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Preliminary observations on the flowering and seed production of the thistle *Carlina onopordifolia* Besser

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Abstract

The paper deals with the flowering and seed production of thistle *Carlina onopordifolia* Besser, one of the rarest species in the flora of Poland. Bad weather conditions and shading were found to exert an unfavorable effect on the flowering, fruiting and seed viability. Isolation of the inflorescences had a similar influence, however it did not prevent seed setting. The results obtained including the lack of pollen germination on the stigma and the lack of pollen tubes in the styles, give the basis for supposition that *Carlina onopordifolia* reproduces apomictically.

Key words: apomixis, flowering biology, seed production, thistle

INTRODUCTION

Carlina onopordifolia Besser belongs to the *Compositae* family. It is one of the rarest species in the flora of Poland. Its range is dispersed over the Uplands of Małopolska, Lublin, Wołyń and Podole (Łapczyński 1882, Szafer 1923, Jasiewicz and Pawłowski 1956, Fijałkowski 1959, Zavierukha 1981). At present only eleven sites with this plant within this area are known. In Poland *C. onopordifolia* has been recorded on four sites: in the vicinity of Racławice, on hills between Pińczów and Skowronno, on Stawska Góra (mt) near Chełm and in Rogów near Zamość. Only the population near Racławice is rather numerous, it comprised in 1980 about 12 000 individuals. The remaining populations consist of as few as several score to several hundred specimens (Poznańska 1978). C. *onopordifolia* grows almost exclusively in loose xerothermic swards of the *Inuletum ensifoliae* association developing on cretaceous skeleton rendzinas which in the course of succession are overgrown with shrubs. *Carlina* multiplies exclusively by seeds and is a monocarpous species. The age of the particular specimens ranges from three to over ten years (Poznańska 1984).

The rare occurrence of *C. onopordifolia* and the low durability of the communities in which it grows are a threat to this species, therefore, only active protection, based on a knowledge of its biology and habitat requirements may prevent its complete extinction. The knowledge of the biology of flowering, especially of diaspore formation is not only important cognitively in investigations on the fruiting of this plant, but also of practical significance in protective measures. The subject of the present paper was tackled, owing to observations of relatively frequent fruitless specimens.

MATERIAL AND METHODS

The investigations were conducted in the period 1980-1982 on *Carlina onopordifolia* plants from the site near Racławice where they occupy an area of about three hectares. The studies included characterisation of flowering and fruiting, evaluation of pollen fertility by means of staining and germination tests on nutrient medium, attempts to evaluate the penetration of pollen tubes through the styles by means of the "*semi-vitro*" and fluorescence techniques, as well as evaluation of seed setting and viability in anthodia open-pollinated and isolated.

