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Helleborus niger

Systematics, Ecology, Pollination and Production Technology

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Faculty of Agriculture
and Life Sciences

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1 Introduction

Modern markets require highly competitive production of ornamental plants. It is possible to find many new species on the market. Some of these are wild plants. Diversification of production is becoming very important and involves new species, new varieties, and new production technologies.

Recent studies have indicated that the Christmas rose (*Helleborus niger* L.) is becoming increasingly popular on the market as an ornamental plant. They can also be grown as cut flowers or potted plants, and can be forced to flower under greenhouse conditions around Christmas or New Year.

The Christmas rose is one of the earliest flowering plant species from the genus *Helleborus*. Its ability to bloom during the 'darker' months of the year, when everything else is frozen, makes it highly valuable. As evident from the natural flowering time (from November to April), it does not need high temperatures when beginning to flower, which is very important for producers in a moderate continental climate. Greenhouses in moderate continental climates, owing to their specific structure, are significant energy-consumers and therefore very expensive for producers. The Christmas rose does not need high temperatures, and for this reason can be considered both cost-effective and environmental-friendly.

The Christmas rose is a well-known ornamental plant; however, it is relatively new to intensive production technologies. There is insufficient data about many of the agronomic aspects associated with the cultivation of the Christmas rose. The problems have always centred on its propagation, which can be either vegetative or through dissemination. The methods of generative propagation have generally tended to create plants with high degrees of variation in terms of their flowering periods and both the sizes and colours of the flowers. Vegetative propagation is very important during both commercial production and artificial plant cultivation: the parental lines have to be maintained in vegetative propagation for seed production; cloning is often required for setting up gene banks; advantageous shoot-formation is needed for obtaining solid mutants after mutagenic treatment.

The classical vegetative production, which is based on rhizome cuttings, is time-consuming and cannot always guarantee success. In many cases, it is combined with '*in vitro*' micropropagation, which is becoming the more frequently used technique in large-scale production of the Christmas rose.

The regeneration of plants and their growth is closely associated with their root systems. Roots have several important functions, such as anchorage, absorption of nutrients and water, and the production of exudates with growth-regulatory properties. Inadequate root development and function are two of the more common causes of failure in young plants subjected to vegetative propagation.

Plants that have originated '*in vitro*' appear to be vulnerable and do not function properly '*in vivo*'. They quickly die off and must be replaced by newly-formed subterranean roots. Those plants that under normal growth conditions would live symbiotically with fungi or bacteria, lack these symbiotic organisms when transferred from test tubes to soil.

The microbial activity within the plant rhizosphere has substantial effects on plant performance and productivity. Plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi are able to colonise plant roots and stimulate plant growth when applied to seeds, tubers or roots. They represent integral parts of many cultivated plants and are also an essential component of soil fertility because of their roles as bio-regulators, bio-fertilisers and bio-control agents. Plant biotisation represents a very promising technology for improving the quality of plants and ensuring more

appropriate development in the context of sustainable horticulture. Some recent studies have indicated that improvements in the growth and health of plants can be achieved by either inoculating the selected strains of beneficial microorganisms (separately or in combination) or by applying cultural practices that favour the development of indigenous beneficial rhizosphere and microbial populations in soils, whilst suppressing pathogenic ones.

The Christmas rose is a predominantly cross-fertilising species. Flowers are hermaphrodite (having functional male and female sexual organs) and protogynous. Following fertilisation by February or March, the fruit starts to develop and is mature by May or June (depending on ambient temperatures). Simultaneously, the sepals that are white or pink at anthesis persist until the seeds are ripe, and become intensely green (in shaded plants) or dark red (in sun-exposed plants). The more important pollinators are insects, such as bees and flies. Their activities depend on several factors, such as insect species, location, and the time of day.

The number of genetically improved varieties of the Christmas rose in the market is limited. Naturally grown populations are probably the most valuable and the biggest sources of genetic variation, as well as sources of specific genes for resistance against pests and diseases. For successful breeding, it is essential to have reliable data about existing natural variations and variations in germplasm collections. The Christmas rose germplasm collections are rare and often include a limited number of genotypes.

Slovenia, one of the smallest European countries, appears to be very rich in terms of its biotic diversity, with over 3000 vascular plant species. The main reasons for such diversity are its specific geographical position, as well as its diverse climate, relief and bedrock. In Slovenia, Christmas roses can grow in different geographical areas, as well as in diverse climates, reliefs, and bedrocks, and for these reasons it may play an important role as it may be one of the centres of diversity.

