INFLUENCE OF CORMORANT *Phalacrocorax carbo* COLONY ON BIOMETRICAL PARAMETERS OF THREE-NERVED SANDWORT *Moehringia trinervia* (*Caryophyllaceae*) LEAVES AND SEEDS

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Abstract


Investigation on the influence of fertilization on enlargement of leaf dimensions and seed production of *Moehringia trinervia* was carried out in July 2006 on Vistula spit, within the largest European breeding colony of cormorant *Phalacrocorax carbo*, where multiple enhancement of NPK in the soil due to deposition of guano was noted. The control sample was taken from place located ca. 500 m away from the colony, where cormorants never nested. Leaves from the area within the colony were significantly larger and more rounded than leaves of plants growing outside the colony. Enlargement of leaf blade area allows plant to develop assimilation apparatus, which in turn affects on improvement of ability to absorb and transform greater amounts of nitrogen into organic compounds. It had probably an influence on extension of seed number, because plants within the colony area produced significantly more seeds than plants outside the colony. However seeds from the colony were twice lighter seed from the control sample, which may facilitate their dispersal and occupancy on larger area. Probably in this way *Moehringia trinervia* adapts to the unlimited availability of nutrients coming from bird excrements.

Key words: bird colonies, soil fertilization, nitrogen, nitrophilous species, leaf blade morphology, seed production

Introduction

Natural or anthropogenic changes, that take place in nature, lead to formation of new habitat conditions. A good example of such influence may be functioning of bird colonies. In that case habitat changes are mainly connected with deposition of guano as well as undigested and leftover bits of food, which at first affect soil enrichment, but further cause its over-
fertilization. Long-term pressure of large colonies causes extremely high concentration of nutrients in soils (Abbot et al., 2000; Ligeza, Smal, 2003).

Changes in habitat caused by bird colonies result in transformations of flora and vegetation within the whole occupied area. That phenomenon was observed and described mostly from maritime and oceanic islands as well as coastal areas of different parts of the world (Smith, 1978; Paradis, Larenzoni, 1996; Norton et al., 1997; Vidal et al., 1998; Abbot et al., 2000; Garcia et al., 2002), less often from forest ecosystems (Maesako, 1991, 1999; Ishida, 1996; Mun, 1997; Żółkoś, Meissner, 2008). It was indicated that within bird colonies the indigenous flora is replaced by nitrophilous species (described by some authors as “ruderal” (Grime et al., 1988). *Moehringia trinervia* may be ranked to the group of such species, as it is a regular component of the undergrowth layer in colonies of grey heron *Ardea cinerea* and cormorant *Phalacrocorax carbo* in Poland (Żółkoś, Meissner, 2008, authors’ unpublished data). It is an annual, less often biennial dicotyledonous plant of Eurasian distribution range (Grime et al., 1988), connected with undergrowth layer of mesotrophic mixed and broadleaf forests. The species is frequently observed in changed places – in the surroundings sites of tree falls, rabbit scarpes and on tree plantations (Grime et al., 1988). *Moehringia trinervia* is thought to be a plastic species, resistant to environmental stress, or even a ruderal one (Grime et al., 1988).

Most papers, concerning the impact of bird colonies on flora and vegetation, present changes in floristic composition (Hogg, Morton, 1983; Hogg et al., 1988; Vidal et al., 1998), also plant productivity, expressed usually by a total biomass, was analyzed (Smith, 1978; Mun, 1997; Anderson, Polis, 1999; Sanchez-Piñero, Polis, 2000). However, there is no information about the influence of colony on production, size and seed quality of plants growing within the colony. Only few papers demonstrate results of the investigations on impact of colony on germination and development of particular species (Sobey, Kenworthy, 1979; Smith, 1978; Ishida, 1997; Garcia et al., 2002). It was also shown that overfertilization within the colony area causes changes in dimensions of the whole plants or their particular organs (Żółkoś, Meissner, 2008).

The main aim of presented work was an attempt to explain how the excess of nutrients, coming from the bird colony, may cause changes of leaf blade morphology as well as size and number of seeds in *Moehringia trinervia*.

