Introduction

Valley meadows are characterised by a high differentiation of soil and water conditions. The area of a relatively small valley may also be covered by flooded habitats, such as the swampy and periodically or inordinately wet, and dry ones on the edges. The majority of such habitats are protected by the Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora (OJ L 206 of 22.07.1992, pp. 7–50). Those habitats are enclaves for an important part of European biological diversity (Warda & Kozłowski, 2012; Dengler, Janišová, Török & Wellstein, 2014). However, 86% of semi-natural grasslands, being a topic of European interests, are nowadays estimated to be ones of disadvantageous protection conditions according to European Environment Agency (EEA, 2015). Results of studies indicate a consistent trend towards much more species-poor communities (Trąba & Wolański, 2012; Wesche, Krause, Culmsee & Leuschner, 2012; Warda, Stamirowska-Krzaczek & Kulik, 2013; Kucharski, 2015). The present state of protection for meadows and grassland habitats of the continental region in Poland according to the data presented by Polish Leadership of Chief Inspectorate of Environmental Protection (GIOŚ, 2018) shows that only 10% (of the codes 6440 and 6210) and 20%
The analysis of the traits determining the development of some plant... 83

(of the codes 6410 and 6510) of the sites are in a favourable state of maintenance (Fig. 1). To make amends for losses to those habitats in the last decades, many compensative activities are being undertaken (Kiehl, Kirmen, Donath, Rasran & Hörlzel, 2010; Conrad & Tischew, 2011; Török, Vida, Deák, Lengyl & Tóthmérész, 2011; Pawluśkiewicz, Janicka & Piekut, 2017 and 2019). Improving the protective situation for such areas often demands the introduction of representative species because of a lack of the proper sources of those species’ seeds in the soil seed bank and nearby the restitution sites as well (e.g. Janicka, 2016). The studies carried out mainly in Western Europe, show that some species are easier to implement than others (Hölzel & Otte, 2003). The main analysed traits are those linked to reproduction (e.g. the life form, the blooming periods), the seeds’ germination and dispersion, and various combinations of mentioned traits determining the success of implementation of peculiar plant species (Isselstein, Tallowin & Smith, 2002; Hölzel & Otte, 2004; Jensen, 2004; Janicka, Pawluśkiewicz, Małuszyńska & Szydlowska, 2016). In Poland, this type of research has so far rarely been undertaken, especially in dicotyledonous herbaceous species. It was stated that various traits support the establishment of the species under various conditions (Engst, Baasch & Bruelheide, 2017). The type of substrate is also very important (Dąbrowski & Pawluśkiewicz, 2011; Gmitrzuk, Dąbrowski, Pietrzyk & Pawluśkiewicz, 2017). For this reason, it is necessary to know the seed germination capacity and the initial growth and development of the species typical for those meadow habitats which are of great importance to Europe. The study hypothesis assumed that fast growth and development of the seedlings increases their survivability. Moreover, the determination of the peculiar species’ traits mostly influencing their development

![Figure 1. Conservation status of selected meadow habitats (A–D). Overall assessment. Own study based on the results of the state environmental monitoring available at Leadership of Chief Inspectorate of Environmental Protection (GIOŚ, 2018)](image-url)
and survivability in the initial development period was the aim of the study. Knowledge of species’ traits can help in predicting the success of restoration of natural ecosystems (Engst et al. 2016).

**Materials and methods**

The studies were carried out in the years 2015–2018. Eight representative plant species typical for four non-af
forsted habitats of the river valleys (*Cnidium dubium* and *Allium angulosum* – 6440; *Sanquisorba officinalis* and *Galium boreale* 6410; *Campanula patula* and *Tragopogon pratensis* 6510 and *Centaurea stoebe* and *Scabiosa ochroleuca* 6210) were investigated. The diaspores (fruits and seeds) of those species (Table 1) were collected by hand, in the third and fourth weeks of September in the years 2014–2017 in the Special Habitat Protection Area – The Lower Pilica Valley (PLH 140016), near Mniszew (51°51′04.1″ N 21°15′57.2″ E, 51°50′00.3″ N 21°17′05.2″ E). The weather conditions in the vegetation periods 2014–2017 were highly differentiated – from exceptionally unfavourable, determined as catastrophically dry in the years 2015 and 2016, to wet in 2017. They were characterised on the basis of the climatic precipitation index, figured out as the quotient of the total precipitation and the temperature added values (Vinczeffy, 1984): 2014 – 0.132 mm·°C–1 (dry), 2015 – 0.091 mm·°C–1 (extremely dry), 2016 – 0.100 mm·°C–1 (extremely dry), 2017 – 0.194 mm·°C–1 (wet). Inclusion in the study of seeds from years with different weather conditions during seed formation and filling, allows the obtained results to be objectified.

