THESES OF PHD DISSERTATION

MÉSZÁROS TÜNDE

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SZENT ISTVÁN UNIVERSITY

AUTECOLOGY STUDIES ON RARE AND PROTECTED PLANT SPECIES (*PULSATILLA GRANDIS* WENDER. AND *ADONIS VERNALIS* L.)

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1. SCIENTIFIC BACKGROUND OF THE STUDY

Studies on the biology of plant species are emphasized and incomplete at international level as well. Two acknowledged international journals (*Journal of Ecology, Perspectives in Plant Ecology, Evolution and Systematics*) also have "Biological flora" section to provide opportunity to publish synthesis papers on plant species. It is especially important to study the life cycle of species with considerable conservation importance (WÄRNER et al. 2011), protected species (PAROLO et al. 2011) and invasive species (WEBER and JAKOBS 2005). As a result of the problems investigated by scientists increasing databases are available (KUEFFER et al. 2011, LINDENMAYER and LIKENS 2011) which allows to sort and synthetize information on species. Synthesis play an important role in scientific understanding, especially in the case of ecological and environmental questions (HAMPTON and PARKER 2011).

In the conservation of rare and/or endangered species is necessary to have detailed knowledge on the biology and ecology on the given species. Furthermore it is also important to assess their actual population size and the extent of its decrease. Some synthesis were published on elements of the Hungarian flora as well (BÓDIS 1997, DOBOLYI 2007, LENDVAY and KALAPOS 2014, BÓDIS et al. 2019).

2. OBJECTIVES

The aim of my study was to enhance our knowledge on the biology of two high nature conservation value species with the investigation of the followings:

1. Change of morphological characteristics of *Pulsatilla grandis* depending on study area and number of shoots per individuals
2. Flowering phenology of *Pulsatilla grandis* and game damage in its populations
3. Flower visitors of *Pulsatilla grandis* (Aculeata, Diptera, Thysanoptera)
4. Variability of morphological characteristics of *Adonis vernalis* (vegetative and generative shoots, achenes)
5. Species composition of flower visitors of *Adonis vernalis* (Aculeata, Diptera, Thysanoptera, Coleoptera, Heteroptera)

3. MATERIALS AND METHODS

3.1. Study species

Two protected plant species, *Pulsatilla grandis* Wender. and *Adonis vernalis* L. were studied.

3.2. Study areas

The studied populations of *Pulsatilla grandis* can be found on the Csatár Hill near Veszprém, at Balatonalmádi and Bakonykoppány. Populations of *Adonis vernalis* were studied on the Csatár Hill near Veszprém, at Veszprém-Kádárta, Szentkirályszabadja and Csajág.

3.3. Morphological studies

Length of shoots, number of viable and non-viable achenes were measured in place. Length of shoots was measured from soil surface to the base of tepals. Achenes were grouped in two categories: viable and non-viable achenes. 5 viable and 5 non-viable achenes were randomly collected from each achene aggregates (in the case of *Pulsatilla grandis* with flying hair) for further measurement. Achenes were dried on room temperature for at least 3 weeks before the measurements. Length and width of achenes, length of feathery style were measured with digital caliper. Means were compared with standard deviation analysis (One-Way ANOVA) or two independent samples T-test, from Post Hoc tests the Tukey HSD was used. Data were analysed with SPSS Statistics.

3.4. Flowering phenology of *Pulsatilla grandis* and investigation of game damage

We used 10 1x1 m fix relevés (each containing *Pulsatilla grandis*) to follow the flowering phenological phases of the clumps and record game damage. The survey was carried out ten times. Flowering phenology phases were categorized in 4 groups.

3.5. Studies on Aculeata, Diptera, Coleoptera and Heteroptera taxa

The whole area of study sites was scanned continuously by 1-3 people. Insects were collected with a butterfly net 30 cm in diameter. Insects were collected one by one and put into glasses in every hour. The individuals were identified by Józan Zsolt (Aculeata), Tóth Sándor (Diptera) and Kondorosy Előd (Coleoptera and Heteroptera) with binocular microscope.

In the case of dominance the following categories were used according to SCHWERDTFEGER (1977): eudominant (>10%), dominant (5-10%), subdominant (2-5%), recent (1-2%), subrecent (1%>). Community structure indicators were analysed with PAST software (HAMMER 2012). The index of SHANNON and WEAVER (1949) was chosen to describe diversity. Evenness was measured according to PIELOU (1966), which index shows the distribution of the species in a community. The dominance index was calculated with the Berger-Parker index (SOUTHWOOD 1984).

3.6. Thysanoptera species

Thysanoptera species were collected twelve times from 15-100 flowers (later aggregate of achenes). The flowers (fruits) were shaken over a sheet of white paper. Collected thrips were put in the solution of 80% alcohol and some drops of glycerine. Mounts were made with Berlese's fluid. Species were identified by Czencz Kornélia according to SCHLIEPHAKE and KLIMT (1979), and JENSER (1982), with the help of comparative specimens sent by Jenser Gábor and Irene Zawirska at 32x, 100x and 400x magnification.