**Material and methods**

**Study area**

The research was carried out on two plots, covered by a substitute, two-layer tree stand in the habitat of acidophilous oak forest. The crown canopy layer covered ca. 50% and was mainly built by Scots pine, with admixture of *Picea abies* and *Betula pendula*. The subcanopy layer, with 30% cover, was built by *Quercus robur*, *Picea abies* and *Sorbus aucuparia*. In the shrub layer there were tree saplings of *Quercus robur* and *Picea abies*, altogether with *Frangula alnus* and *Sorbus aucuparia*, and the cover reached 20%. Additionally, in the area within the colony, there were few exemplars of *Sambucus racemosa* in shrub layer. Differences between two studied plots were noted mostly in species abundance of the undergrowth layer. In the control plot *Vaccinium myrtillus* was a dominant component, and among regular and relatively abundant species there were: *Trientalis europaea*, *Vaccinium vitis-
idaea, Deschampsia flexuosa, Moehringia trinervia, Pteridium aquilinum and some mosses typical for coniferous forests. At the same time in the plot within colony the undergrowth was dominated by Moehringia trinervia, with accompanying participation of Trientalis europaea and less frequent appearance of Vaccinium myrtillus and Calamagrostis epigeios. Also the moss cover in the second plot was significantly lower.

Sampling methods

Material was collected in July 2006 on Vistula spit close to Kąty Rybackie village, within and outside the largest European breeding colony of cormorant (Glaz et al., 2003). Within the colony, samples were collected in place, where cormorant had been nesting for four years. In that part of colony there was no visible changes of community structure, although strong enrichment in chemical composition of soil was already observed (Glaz et al., 2003). The control sample was taken from place located ca. 500 m away from the colony, where cormorants never nested. In the studied areas (within colony and control one) habitat conditions and vegetation were very similar. Both plots were situated on old, hilly, irregular coastal dunes covered by acidophilous oak forest, strongly deformed by Scots pine Pinus sylvestris and spruce Picea abies plantations. The main type of soils within the studied areas was Podzol (Glaz et al., 2003). The analyses of chemical composition of soils from the colony area were carried out and published by Glaz et al. (2003) and Ligeza, Smal (2003). They indicate a multiple enhancement of particular nutrient contents in the surface, mineral-organic soil horizons. The observed values were: total nitrogen – within colony 13850 mg/kg (NH₄ 943 mg/kg, NO₃ 533 mg/kg), outside 5100 mg/kg (NH₄ 93.7 mg/kg, NO₃ 5.7 mg/kg); phosphorus – within colony 641 mg/kg, outside 12.1 mg/kg; total potassium (exchangeable and available) within colony 1221 mg/kg, outside 59.9 mg/kg (Ligeza, Smal, 2003).

Biometrical methods

In order to collect samples for biometrical research both plots (10x3 m each) were divided into 1 m² square grid. From each square two undamaged, fruiting individuals of Moehringia trinervia were sampled. All samples from each plot were gathered and then a biggest leaf of 30 randomly picked individuals was taken. Individuals with damaged biggest leaf were omitted. All leaves were immediately, in fresh condition, scanned with 300 dpi resolution. All measurements were made in CorelDraw 9.0 (Corel Corporation), using its standard procedures to the nearest 0.1 mm.

Measurements of leaf blade were made according to the method of dividing leaf into 10 equal parts perpendicularly to the midrib (Meissner, Żółkoś, 2008). Along the division line the distances between midrib and the leaf blade edge were measured, separately for the left and right side (Fig. 1).

The sum of all 18 measurements (9 per each side of leaf blade) was defined as a leaf blade roundness index. Due to a strict dependence of that index on leaf blade size, the values of leaf blade roundness index were standardized by dividing the index calculated for particular leaf by the leaf blade length:
The middle pair of leaf blade measurements was used for calculation of the fluctuating asymmetry index, according Palmer’s (1994) formula:

$$W = \frac{\sum_{i=1}^{n} Li + \sum_{i=1}^{n} Ri}{\text{leaf length}}$$

Furthermore, a total length of leaf blade, altogether with petiole, and the width of the blade in the half of its length were measured.

Additionally, mature capsules of all collected individuals were gathered. Altogether 228 capsules from the within colony area and 226 from the control area were collected. All seeds were extracted from capsules and counted. In order to compare seed weight in both studied plots, all seeds from each plot were pulled together and then they were divided into groups of 10 specimens, rejecting immature ones. Seeds were dried for 6 hours in temperature of 70 °C and then 50 samples of 10 seeds, from each studied area, were weighted with an accuracy of 0.1 mg.

Statistical analyses were based on statistical methods according to Zar (1996), using STATISTICA 6.0 (StatSoft, 2001).

**Results**

Leaves from the area within the colony were significantly longer and wider than leaves of plants growing outside the colony (Table 1) and the difference was more distinct in case of width than length. Moreover, individuals from the cormorant colony had also more rounded blades (Table 1) and they were characterized by significantly lower variation of the roundness index values (F-test, $F = 3.0, p = 0.004$) than plants from the area outside the colony (Fig. 2). Considerable range of the roundness index values for leaves outside the colony points at high diversity of their shape: from very rounded leaves to slender, oval ones.