**The laboratory studies.** The thousand-seed weight was calculated on the

<table>
<thead>
<tr>
<th>Habitat – code</th>
<th>Species1</th>
<th>Family2</th>
<th>Durability2</th>
<th>Life form3</th>
<th>Diaspore4</th>
<th>TSW5 [g]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cnidion dubii – 6440</td>
<td><em>Allium angulosum</em> L.</td>
<td>Amaryllidaceae</td>
<td>B</td>
<td>G</td>
<td>seed</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td><em>Cnidium dubium</em> (Schkuhr) Thell.</td>
<td>Apiaceae</td>
<td>B</td>
<td>H</td>
<td>fruit</td>
<td>0.44</td>
</tr>
<tr>
<td>Molinion – 6410</td>
<td><em>Galium boreale</em> L.</td>
<td>Rubiaceae</td>
<td>B</td>
<td>H</td>
<td>fruit</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td><em>Sanquisorba officinalis</em> L.</td>
<td>Rosaceae</td>
<td>B</td>
<td>H</td>
<td>fruit</td>
<td>1.24</td>
</tr>
<tr>
<td>Arrhenatherion elatioris – 6510</td>
<td><em>Campanula patula</em> L.</td>
<td>Campanulaceae</td>
<td>D, B</td>
<td>H</td>
<td>seed</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td><em>Tragopogon pratensis</em> L.</td>
<td>Asteraceae</td>
<td>D</td>
<td>H</td>
<td>fruit</td>
<td>5.53</td>
</tr>
<tr>
<td>Festuco-Brometea – 6210</td>
<td><em>Centaurea stoebe</em> L.</td>
<td>Asteraceae</td>
<td>D, B</td>
<td>H</td>
<td>fruit</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td><em>Scabiosa ochroleuca</em> L.</td>
<td>Dipsacaceae</td>
<td>D, B</td>
<td>H</td>
<td>fruit</td>
<td>1.53</td>
</tr>
</tbody>
</table>

1Species names by Mirek, Piękoś-Mirkowa, Zając & Zając (2002).
4Diaspore according to Cappers, Bekker & Jans (2006).
5*TSW* – thousand-seed weight (own results).
basis of the weight of 100 seed units in four replications. The germination capacity was carried out in accordance with International Rules for Seed Testing (ISTA, 2019) and three replicates of 50 diasopores were placed in plastic boxes on moistened filter paper. After sowing, for breaking dormancy, the majority of the species needed pre-chilling for five days in 7°C, while G. boreale needed a longer time of 84 days (Ellis, Hong & Roberts, 1985). Those species which were not pre-chilled were only T. pratensis and S. ochroleuca. After that, the samples were placed in the thermostat, at various temperatures (20°C for 16 h in the dark, and 30°C for 8 h in the light). After 28–63 days, germination analysis was carried out, depending on the species, and the normal seedlings (showing all the structural elements of the peculiar species), abnormal seedlings (not showing all the structural elements of the peculiar species, for example with lack of properly developed roots), dead seeds (not germinated but with a soft seed coat and/or strongly infected by fungi) and fresh non-germinated seeds (not germinated and not infected by fungi) were evaluated.

The pot experiments. The development of plants was determined on the basis of a number of the fully developed leaves, while the plants’ growth was determined on the basis of the specimen’s height measured from the base of the shoot to the highest fully developed leaf. The measurements were made at seven day intervals over about three months. The maintenance capacity of the investigated species’ seedlings in the initial phase of their development was determined on the basis of the number of the specimen which subsisted during the study period (12–14 weeks depending on the species) in relation to the number of the sprouted seeds put in the soil. The total number of seedlings was assumed to be 100%. This number for individual species was as follows: A. angulosum – 56, C. dubium – 29, G. boreale – 67, S. officinalis – 36, C. patula – 38, T. pratensis – 119, C. stoebe – 81 and S. ochroleuca – 50. The temperature in the room where the pots were placed during the first three weeks oscillated between 18–22°C in the day and 13–15°C at night, but was later higher and fluctuated between the range of 20–26°C in the day and 15–17°C at night. The pots were regularly watered with distilled water to optimize moisture conditions. The investigators tried to ensure optimal moisture conditions for the plants’ development. The frequency of watering was differentiated for the peculiar species and depended on the soil moisturizing and plant conditions.