The characterisation of flowering in the period of study was based on direct observations of all the particular flowering *Carlina* specimens in the field. The observations concerned mainly the date of appearance of the first flowers in the inflorescences and wilting of the last flowers in the anthodia. The influence of the habitat conditions on the course of flowering and fruiting was studied in the seasons 1980 and 1982 which differed widely as regards atmospheric conditions. The mean temperatures and sums of precipitation from the onset of *Carlina* vegetation up to the end of its flowering are given on the basis of data obtained from the nearest meteorological stations to the site of its growth. Pollen fertility was examined on four unshaded plants in 1981. Pollen viability was evaluated by staining with Aleksander's solution (1969) which differentiates viable and nonviable grains better than carmine. As basic medium for pollen germination 1 per cent agar was used with 10 per cent sucrose added. Boric acid was also tried in a $10\,mg \cdot dm^{-3}$ concentration and media without sucrose. Pollen growth on the media was checked at several hour intervals over several successive days. For application of the "*semi-vitro*" technique which proved effective for some species with a selfincompatibility reaction localised in the style (Spiss and Paolillo 1969), pistils were excised and cut with a sharp blade at the height of 1 cm below the stigma. Then they were pollinated by transfer of pollen onto the stigma which was pricked with a sectioning needle and the styles were placed vertically in the medium. One- and 0.5-per cent agar was tried with and without 10 per cent sucrose added. The pistils were checked under a stereoscopic microscope 24 and 48 h after pollination. Penetration of the pollen tubes through the style was studied on unshaded specimens. For investigation by the fluorescence method, which proved useful for observation of pollen tubes in the pistils of many plants, various modifications were applied of the technique used recently with good results for white clover (Babik et al. 1981). The pistils were excised out 24 and 48 h after pollination and fixed in FAA. They were then macerated in 8 N NaOH and clarified with 6 per cent H_2O_2. After thorough washing in distilled water the pistils were stained with 0.2 per cent aniline blue in a 2 per cent K_3PO_4 solution, squashed on a microscopic slide and inspected under a fluorescence microscope (MB 30FL) under blue light. The modifications introduced concerned above all the use of various concentrations of the macerating and clarifying solution and the period of exposure to these solutions. The fluorescence technique was applied both for the excised out pistils pollinated by the "*semi-vitro*" technique in 1981 and to those isolated or not isolated remaining *in situ* in 1982. Inflorescences were isolated with dense linen cloth (5 plants) and a 1-mm mesh (4 plants). The isolators were placed over the whole plants just before anthesis, in the shape of tight tents about 20 cm high and they were removed immediately after the end of flowering. From each plant, both isolated and not, 30 pistils were collected at 3 phases of flower development (buds, pollen shedding and mature stigma) and examined by the fluorescence technique. Seed setting was evaluated by calculating the percentage of normally developed fruits in reference to the number of all flowers in the inflorescence. Germination energy and capacity were determined under optimal laboratory conditions, that is under light in a Jacobsen germination apparatus at 30°C. Germination energy and capacity were recorded after five and seven days, respectively.

FLOWER STRUCTURE

The inflorescence of *Carlina onopordifolia* is an anthodium (Fig. 1) containing about 400 to 1500 florets. Single florets last for about 4 days. All the florets in the inflorescence are bisexual (Fig. 2A), the calyx is developed as pappus. The corolla is tubular 15-20 mm long with 5 to 3 mm long lobes. It is yellowish in colour. There are five stamens,