Leaf blades in both studied plots of three-nerved sandwort show significant fluctuating asymmetry (values of the fluctuating asymmetry index significantly differed from 0: for

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Outside colony</th>
<th>Within colony</th>
<th>t-test or Cochran-Cox test (t')</th>
<th>Relative difference [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf length</td>
<td>22.3 ± 2.08</td>
<td>111.1 ± 3.35</td>
<td>t = 16.0, p &lt; 0.001</td>
<td>51.6</td>
</tr>
<tr>
<td>Leaf width</td>
<td>9.5 ± 1.09</td>
<td>16.2 ± 1.35</td>
<td>t = 21.1, p &lt; 0.001</td>
<td>70.5</td>
</tr>
<tr>
<td>Roundness index</td>
<td>2.9 ± 0.47</td>
<td>3.3 ± 0.27</td>
<td>t' = 3.5, p &lt; 0.001</td>
<td>13.8</td>
</tr>
</tbody>
</table>

Abbreviations as on Fig. 1.
the within colony area t-test, \( t = 6.22, p < 0.001 \); for the outside colony area t-test, \( t = 7.19, p < 0.001 \). However, mean values of the asymmetry index in both samples did not differ significantly (within the colony \( FA = 0.29 \), outside \( FA = 0.20 \), t-test, \( t = 1.76, p = 0.08 \)).

Plants within the colony area produced from 6 to 26, on average 14.2 seeds, (SD = 2.93, \( N = 228 \)) in one capsule. That is significantly more than plants outside the colony, which had from 4 to 18 seeds in one capsule, with mean value 12.0 (SD = 2.83, \( N = 226 \)) (t-test, \( t = 7.9, p < 0.001 \)) (Fig. 3). The seed mass of plants growing within the colony was twice lower than seed mass of plants from the outside colony area (t-test, \( t = 54.6, p < 0.001 \)) (Fig. 4). The heaviest seeds (10 specimens) collected within the colony weighted 2.4 mg, while the lightest seeds from the outside area weighted 3.5 mg. Thus the difference was so big that mass ranges of weighted samples from the studied areas did not overlap (Fig. 4).

**Discussion**

Significant enlargement of the leaf blade in plants growing within the cormorant colony was observed. *Moehringia trinervia* has two meristems that take part in the growth and development of leaves: an intercalary meristem, which is responsible for gain of leaf blade length and a plate one, responsible for the blade width (Bell, 1993). In case of three-nerved sandwort individuals that grew within the colony, the greatest relative extension concerned the leaf width, although its length also changed significantly. It was confirmed by the analysis of leaf blade shape, as leaves of plants growing within colony were more rounded comparing to those from the outside colony area. It indicates enlargement of assimilation apparatus not only due to improvement of linear dimensions, but also by tendency of rounding the ovate leaf blade. All leaves from the colony area were similarly rounded, which was confirmed by relatively high value and small diversity of the roundness index. That is probably a way in which the species adapts to the unlimited availability of nutrients coming from bird excrements. Maximizing of leaf blade area, through its rounding, allows plant to develop assimilation apparatus, which in turn affects on improvement of ability to absorb and transform...
greater amounts of nitrogen into organic compounds (Mohr, Schopfer, 1995). Additionally, increased availability of nitrogen causes higher photosynthesis rate (Doescher et al., 1990). Cormorant guano is decomposed in soil into two forms of nitrogen: ammonium (NH$_4^+$) and nitrate (NO$_3^-$) (Ligeza, Smal, 2003). It is profitable for plants, which absorb nitrogen in both ionic [NO$_3^-$ and NH$_4^+$] forms (Chapin, 1980; Mohr, Schopfer, 1995). The kind of absorbed ion depends on its concentration in soil as well as on environmental conditions, such as: temperature, moisture and soil pH (Mohr, Schopfer, 1995; Paul, Clark, 2000). In case of acidic soil, which was detected within the studied cormorant colony, plants absorb nitrogen mainly in NH$_4^+$ form (Brady, 1984). Absorption of ammonium ions is more profitable for plants, as those ions are in cells transformed into amino acids in more energy-efficient way (von Wiren et al., 2001). The nitrate form requires energetically expensive reaction of reduction to be integrated into protein chains (Paul, Clark, 2000; Miller, Cramer, 2004). However, NO$_3^-$ ions are also absorbed, because nitrates are those, who regulate activity of
metabolic processes in plants. They are essential, among others, for a proper photosynthesis run (Mohr, Schopfer, 1995).