The statistical analyses. The increments of the plants’ height and the number of leaves in peculiar study periods (per day) were figured out and the efficiency (in percentage) of the seedlings’ maintenance during the study period was determined. The data were analysed statistically using variance analysis (ANOVA). The verification of the significance of differences was based on Tukey’s test ($p \geq 0.05$) and multiple range tests. The linear dependency of the seeds’ germination capacity on the dead seeds’ share for peculiar species was determined, and the regression equations ($R^2$) showing that parameter were carried out. To characterize the growth rate of seedlings, the logistic function was used according to the formula:
where:

- \( h \) – height of seedlings [cm],
- \( t \) – time [day],
- \( a \) – upper asymptote of growth [cm],
- \( b, c \) – parameters of logistic curve shape [-],
- \( e \) – natural logarithm base.

The rate of leaf development was determined by using the same logistic function. The parameters of the logistic function for the growth rate of seedlings and the rate of leaf formation are shown in Table 2.

In order to group the tested species with similar characteristics of initial growth and development, the dendrogram using Ward’s method, city-block was calculated, taking into account the following features: capacity of seed germination, share of dead seeds, share of fresh non-germinated seeds, daily growth rate of seedlings, daily number rate of leaves and efficiency of seedling maintenance.

**Results and discussion**

The high differentiation of the 1,000-seed weight in relation to the species was stated. *T. pratensis* produced the biggest seeds; the average weight of 1,000 diaspores of that species in the three-year period was equal to approximately 5.53 g (Table 1). The diaspores of *S. officinalis*, *A. angulosum* and *S. ochroleuca* turned out to be much lighter (1.24–1.53 g). The tiniest seeds (the weight of 1,000 ones equal to 0.02 g) are typical for *C. patula*.

The seeds of *A. angulosum* were characterised by the best germination capacity (72.5%), while a good capacity (above 50%) was typical for *T. pratensis* and *S. ochroleuca*, and significantly the lowest (25.7%) for *C. dubium* and *S. officinalis* (Table 3). The negative dependency of the germination capacity on the number of dead seeds was stated for the majority of investigated species. Moreover, the linear dependence of germination capacity on the share of dead seeds, was calculated altogether for all five species (*C. dubium*, *S. officinalis*,

\[
h(t) = \frac{a}{1 + b \cdot e^{-c \cdot t}}
\]

**TABLE 2. Parameters of the logistic function for the growth rate of seedlings and the rate of leaf formation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>Allium angulosum</em></th>
<th><em>Cnidium dubium</em></th>
<th><em>Galium boreale</em></th>
<th><em>Sanguisorba officinalis</em></th>
<th><em>Campanula patula</em></th>
<th><em>Tragopogon pratensis</em></th>
<th><em>Centaurea stoebe</em></th>
<th><em>Scabiosa ochroleuca</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth rate of seedlings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( a )</td>
<td>28.00</td>
<td>18.54</td>
<td>35.76</td>
<td>19.13</td>
<td>10.78</td>
<td>31.36</td>
<td>13.02</td>
<td>11.87</td>
</tr>
<tr>
<td>( b )</td>
<td>7.917</td>
<td>29.040</td>
<td>23.970</td>
<td>7.391</td>
<td>13.270</td>
<td>4.060</td>
<td>4.593</td>
<td>15.482</td>
</tr>
<tr>
<td>( c )</td>
<td>0.361</td>
<td>0.499</td>
<td>0.447</td>
<td>0.356</td>
<td>0.265</td>
<td>0.330</td>
<td>0.522</td>
<td>0.478</td>
</tr>
<tr>
<td><strong>Rate of leaf formation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( a )</td>
<td>9.26</td>
<td>7.51</td>
<td>11.41</td>
<td>7.059</td>
<td>11.55</td>
<td>10.61</td>
<td>11.09</td>
<td>12.60</td>
</tr>
<tr>
<td>( c )</td>
<td>0.326</td>
<td>0.3613</td>
<td>0.569</td>
<td>0.393</td>
<td>0.477</td>
<td>0.325</td>
<td>0.358</td>
<td>0.475</td>
</tr>
</tbody>
</table>
The analysis of the traits determining the development of some plant species, including *T. pratensis*, *C. stoebe*, and *S. ochroleuca*, for which germination ability depended on the share of dead seeds ($y = -0.903x + 89.266; R^2 = 0.802$).