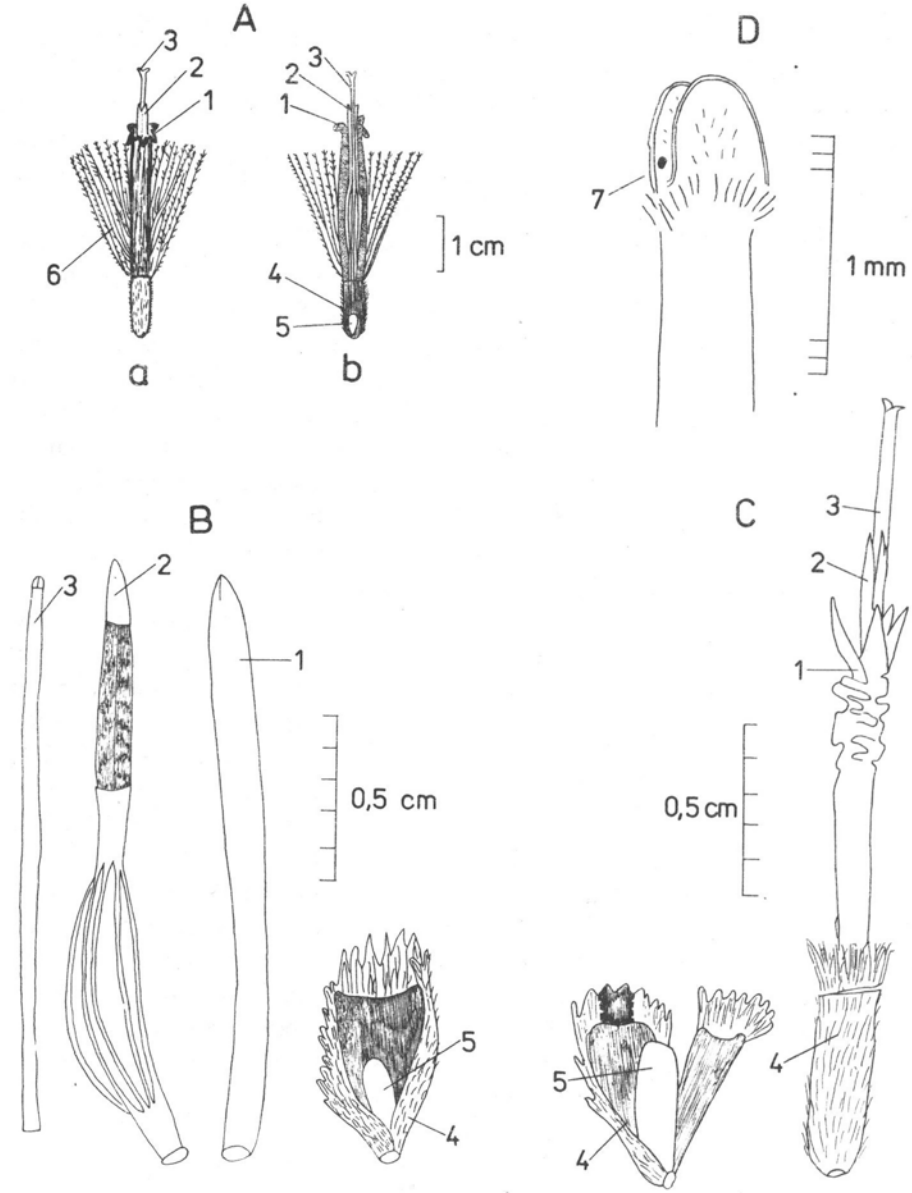

Fig. 2. Flower structure in *Carlina onopordifolia*. A — diagram of flower in phase of anthesis, a — tubular floret, b — longitudinal section through floret. B — flower element in bud phase, C — flower elements in phase of browning of corolla. D — stigma, 1 — corolla, 2 — stamens, 3 — pistil, 4 — ovary, 5 — ovule, 6 — pappus, 7 — pollen grain

