lowest number of the lichen taxa belonged to the constancy degree IV and V, 4% and 2%, respectively (Table 1).

The number of taxa found in each of the three main study sections increased with increasing distance from the city centre and was the largest in green areas, mainly in forests (Fig. 1).

Lichens with crustose thalli predominated on the bark of birch in forests, with foliose thalli in the city centre, on the periphery, in parks, cemeteries and on exit roads. Lichens with fruticose thalli were not recorded in the city centre, in parks and on exit roads (Fig. 2).

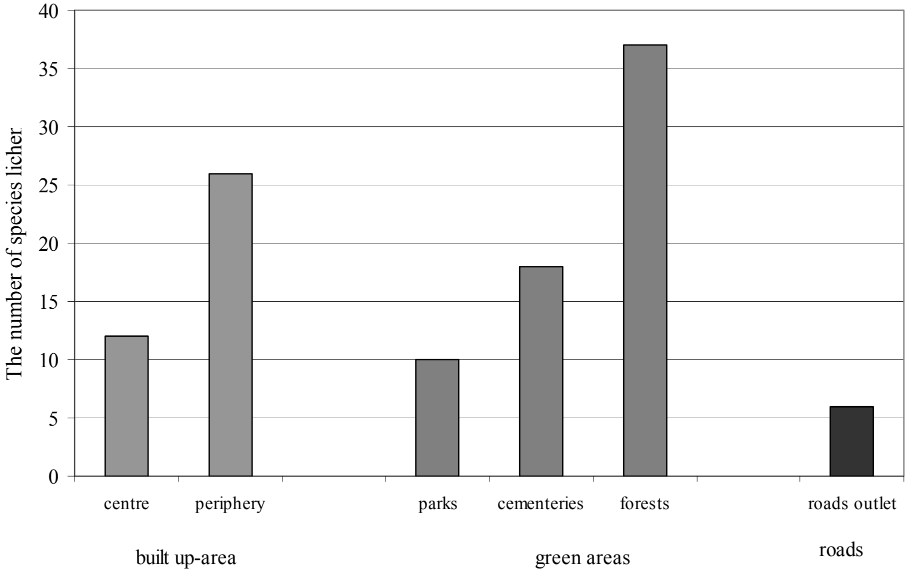

Figure 1. The number of lichens species on birch trees in different sections of Bialystok

Rycina 1. Liczba gatunków porostów brzozy w różnych obszarach Białegostoku

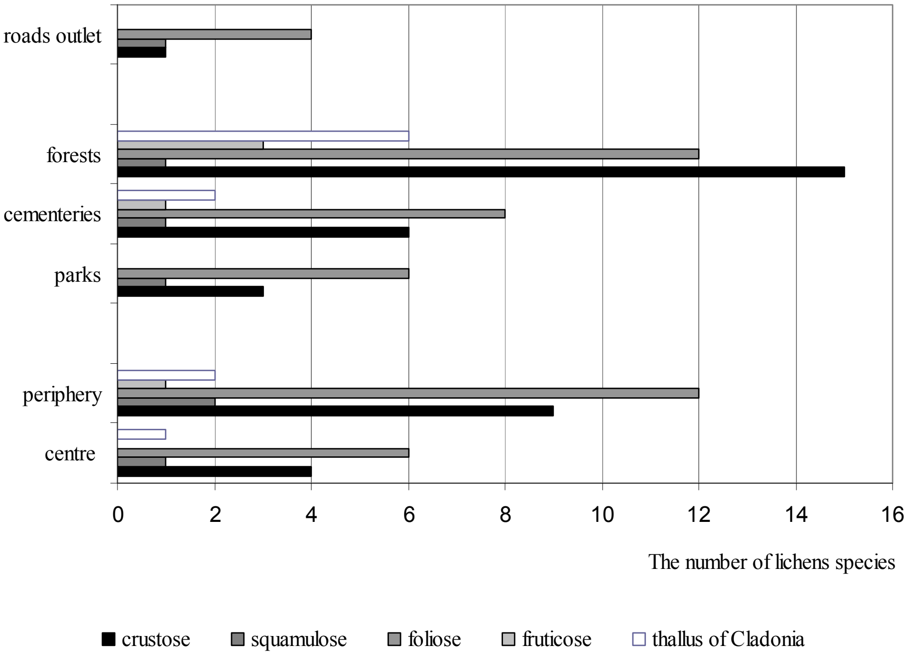

Figure 2. The contribution of individual forms of growth of lichens in different sections of the city