3.7. Studies on vegetative and generative shoots of *Adonis vernalis*

Shoot number of *Adonis vernalis* was counted in four slope steppes to investigate vegetative and generative shoots. 100 *Adonis vernalis* groups (genets) were studied along a transect in each study area. The number of vegetative and generative shoots was counted and recorded. Generativity was described with the generativity index by MÁTHÉ (1977).

Data were analysed with SPSS Statistics. Pairwise comparison was used in the case of every statistical analysis. Means were compared with standard deviation analysis, from Post Hoc tests the Tukey HSD was used. To calculate G-index we defined the G-index of each group, then take the mean of G-index values, so we got the mean of G-index of the 100 groups of every study areas.

4. RESULTS AND DISCUSSION

Achenes were measured to investigate the morphology and reproductive success of *P. grandis*. During our morphological studies we found significant differences in the number and size of achenes among populations. The mean number of achenes, the viable/total number of achenes ratio, the length and width of viable and non-viable achenes, the length of plume of viable and non-viable achenes were significantly different. The number of achenes heads per individuals had no significant effect on the mean number of achenes and mean length of the stem.

In our studies we found new differences in the morphological characteristics of *P. grandis* and *P. vulgaris*: the number of viable and non-viable achenes, and the total number of achenes per head is considerably higher in the case of *P. grandis*. We found differences in the length of achenes as well: this value was higher in the case of *P. grandis* achenes. This investigation support the differentiation of the two species.

Our studies on flowering phenology showed that the *P. grandis* population reached the peak of flowering in the first third of March, while fruits ripened around 10th May. Although every part of the plant is poisonous game species often feed the *P. grandis* shoots with buds and flowers, and preferred the former phenological stage. This damage is especially harmful as generative organs are very important in the reproduction of the species.

During our pollinator studies we have found 40 Aculeata species on *P. grandis* flowers. *Apis mellifera* was the most abundant pollinator, followed by species from *Lasioglossum* and *Andrena* genera. Though our main aim was to study wild bee species, we concluded honeybees played a key role in the pollination of the species. The diversity of wild bee species was remarkable in both study areas. Considerable number of individuals were collected from *Bombus*, *Colletes*, *Osmia* genera, while *Polistes*, *Nomada, Priocnemis*, *Halictus* and *Chysura* genera were represented by a low number of individuals. Most individuals were females, but notable number of males were collected as well, which proves combined functions of flowers. This means that insects visit flowers not only for forage but flowers also provide shelter and place for mating and warming. Insects were most active between 12–2 p.m. The fact that only 13 of the 40 collected species were present on both sites can indicate that the species composition of Aculeata pollinators of *P. grandi*s is not constant.

During our studies on Diptera taxa we collected 15 species on *P. grandis*. Our results showed that *P. grandis* flowers were most frequently visited by Syrphidae species. We also recorded Calliphoridae, Tachinidae, Stratiomyidae, Empididae and Muscidae species. The most abundant Diptera visitor was *Brachypalpus valgus*, playing an eudominant role. The number of individuals/hour was the highest around 12 noon, and decreased during the afternoon. We recorded 9 new hoverfly visitors of *P. grandis* in the Bakony Hills. From the representatives of the Diptera order mainly hoverflies take part in pollen transfer, so our studies proved that Diptera taxa also can play an important role in the pollination of *P. grandis*.

We found 13 Thysanoptera species (adults and larvae) on *P. grandis* flowers and fruits, which were members of the Thripidae and Phlaeothripidae families. We collected the highest number of individuals from *Thrips minutissimus*. Thrips can cause damage in the reproductive organs of *P. grandis* during feeding, but can be sustainer elements as well, though their role in pollination is small.

The shoots of *Adonis vernalis* were counted. The ratio of vegetative shoots (67.27) was significantly higher than of generative shoots (32.73). The mean number of vegetative and generative shoots per clumps and populations was significantly different among study areas. The mean values of generativity indexes were also significantly different.

A. vernalis achenes and shoots were studied at different study areas. The mean of the total number of achenes (viable and non-viable), the number of viable achenes, the number of non-viable achenes, the viable/total number of achenes ratio and the length and width of achenes were significantly different among sites. The number of flowers per individuals (genets) has a significant effect on stem height, number of viable achenes, number of non-viable achenes and on the viable/total number of achenes ratio. From our results we can conclude that *A. vernalis* shows different reactions on different habitats, so the characteristics of the areas appear in the traits of achenes.

During our studies on Aculeata pollinators of *A. vernalis* we recorded 37 species. *Lasioglossum* species were the most frequent pollinators, followed by *Apis mellifera*. In the case of *P. grandis* honey bee was the main pollinator but *A. vernalis* flowers later than *P. grandis* so honey bees can find other pollen sources as well. Insects were the most active between 10 a.m. and 14 p.m. Other collected pollinators were members of the *Andrena, Bombus, Osmia, Nomada, Halictus, Polistes, Vespula* and *Chelostoma* genera.