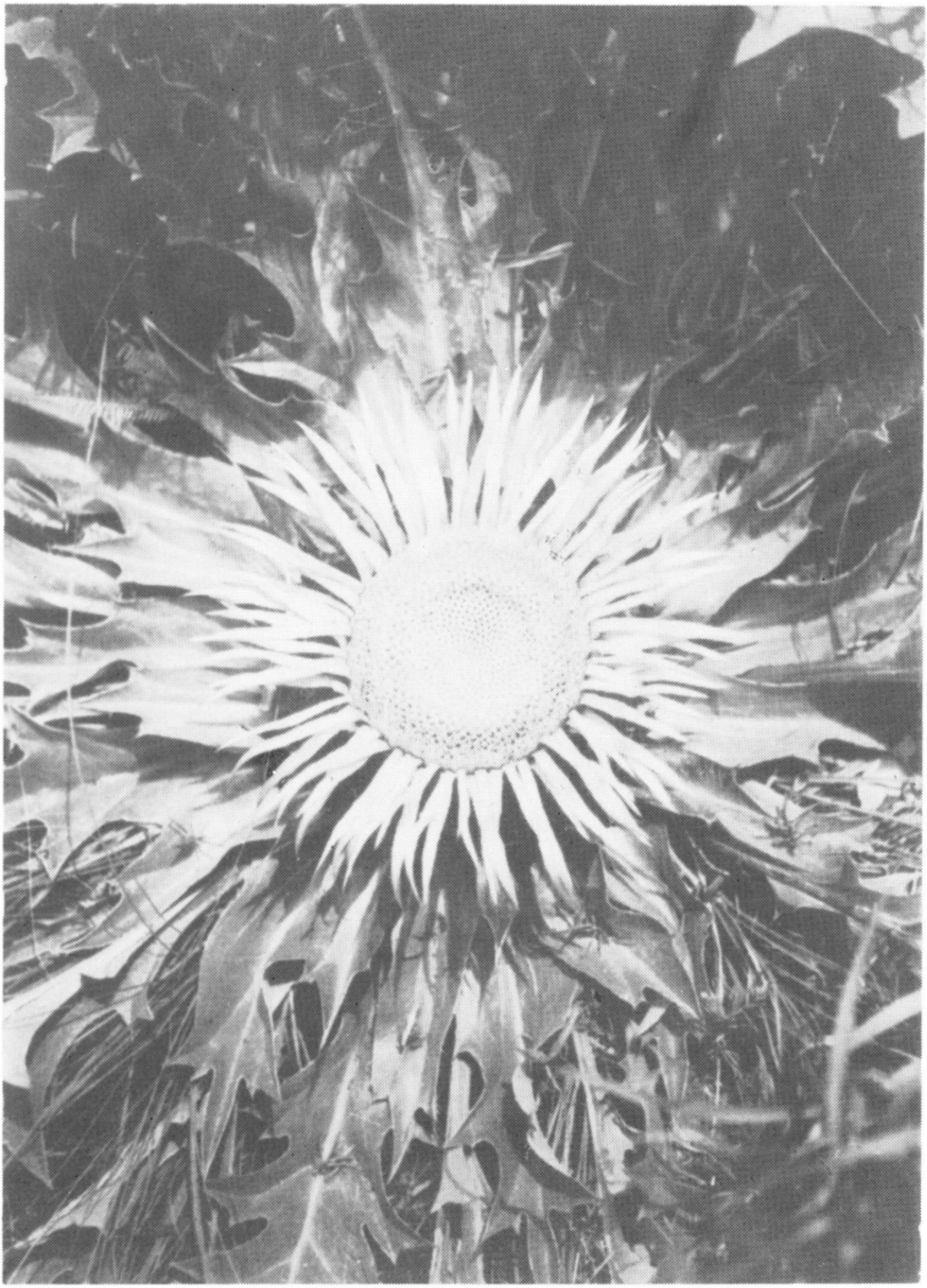

Fig. 1. The thistle *Carlina onopordifolia* Besser (photo by Z. Poznańska)

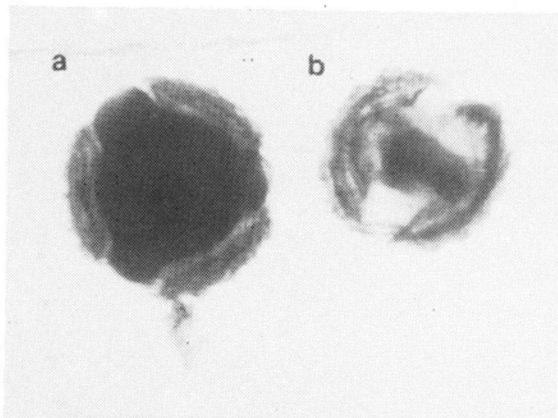

Fig. 3. Pollen grains of *Carlina onopordifolia*: a — viable grain, b — nonviable grain (photo by J. Pirecki)

their filaments join into one tube (Fig. 2B). The pistil is inferior unilocular and has only one ovule at the base which is already well visible in the flower bud. The external surface of the stigmas and the style just under them are covered with hairs (Fig. 2D). During elongation the style sweeps out the pollen from the anthers. The emerging pollen is clotted. For several hours it remains on the stigma in the form of a cap. At this time the stigma is still folded. The pollen after drying becomes loose. It is then blown off by winds and shaken off by insects — mainly by bumble-bees and bees. The anthers produce very much pollen. The pollen grains are yellow and their surface is not smooth. They have a rounded outline (Fig. 3). The stigma lobes open when the pollen is shaken off and expose the internal surface covered with hairs, mucilaginous and sticky. Thus, the flowers are protandrous. Mature stigmas reach beyond the corolla for up to 8 mm, thus they are adopted to wind pollination. The development of the ovules is very rapid. When the corolla begins to turn brown, that is about ten days after opening of the flower, the ovules are already almost of the size of normal seeds (Fig. 2C). They are white at the time and of soft consistence. The fruit is an achene.