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2 Classification and botany

2.1 Botanical classification

In the classifications of the Ranunculaceae family, Tamura (1993) placed *Helleborus* with *Beesia* Balf. f. & W. Smith, *Calathodes* Hook f. & Thomson, *Caltha* L, *Eranthis* Salisb, *Megaleranthis* Ohwi and *Trollius* L. in the subfamily Helleboroideae. According to the results of serological studies (Jensen, 1968) and molecular data obtained for the Ranunculaceae family (Hoot, 1995; Ro et al., 1997; Wang et al., 2005), *Helleborus* has been classified in the subfamily Rununcoloideae, which comprise all genera with Ranunculus-type chromosomes (Ro et al., 1997) (Table 1). All hellebores have the same number of chromosomes, 2n = 32 (Castro and Rossello, 2007; Meiners et al., 2011).

Table 1: Botanical classification of the genus *Helleborus* L.

Kingdom: Plantae (plants)
Subkingdom: Tracheobionta (vascular plants)
Superdivision: Spermatophyta (seed plants)
Division: Magnoliophyta (flowering plants)
Class: Magnoliopsida (dicotyledons)
Subclass: Magnoliidae
Order: Ranunculales
Family: Ranunculaceae Juss. (buttercups)
Subfamily: Rununcoloideae Arnott
Genus: *Helleborus* L.

Within the genus *Helleborus*, several authors have proposed various groupings of the related species based on different criteria. Two morphological groups have been distinguished in the genus according to caulogenesis: the Caulescentes and Acaules (Schiffner, 1890a, 1890b; Braun and Bouche, 1861). Mclewin and Mathew (1995) argued that since all hellebores are caulescent to some degree, the caulescent/acaulescent (stem bearing both leaves and flowers/leaves and flowers not carried on the same stems) separation is not strictly valid, but is useful for horticultural purposes. Various authors divide hellebores into groups in different ways (Bavcon et al., 2012):

1. Spach (1839, cited after Mathew 1989) – three subgenera
   Chionorhodon (*H. niger*)
   Helleborastrum (green 'acaulescent' hellebores)
   Griphophus ('caulescent' hellebores)
2. Braun and Bouche (1861) and Baker (1877, cited after Mathew 1989) – two subgenera
   Caulescentes – species with leaves on the aboveground part of the stem
   Scapigeri (Acaules) – floral stems and basal leaves emerge separately from an underground rhizome
3. Schiffner (1890a) – two subgenera and five sections
   Caulescentes Braun & Bouche
   Section 1 – *Syncarpus* Schiff.
   Section 2 – *Griphopus* Spach
   Section 3 – *Chenopus* Schiff.
   Acaules Baker
   Section 4 – *Chionorhodon* Spach
   Section 5 – *Euhelleborus* Schiff.
4. Ulbrich (1938) – two subgenera and six sections
   Caulescentes Braun & Bouche
   Section 1 – *Syncarpus* Schiff.
   Section 2 – *Griphopus* Spach
   Section 3 – *Chenopus* Schiff.
   Acaules Baker
   Section 4 – *Chionorhodon* Spach
   Section 5 – *Dicarpon* Ulbrich
   Section 6 – *Helleborastrum* Spach

5. Zimmermann (1974) – two subgenera and six sections (adaptation to nomenclature rules –
   autonyms, typification, the type species of the genus is *H. niger* L.)
   Caulescentes Braun & Bouche
   Section 1 – *Syncarpus* Schiff.
   Section 2 – *Griphopus* Spach
   Section 3 – *Chenopus* Schiff.
   Acaules Baker
   Section 4 – *Helleborus*
   Section 5 – *Dicarpon* Ulbrich
   Section 6 – *Helleborastrum* Spach
6. Mathew (1989) – abandonment of subgenera, just six sections
   Section 1 – *Syncarpus* Schiff.
   Section 2 – *Griphopus* Spach
   Section 3 – *Chenopus* Schiff.
   Section 4 – *Helleborus*
   Section 5 – *Dicarpon* Ulbrich
   Section 6 – *Helleborastrum* Spach
7. Werner and Ebel (1994) – merging the sections of Matthew (1989) into two subgenera
   Subgenus *Helleborus* – sections *Helleborus, Griphopus, Chenopus*
   Subgenus *Helleborastrum* (Spach) Werner & Ebel – sections *Helleborastrum, Dicarpon* and *Syncarpus*