We collected 58 Diptera species on *A. vernalis* flowers. Only 2 individuals represented the Nematocera suborder and all the other individuals were members of the Brachycera suborder. Most individuals were hoverflies (Syrphidae) on all of the three study areas.

Anthomyidae, Tachinidae, Sepsidae, Empididae, Bombyliidae, Calliphoridae, Culicidae, Muscidae, Platystomatidae, Stratiomyidae and Tephritidae species were collected as well. The highest number of individuals were collected from *Sphaerophoria scripta*, *Chrysotoxum vernale* and *Pipizella viduata*. Tephritidae larva was also found in the flowers. The number of individuals/hour was the lowest between 10–11 a.m. and the highest between 11–12 a.m. New flower visitation data from the Bakony Hills were recorded in the case of 17 hoverfly species. The number of individuals and species was significantly different among study areas. Our results proved that Diptera species prefer areas near forests.

We collected 6 Thysanoptera species on *A. vernalis* flowers and fruits which were members of the Thripidae and Phlaeothripidae families. We collected the highest number of individuals from *Tenothrips frici* and *Haplothrips acanthoscelis* adults. The only collected larva was found on a fruit. 91% of thrips species was recorded on *P. grandis* individuals. This can be only partly explained by the fact that *A. vernalis* is nectarless and offers only pollen for pollinators. The difference is presumably influenced by plant morphological factors and insect behaviour as well. Thrips species prefer tactile stimuli and shelters. The plumed *P. grandis* satisfies these conditions but the relatively smooth *A. vernalis* does not quite fulfil them.

During our studies on Coleoptera and Heteroptera visitors of *A. vernalis* we identified 10 species. The most frequent was *Tropinota hirta*, representing 55% of all individuals. Most visitors were members of the Coleoptera order (73%), while the ratio of the Heteroptera order was 27%. Beetles were collected from the *Tropinota, Cantharis, Clanoptilus, Malachius, Coccinella* orders and Mordellidae family, the Heteroptera order was represented by *Lygaeus, Dimorphopterus, Pyrrhocoris, Canthophorus* and *Eurydema* individuals. Though these species can take part in pollination as they are moving in the flower, their effectiveness is questionable. *Tropinota hirta*, which was collected in significant number can cause damage as it feeds on the reproductive organs of plants.

The above-mentioned results contribute to the development of conservation strategies of two rare and protected plant species (*P. grandis*, *A. vernalis).* Our investigations also proved that the diversity of insects and plants is still high in Hungary. Our results confirm that insect-plant interactions are very complex and their investigation is indispensable to help the survival of these multiple systems.

5. NEW SCIENTIFIC RESULTS

1. Our results evinced, that in the case of *Pulsatilla grandis* the mean number of achenes, the viable/total number of achenes ratio, the length and width of viable and non-viable achenes, the length of plumes of viable and non-viable achenes were significantly different among study areas, which shows that the species can change its reproductive characteristics between different environmental conditions.

2. In our studies we found new differences in the morphological characteristics of *P. grandis* and *P. vulgaris*: the number of viable and non-viable achenes, and the total number of achenes per heads is considerably higher in the case of *P. grandis*. The achenes of this species are longer as well. These results are important in the differentiation of the two species.

3. In our pollinator studies we found 40 Aculeata species on *P. grandis* flowers. *Apis mellifera* was the most abundant pollinator, so we conclude that honey bees can play a key role in the pollination of the species. During our studies on Diptera taxa we collected 15 species on *P. grandis, Brachypalpus valgus* was the most abundant of them. *P. grandis* flowers were most frequently visited by Syrphidae species, which play important role in pollination. We recorded 9 new hoverfly visitors of *P. grandis* in the Bakony Hills. We found 13 Thysanoptera species, which feed on the generative organs and this presumably have influence on the number of achenes per aggregates.

4. In the case of *Adonis vernalis* significant differences were found in the number of vegetative and generative shoots, number and size of achenes among its populations. This shows that the species can adapt to different environmental conditions with changing morphological characteristics.

5. We recorded 37 Aculeata visitors on *Adonis vernalis.* Most individuals were members of the *Lasioglossum* genus, which evinced that wild bee species play an important role in the pollination of the species. We collected 58 Diptera species. The number of hoverflies (Syrphidae) was the highest, which shows that Diptera taxa are important pollinators of *A. vernalis* as well. New flower visitation data from the Bakony Hills were recorded in the case of 17 hoverfly species. During our study on Thysanoptera we

collected 6 species on flowers and fruits. The most individuals were *Tenothrips frici* and *Haplothrips acanthoscelis* adults. Considerable number of *Tropinota hirta* was collected from flowers. This species can decrease the reproductive success of the plant with its feeding on generative organs.

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