Rycina 2. Udział poszczególnych form wzrostu porostów w różnych regionach miasta

On the basis of floristic-ecological analysis of lichens on birch trees in Bialystok, it is possible to demarcate four lichen occurrence zones. Zone I (absolute lichen desert) was not found in the city centre. On anthropogenic habitats (built-up areas in the centre and on the outskirts of the city) there were species characteristic of zone II (relative lichen desert) – *Amandinea punctata*, *Lecanora conizaeoides*, *Lepraria* sp., *Scoliciosporum chlorococcum* and III (internal fight zone) – *Hypocenomyce scalaris, Hypogymnia physodes, Phaeophyscia orbicularis, Physcia adscendens* and *Xanthoria parietina* and on the green areas with a high degree of tree cover and in urban forests there were sentinel species characteristic of zone IV (central fight zone) – *Hypogymnia tubulosa, Pseudevernia furfuracea, Usnea hirta* and V (outer fight zone) – *Bryoria vrangiana, Chaenotheca chrysocephala, Cladonia* sp., *Platismatia glauca, Usnea subfloridana* and *Vulpicida pinastri.*

5.2. Vertical distribution of lichens on a phorophyte

In the woods of Bialystok vertical structures in the distribution of lichens on birchbark are noticeable. There is an essential relationship between the height on the trunk and the number of species, with increasing height (more than 2 m) the number of taxa gradually decreases.

The largest group of lichens occupied the central part of the stem. Among them crustose and foliose lichens predominated. These were light-loving species, which found the best conditions for growth in this part of the trunk. The species colonizing the base of the trunk were those preferring moisture and shade. The lowest number of lichens was identified in the crown of trees.

At the base of the trunk, in places where humus accumulates in cracks, there were *Cladonia* (*C. coniocraea, C. fimbriata* and *C. digitata*), often visible only in the form of squamules of primary thallus. *Lepraria* sp., *Hypocenomyce scalaris* also showed preference for trunk bases, occasionally there were *Vulpicida pinastri* with foliose thallus of lemon yellow colour, with brightly yellow soralia present at the edges of lobes and *Parmelia sulcata*. In the higher parts of trunks (not higher than 2 m) and in the zone under the tree crown there was only *Hypogymnia physodes,* occasionally there grew *Hypogymnia tubulosa* (Pietrasze Forest, Solnicki Forest), *Tuckermanopsis chlorophylla, Platismatia glauca* (Bagno Forest, Solnicki Forest), *Melanelixia fuliginosa, Melanohalea exasperatula, Bryoria crispa* (Bagno Forest). The scars resulting from sprouting shoots were mostly colonized by crustose lichens. The percentage of cover varied.

In built-up areas at the base of the trunks of birch mainly synanthropic species grew such as *Phaeophyscia orbicularis, Physcia dubia, P. adscendens, X. parietina* and *Scoliciosporum chlorococcum* and *Lepraria* sp.

5.3. Participation of vulnerable and protected lichens

Of the 45 lichen species identified in Białystok on bark of birch trees, 6 species have been included in the Red List of extinct and vulnerable lichens of Poland (Cieśliński et al**.** 2006), 1 species in the critically endangered category – CR (*Bryoria vrangiana*), 2 species in the endangered category – EN (*Bryoria crispa, Usnea subfloridana*), 1 species in the vulnerable category – VU (*Tuckermanopsis chlorophylla*), 2 species in the category of near threatened – NT (*Hypogymnia tubulosa, Vulpicida pinastri*), as well as 1 species on the Red List of lichens vulnerable in north-eastern Poland (Cieśliński 2003), including 1 – CR (*Bryoria vrangiana*). The level of threat for lichens in north-eastern Poland, compared to other regions in lowland Poland is lower, which is reflected in a small number of vulnerable lichens of birch trees from Białystok in the local Red List (Cieśliński 2003) compared to the national Red List (Cieśliński et al. 2006).

Of all the 45 lichen species of birch in Białystok, 10 have been put under legal protection.