RESULTS

FLOWERING AND FRUITING

The data of flowering of *Carlina onopordifolia* is distinctly dependent on the weather conditions (Table 1). In dry and warm summers, in 1981 and 1982 for example, anthesis began about two weeks earlier and lasted about ten days shorter than in the cool and wet year 1980. Data obtained in both years for specimens growing in swards without any shrubs indicate rather wide reproductive possibilities of this species (Table 2, Fig. 4). Unfavorable weather conditions in 1980 were reflected only in about 11 per cent lower seed production and 25 per cent lower seeding value. In the same year in swards overgrown with shrubs most of the specimens

Table 1

Dates of flowering of *Carlina onopordifolia* plants not shaded by shrubs

Year of observation	1980	1981	1982
Beginning of bud phase	June 18	June 4	June 1
Beginning of flowering	Aug. 5	July 25	July 23
End of flowering	Sept. 4	Aug. 15	Aug. 12
Duration of flowering, days	30	22	21
Mean temperature from May to August, °C	13.8	15.8	15.9
Sum of precipitation from May to August, mm	317.3	222.9	188.4

Table 2

Flowering and fruiting of *Carlina onopordifolia* plants shaded and not shaded by shrubs

Conditions of appearance	Not shaded		Shaded (area covered in 60% by shrubs up to 1.5 m high)	
Year	1980	1982	1980	1982
Number of plants	10	15	14	15
Plants which ended flowering, %	90	100	14.2	100
Plants rotted, %	0	0	85.7	0
Plants developing seeds, %	90	93.3	0	46.6
Seed setting in fruiting anthodia, %	77.2±6.5*	88.5±0.95	0	73.8±6.13
Germination capacity, %	74.4	98.7	–	98.7

* Mean ±SE.

rotted before the end of flowering, and no inflorescence produced fruits. On the other hand, in 1982, although all plants growing in shade ended flowering, only part of them fruited. Seed setting in the anthodia was in this year quite profuse, however, significantly lower than in unshaded specimens. The values of the mean errors show that under unfavorable conditions, in addition to the low seed setting, also the random variability of this character was manifoldly increased as compared to that under favorable conditions. Noteworthy is the fact that part of the shaded specimens, classified as not fruiting, set seeds, which, however, did not attain morphological ripeness. The course of flowering and fruiting of *Carlina onopordifolia* on an area shaded by shrubs constitute an evidence of the high sensitivity of this species to habitat conditions.

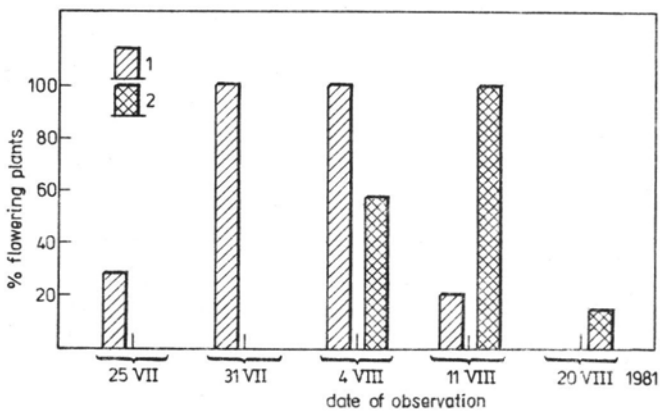

Fig. 4. Course of flowering of *Carlina onopordifolia* specimens unshaded (1) and shaded (2) by shrubs