A different relationship to the germination capacity was observed in the case of *A. angulosum*, for which that trait was linked to the share of fresh, non-germinating seeds (Table 3). But for two other species (*G. boreale* and *C. patula*), the germination was dependent on the number of dead seeds and the fresh, non-germinated ones as well (Table 3). The share of abnormal seedlings was minor and ranged from 0.7% (*A. angulosum* and *T. pratensis*) to 5.3% (*S. officinalis*), and the differences between the species were not statistically significant. The share of abnormal seedlings was significant only in 2014 for *S. officinalis* and in 2016 for *C. dubium* and was equal to 25 and 11%, respectively.

The tested species differed in their growth rate during the first three months (Fig. 2). The following three species were distinguished by the fastest growth rate: *T. pratensis*, *A. angulosum* and *G. boreale*. Of these, seedlings of *T. pratensis* (with the largest seeds) grew the fastest for the first five weeks, followed by *A. angulosum*, and the slowest were seedlings of *G. boreale*. In the following weeks, *G. boreale* seedlings were characterized by a very fast growth rate. As a result, from the 10th week, the seedlings of this species were the highest, reaching a height of 35 cm in the 14th week. The second group consisted of two species:

---

**TABLE 3.** The germination capacity [%], the share of dead seeds [%] and the share of fresh non-germinated seeds [%] of tested species and statistical results

<table>
<thead>
<tr>
<th>Species</th>
<th>Germination capacity</th>
<th>Dead seeds</th>
<th>Fresh non-germinated seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\overline{x}$</td>
<td>$SD$</td>
<td>max</td>
</tr>
<tr>
<td>Allium angulosum</td>
<td>75.5 a*</td>
<td>14.2</td>
<td>92</td>
</tr>
<tr>
<td>Cnidium dubium</td>
<td>25.7 c</td>
<td>18.3</td>
<td>52</td>
</tr>
<tr>
<td>Galium boreale</td>
<td>44.4 bc</td>
<td>12.3</td>
<td>68</td>
</tr>
<tr>
<td>Sanguisorba officinalis</td>
<td>25.7 c</td>
<td>17.8</td>
<td>50</td>
</tr>
<tr>
<td>Campanula patula</td>
<td>37.9 bc</td>
<td>18.2</td>
<td>68</td>
</tr>
<tr>
<td>Tragopogon pratensis</td>
<td>57.0 ab</td>
<td>33.7</td>
<td>96</td>
</tr>
<tr>
<td>Centaurea stoebe</td>
<td>32.7 bc</td>
<td>15.2</td>
<td>56</td>
</tr>
<tr>
<td>Scabiosa ochroleuca</td>
<td>53.5 ab</td>
<td>18.6</td>
<td>88</td>
</tr>
</tbody>
</table>

* homogenous groups in columns.
S. officinalis and C. dubium. These species grew at a slower rate than species of the first group, and after 10 weeks they reached a height almost half the size. The slowest growth rate was characterized by S. ochroleuca, C. stoebe and C. patula. These species formed a rosette of leaves near the ground. After
10 weeks, the height of the seedlings of these species was only 5 cm (C. patula) to 13 cm.

Amongst the tested species, G. boreale was characterized by having the fastest daily increase in seedling height growth, but it showed a significantly faster daily growth rate only in comparison to C. patula (Fig. 3). The differences in the daily growth rates amongst the other species turned out to be statistically insignificant. A. angulosum, T. pratensis, C. dubium and S. officinalis, in order, were characterised by a little slower (statistically not significant) daily growth rate. The study results showed the slowest initial daily growth rate of C. stoebe, S. ochroleuca and C. patula.

An analysis of the results showed statistically insignificant differences in the rate of successive development of the leaves of investigated species. However, it is worth noticing the development of this feature, especially in the case of C. patula. This species, despite a very slow increase in plant height, was distinguished by the fast rate of its leaves’ development and, in the final days of the studies, was characterised by the highest number of them, close to the number of whorls of G. boreale (Fig. 2). The slowest rates of leaf development were typical for C. dubium and S. officinalis. Those dependencies are shown in Figure 2.

The statistical analysis (multiple range test) of the effectiveness of the plants’ maintenance in the initial stage of their development revealed the presence of four homogenous groups. Significantly the highest, at the level of 90% capacity of maintenance of seedlings was typical for A. angulosum, G. boreale and T. pratensis (Fig. 4). These are species that were characterized by the fastest growth rate, which confirms the hypothesis. The second group consisted of C. stoebe, S. officinalis and C. patula (57.5% on average), which, as well as the species mentioned above, maintained themselves better than S. ochroleuca (39%) and C. dubium (20%). In respect of that parameter, C. patula (54%) was not significantly different from C. stoebe, S. officinalis and S. ochroleuca.