6. Discussion

In Europe, two birch species occur naturally: silver birch (*Betula pendula* Roth) and downy birch (*Betula pubescens* Ehrh.). Both species have a wide natural distribution area on the Eurasian continent, ranging from the Atlantic to eastern Siberia. Birches are light-demanding early successional pioneer species. Their bark is white, often with black diamond-shaped marks or larger patches, particularly at the base. Birch has acidic bark (under natural conditions the pH of bark varies from 3.5 to 5), poor in nutritional compounds and of small water capacity.

The lichen biota of birch has not been studied extensively in Poland. Epiphytic lichens are one of the most numerous habitat groups in cities. At the premises of Bialystok city on the bark of birch *Betula* sp. 45 lichen species have been identified. In other cities of Poland 41 lichen species have been recorded in Łódź (Kuziel, Halicz 1979), 26 in Kielce (Toborowicz 1976), 22 in Słupsk (Śpiewakowski, Izydorek 1981), 20 in Przemyśl (Kiszka 1999), 18 in Lublin (Rydzak 1953), 11 in Rzeszów (Pustelniak 1991).

The most frequently reported species on the bark of this phorophyte are crustose lichens: *Lepraria incana, Lecanora conizaeoides, Amandinea punctata* and the most toxitolerant foliose lichens: *Hypogymnia physodes*, *Parmelia sulcata* and squamulose *Hypocenomyce scalaris.* These are common lichens, which constitute the main backbone of lichenbiota in many cities. The richest lichenbiota in cities has been noticed on trees growing in green areas, e.g. in forests or parks.

In the 1970s on the bark of birch in cities, the species which are currently very rare used to grow, such as *Chrysothrix candelaris* (in category CR), *Cladonia botrytes* (EN) in Łódź (Kuziel, Halicz 1979), *Chrysothrix chlorina, Flavoparmelia caperata* (EN) in Lublin (Rydzak 1953). In parks of Kyiv on the bark of *Betula pendula* 30 lichen species have been recorded (Dymytrova 2009). The highest richness of epiphytic lichen species (mean 10.6) was found on the bark of *Betula pendula*.

As well as having many biocoenotic functions, birch may play an important role in preserving lichen biodiversity in the forest environment in Poland and Europe.

The epiphytic biota of *Betula* sp. as a phorophyte is characteristic, diverse and rich, and it comprises 82 lichen species in Knyszyńska Forest (Bystrek, Kolanko 2000), 78 in Białowieża Primeval Forest (Cieśliński, Tobolewski 1988). On bark of *Betula* in the British Isles 235 lichen species have been recorded (Coppins et al. 1984).

7. Conclusions

1. In the lichenbiota of birch in Białystok non-forest species dominate. The participation of species which are natural constituents of forest biocenoses is negligible.
2. City forests exhibit a much greater diversity in lichen species and higher degrees of frequency in relation to other urban areas.
3. Forest areas of Białystok should be treated as an important refuge of many endangered lichen species in the country.
4. On birchbark the dominant presence of widespread lichens reaching high frequency levels was established, e.g. *Hypogymnia physodes, Parmelia sulcata.*
5. Around 80% of lichen taxa occurred with degree I of phytosociological constancy, and the lowest number of lichen taxa occurred with degrees IV and V, 4% and 2%, respectively.

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Porosty brzozy (*Betula* sp.) na obszarze o zróżnicowanej antropopresji w granicach miasta Białystok – studium florystyczno-ekologiczne

Streszczenie

Praca przedstawia listę 46 gatunków porostów rosnących na korze brzozy *Betula* sp. w Białymstoku. Około 80% taksonów występowała z I stopniem stałości fitosocjologicznej, natomiast najmniej (po 4% i 2%) należało do stopni IV i V. Liczba taksonów znalezionych na korze brzozy była największa na drzewach rosnących na terenach zielonych: w lasach – 35 gatunków, a najmniejsza w centrum miasta – 9 i przy drogach wylotowych – 6. Dla wielu gatunków porostów stwierdzono różnice w częstości występowania i obfitości na poszczególnych obszarach. W lasach w rozmieszczeniu porostów na korze brzozy stwierdzono wyraźnie struktury pionowe.