POLLINATION

Pollen fertility determined by the staining method at the stage of flower bud and in the period of pollination in all the examined specimens did not show any differences and was very high (ca 98%). Pollen germination, however, collected from flower buds and in the pollination period did not give on agar medium positive results. Neither was pollen germination observed on excised stigmas at various developmental phases of the flowers, on which selfpollination, pollination by neighbouring flowers and cross pollination were performed. Neither were positive results obtained in examination by the fluorescence technique of pollen tube penetration through the styles of plants on which selfpollination, pollination by neighbouring flowers and cross pollination were performed as well as in plants isolated or not (*in situ*) in which the pistils for examination were taken at three developmental stages of flowering. On the stigmas examined by the fluorescence method no pollen grains were found. They had been washed off by the fixative. The pollen in the vials did not contain germinating grains. The failure to produce pollen tubes was also indicated by the microscopic picture of the styles of the pistils taken at various developmental stages (flower bud, pollen shedding and mature stigma) from isolated and nonisolated plants. Fluorescence of callose elements of the vascular bundles was rather distinct, but fluorescent callose plugs, characteristic for growing pollen tubes were not noted.

FRUITING AND SEEDING VALUE OF SEEDS FROM ISOLATED AND NONISOLATED SPECIMENS *IN SITU*

All individuals, both isolated and nonisolated fruited (Table 3). Seed setting and seeding value in particular groups of specimens differed, however, markedly. Most normally developed achenes were produced by nonisolated plants where there was full possibility of pollination and the microclimatic conditions were desirable. Individuals isolated by mesh eliminating the contribution of insects to pollination and limiting somewhat light access fruited less profusely. The number of developing seeds was the lowest in the anthodia isolated with linen cloth. The dense linen cloth limited markedly light and wind access, and favoured the maintenance of a higher temperature and moisture. It should be mentioned that during flowering the weather was hot, sunny and without rain, and flowering of isolated and nonisolated plants occurred without any disease symptoms. The seeding value of achenes responded to isolation in similar way as their setting (Table 4). Both the germination energy and capacity of nonisolated seeds were the highest. Seeds from plants isolated with mesh showed lower germination, as compared to those from nonisolated plants, and the lowest germination was exhibited by seeds isolated with linen cloth. Interpretation of the results is difficult. The fact that production of diaspores by the specimens isolated with mesh was only slightly lower than that of the nonisolated ones, with their seeding value being considerably depressed, seems to indicate that the data obtained in this experiment result from of the unfavorable influence of the enclosures.

Table 3

Seed production by *Carlina onopordifolia* plants isolated and nonisolated (as % of all flowers in anthodium)

No.	Nonisolated	Isolated	
		mesh	linen cloth
1	84.1	52.6	14.9
2	92.1	75.1	33.6
3	88.8	88.4	57.1
4	91.6	78.5	11.0
5	93.1	—	31.2
6	87.5	—	—
Mean	89.6	73.6	29.6

Table 4

Seeding value of *Carlina onopordifolia* achenes from isolated and nonisolated plants

Isolation	Germination energy	Germination capacity
Nonisolated	84.6	98.7
Mesh	31.0	59.0
Linen cloth	16.7	52.0
$LSD_{\alpha=0.05}$	15.0	13.9

DISCUSSION

Observations on the flowering and fruiting of *Carlina onopordifolia* point to its distinctly xerothermic and heliophilous requirements in the generative phase. In the cool and wet season flowering was retarded, and this led as consequence to poorer fruiting or complete lack of fruits. The effect of partial shading of the plants is similar. Owing to its high specialisation as regards habitat requirements, this species is threatened with extinction in Poland, as indicated by the population size on the particular sites and the fact that it does not occupy new ones.

The sites occupied by this species may be considered as extreme from the point of view of the adaptive abilities of the species. In such cases the supposition may be justified that new forms develop in which the mechanisms making selfpollination difficult disappear. De Nettancourt (1977) quotes four reasons explaining the development and success of self-fertile forms in definite ecological conditions. At least three of these reasons may apply to the species studied by us. Self-fertility ensures reproduction under unfavourable conditions of pollen spread, makes development of population possible after accidental transfer of seeds to large distances and allows to occupy new definite habitats, owing to the regularity and genetic uniformity represented by such forms from generation to generation. To support his argument de Nettancourt (1977) quotes the papers of Stebbins and Baker and gives examples illustrating the changes occurring in the evolution of species.