![Figure 3. Average daily growth rate [mm] of tested plant species (box and whisker plot; a, ab, b – homogenous groups)](image-url)

1. A. angulosum
2. C. dubium
3. G. boreale
4. S. officinalis
5. C. patula
6. T. pratensis
7. C. stoebe
8. S. ochroleuca
reua. *Cnidion dubium* proved to be the species with the significantly lowest effectiveness of seedling maintenance during the study period. The tested species were differentiated in terms of the speed of the seedlings dying out. Seedlings of *C. dubium* and *S. ochroleuca* died out the fastest (60 and 45% after just seven days, respectively). *Sanguisorba officinalis* seedlings maintained its seedlings a little bit longer, but after 14 days their number decreased by half. Other seedlings died out to the level of 45% stepwise, over more than 2 months.

A hierarchical analysis allowed us to group the species in respect of the investigated parameters. Three groups of the species with similar parameters in relation to early plant growth have been identified (Fig. 5). The first one consists of *A. angulosum* and *G. boreale*. These species, although classified by the different units of their natural habitats (Zaluski, 2011; Szwacha, Kącki & Zaluski, 2016), often coexist in transitional patches between these two habitats, which occurred in the area from which the diaspores were taken. Similar humidity conditions are the deciding factor in that case. The results of the studies showed the similar initial development of the two mentioned species, especially similar characteristics of the growth rate, high effectiveness of seedling maintenance and high share of fresh, non-germinated seeds. It proves that not only the humidity conditions, but also similar dynamics of the initial growth determine the co-existence of those species.

The second group distinguished in the hierarchical analysis, consists of both species typical for the *Arrhenatherion elatioris* meadows (*C. patula* and *T. pratensis*). Results showed a significant difference between the growth rates of their shoots, but also a similarity in the daily rate of the development of their leaves. Moreover, they had similar germination capacities and a similar share of dead diaspores. The third distinguished group of plants were the species present in various communities – *S. officinalis*, typical for the *Molinion* habitats and *C. stoebae*, typical for the *Festuco-Brometea* habitat. The similar characteristics of the initial growth and development may be adapta-
The analysis of the traits determining the development of some plant...
the sward, because of bad soil water conditions. The hypothesis put forward was also not confirmed with regard to the development of *S. ochroleuca*. This species was characterized by relatively large seeds (*TSW* = 1.53 g), a good seed germination capacity, but a slow rate of seedling growth and low survival rate of seedlings (39.5%). Poor persistence of *S. ochroleuca* seedlings under laboratory conditions could probably be related to the specificity of ecosystems functioning in the dry habitats in which mycorrhizal fungi are a major factor contributing to the maintenance of plants (Knappová, Pánková & Münzbergová, 2016).

Conclusions

1. *Campanula patula*, *Cnidium dubium* and *Galium boreale* are characterised by the tiniest dias pores, and *Tragopogon pratensis* by the biggest ones.

2. The seeds of *Allium angulosum*, *Tragopogon pratensis* and *Scabiosa ochroleuca* are characterised by a good germination capacity (above 50%), while *Cnidium dubium* is characterised by a poor one (26% on average, large share of dead seeds).

3. *Tragopogon pratensis*, *Allium angulosum* and *Galium boreale* are characterised by the fastest growth rate and the highest ability to maintain seedlings in the early phases of their development.

4. The results of the study showed that the following species indicate greater possibilities of reintroduction:
   - *Allium angulosum* (*Cnidion dubii*, 6440) due to its good seed germination capacity and fast growth rate of its seedlings,
   - *Galium boreale* (*Molinion*, 6410) due to the fast growth rate of its seedlings,
   - *Tragopogon pratensis* (*Arrhenatherion elatioris*, 6510) due to its large seeds, good seed germination capacity and fast growth rate of its seedlings,
   - regarding the tested species of *Festuco-Brometea* (6210), the obtained results do not give a definite answer.

References


Summary

The analysis of the traits determining the development of some plant species typical for the meadow habitats of the Natura 2000 network. The aim of this study was to determine the traits in peculiar species which most influence their survivability during early growth and development. Eight representative plant species typical for four non-afforested habitats of river valleys were tested. The results showed that for the restoration of natural habitats, the following species are best suited:

- Allium angulosum (Cnidion dubii, 6440) due to its good seed germination capacity and fast growth rate of its seedlings;
- Galium boreale (Molinion, 6410) due to the fast growth rate of its seedlings;
- Tragopogon pratensis (Arrhenatherion elatioris, 6510) due to its large seeds, good seed germination capacity and fast growth rate of its seedlings;
- regarding to tested species of Festuco-Brometea (6210), the obtained results do not give a definite answer.

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