Improvement of the ability to absorb and transform large amounts of nitrogen into organic compounds, due to enlargement of linear dimensions and rounding of the leaf blade (resulted in enlargement of leaf area) had probably an influence on extension of seed number in *Moehringia trinervia*. In case of three-nerved sandwort growing within the colony the mean number of seeds in capsules was 14.2 and it was higher than the value known from literature – 12 (Grime et al., 1988). Also the maximum number of seeds in one capsule, noted in this study was 26, which highly exceeded the maximum value of 17, referred to this species (data from http://www.york.ac.uk/res/ecoflora/cfm/ecofl/index.cfm). Greater seed production as a reaction on higher nitrogen concentration in soil has been reported for other plants (Stock et al., 1989; Greipsson, Davy, 1997; Drenovsky, Richards, 2005).

Enlargement of seed number in capsules of plants growing within the cormorant colony probably resulted in reduction of their unit mass. Seeds from the colony area were twice lighter than seeds from the outside area. Similar negative correlation between the number and mean weight of seeds was observed also for *Majanthemum bifolium* (Kosiński, 2008). High diversity of seed size among individuals of the same population is commonly known (e.g. Schaal, 1980; Roach, Wulff, 1987; Ågren, 1989; Kosiński, 2008). Perhaps it is a result of varied fertility of soil or combination of environmental and genetic reasons (Roach, Wulff, 1987). Still, the ability of inheritance of particular seed size is usually very low (Schaal, 1980; Silvertown, 1989), thus environmental conditions play the fundamental role. It is also worth to mention that N and P content in seeds (in mg/seed) increases linearly with the seed mass, which suggests higher production costs of large seed in case of limited availability of nutrients (Vaughton, Ramsey, 1998). For that reason it is thought that if environmental conditions are good, it is more profitable for the plant to product small seeds, while in situation of limited availability of nutrients – to produce large, better equipped seeds (Mc Ginley et al., 1987). Venable, Brown (1988) indicate that variability of seed mass minimalizes the risk of failure in variable environments. Additionally, small seeds provide to more effective dispersion (Hedge et al., 1991). The individuals of three-leaved sandwort that grew within the colony area produced twice lighter seeds, which facilitated their dispersal and occupancy on larger area.

Investigation on the influence of fertilization on enlargement of leaf dimensions and seed production of *Moehringia trinervia* may be treated as an example of adaptation of this plant to changes in environmental conditions. Usually plants invest in enlargement of seed mass as it is important during first stage of plant development, especially in situation of limited resources of the environment (Roach, Wulff, 1987; Grubb, Burslem, 1998; Meyer, Carlson, 2001). Positive influence of seed mass on germination ability was reported for many species (Schaal, 1980; Weis, 1982; Zimmerman, Weis, 1983). Seedlings germinating from large seeds are better equipped in available resources, which allow them to survive longer carbon deficit and consequences of various mechanical damages (Thompson, 1987) as well as they enlarge competition abilities (Turnbull et al., 1999). Within the species, heavier seeds demonstrate a tendency to a shorter time required to germination than the lighter seeds (Barik et al.,
1996), and seedlings grown from larger seeds have a higher survival and growth rate than those developed from the small ones (Bonfil, 1998). However, plants growing within the studied colony used a strategy of investing in seed quantity instead of quality. For several species it was reported that small seeds germinated earlier than the larger ones, due to better proportion of seed area to its mass and quicker water absorption (Stamp, 1990; Zhang, 1993; Khan et al., 1999; Susko, Lovett-Doust, 2000). However seedlings, which grew from small seeds, form their leaves later and achieve smaller biomass than those developed from large seeds (Susko, Lovett-Doust, 2000). Intense fertilization of soil by nitrogen, phosphorus and potassium compounds, observed within bird colonies, probably compensate losses incurred in the earliest stages of individual development, and the increased supply of both nitrogen forms, especially the more easily to absorb ammonium form, affects enlargement of plant dimensions on its further life stages, particularly its assimilation organs. It seems to be a symptom of the species strategy leading to obtain a maximum leaf area, due to unlimited access to nutrients coming from cormorant excrements.

Intense soil fertilization occurring within the cormorant colony is also a specific kind of environmental stress for plants existing there. Even short, few-years long influence of bird colonies affects both quantitative and qualitative changes in phytocoenoses (Żółkoś, Meissner, 2008). Only few forest species such as *Moehringia trinervia* have the ability of adaptation to habitat-phytocoenotical changes. The lack of significant differences between values of fluctuating asymmetry index, established for leaves from the within and outside colony area, suggests such adaptation.

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