The next step of evolutional changes on the way from open recombination (allogamy) to limited recombination (autogamy) is the appearance of agamospermia representing a closed recombination system. According to Grant (1975), the changes described here occur frequently and are generally considered as a general trend in the evolution of higher plants. At another occasion Grant (1981) stresses the adaptive superiority of agamospermia over vegetative propagation, although both ways of reproduction do not differ from one another genetically. If we take into account the foregoing considerations and the character of occurrence of *Carlina onopordifolia*, a similar evolutionary trend cannot be ruled out in this species.

Although the present results do not allow precise determination of the way of propagation of *Carlina onopordifolia* on the studied sites, they suggest, however, the possibility of agamospermia occurence in these plants. It is true that the flowers exhibit adaptation to allogamy and under natural conditions are visited by numerous insects, but isolation of the anthodia from insects and wind did not prevent seed formation. The reduced number of seeds produced may be attributed to unfavorable changes of the microclimate under the enclosure and limitation of light access to the anthodium, as it happens under the shade of shrubs. The present investigations did not demonstrate pollen grain germination either *in vitro* or on the stigma. It was also noted that the pollen was washed out from the stigmas by the fixative, thus indicating that the pollen tubes had not penetrated into the style. Observation under the fluorescence microscope did not demonstrate pollen tubes in the style, although the distinct fluorescence of callose elements in the vascular bundles indicated that the pollen tubes could not penetrate unnoticed.

Since *Carlina onopordifolia* has a narrow ecological scale and the communities in which it grows are not durable, possible agamospermia would be a form of reproduction most independent of unfavorable external conditions. It is also known that apomixis, especially when obligatory, leads to the loss of adaptive abilities of the species. Hence the tendency to such a way of reproduction in some dying out species cannot be excluded. The hybrids between *Carlina onopordifolia* and *C. acaulis* accurring on the investigated area do not exclude the occurrence of apomixis in *C. onopordifolia* or else they indicate that the hypothetical apomixis is not of obligatory character.

For confirming the occurrence of apomixis in *Carlina* and determining its type, embryological investigations on the development of the ovule are indispensable. Information on the chromosome number in various individuals may also be helpful.

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Wstępne obserwacje nad kwitnieniem i wytwarzaniem nasion dziewięćsiłu popłocholistnego (Carlina onopordifolia Besser)

Streszczenie

Praca dotyczy kwitnienia i wytwarzania nasion u dziewięćsiłu popłocholistnego (*Carlina onopordifolia* Besser) — jednego z najrzadszych gatunków naszej flory. Badania prowadzono w latach 1980-1982 na roślinach ze stanowiska koło Racławic. Obejmowały one charakterystykę kwitnienia w zmiennych warunkach siedliskowych i atmosferycznych, ocenę żywotności pyłku za pomocą barwienia i próby kiełkowania na pożywkach, próby oceny przerastania łagiewek przez szyjkę słupka techniką "*semi-vitro*" i obserwacji łagiewek pyłkowych w szyjce słupka przy zastosowaniu techniki fluorescencyjnej oraz ocenę zawiązywania i żywotności nasion w koszyczkach swobodnie zapylanych i izolowanych. Stwierdzono znaczny wpływ niekorzystnych warunków atmosferycznych na kwitnienie, owocowanie i żywotność nasion. Podobny wpływ miała ocienianie przez krzewy a także izolowanie kwiatostanów. Izolowanie okazów nie zapobiegło jednak osadzeniu nasion. Negatywne wyniki obserwacji kielkowania pyłku na znamionach i brak łagiewek pyłkowych w szyjkach słupków nasuwają hipotezę, że dziewięćsił popłocholistny rozmnaża się apomiktycznie.