2.2 Phylogeny

Molecular phylogenies in plants are traditionally based on chloroplast DNA (cp DNA) sequence variation. Johansson (1995), on the basis of cp DNA restriction site data, identified the clade (*Helleborus* (*Trollius* + *Adonis*)) as a sister group to (*Caltha* + *Calliantheum* C. Meyer). Hoot (1995) obtained results where *Helleborus* was sister to *Caltha*, on the basis of cp DNA and nuclear DNA sequences. Jensen et al. (1995) proposed a systematic treatment of Ranunculaceae, based on a comparison between the analyses of nuclear and plastid DNA sequences, cp DNA restriction site data. As a result, *Helleborus* was placed within the monogeneric species *Helleboreae* within the subfamily Ranunculoideae Hutch. Although this approach has proved to be powerful at the family level, the low evolutionary rate limits the power of cp DNA

at the genus or species level (Soltis et al., 1993). Consequently, the relationships amongst closely related taxa have been inferred using non-coding sequences (Clegg and Zurawski, 1991; Gielly and Taberlet, 1996), and have stimulated the development of new molecular approaches.

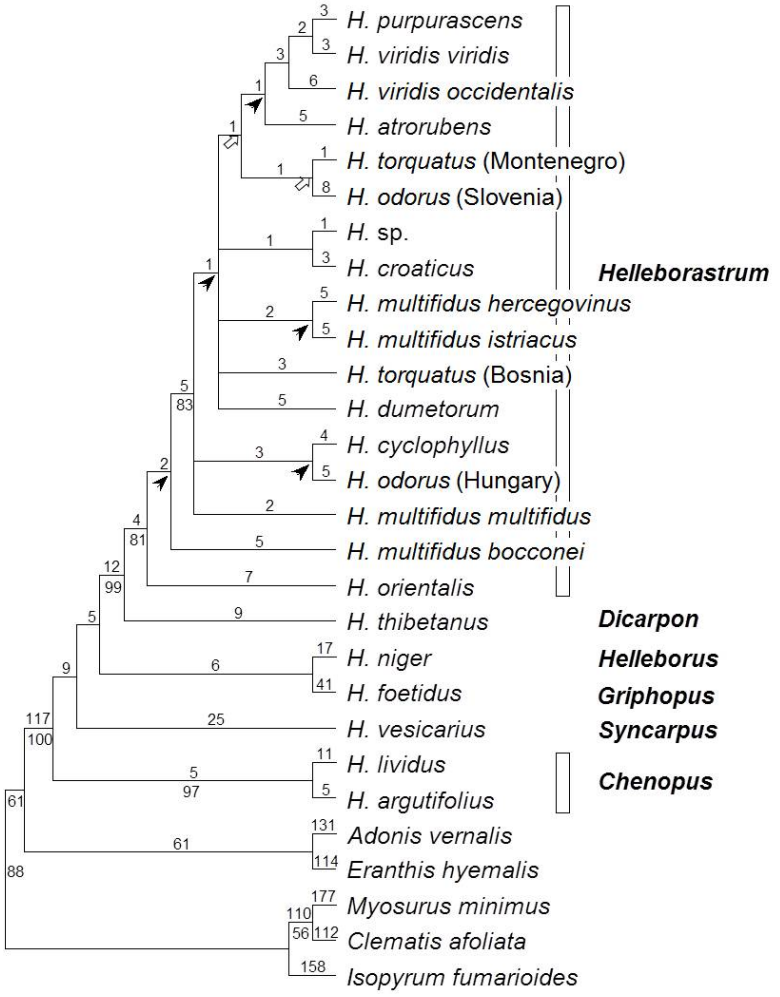

Figure 1: Phylogenetic relationship within genus *Helleborus* obtained with successive weighting based on combined data (*trnL-F*, *matK*, ITS), Fitch length 1310, CI = 0.80, RI = 0.70 (weighted TL = 861.92057, CI = 0.96, RI = 0.92). Arrows indicate branches that were absent during the strict consensus of the Fitch and SW trees

(Source: Sun et al.; © 2001 International Association for Plant Taxonomy (IAPT).

Alternatives include sequencing of the internally transcribed spacer (ITS) region of 18S-26S nuclear ribosomal DNA (rDNA) (Baldwin, 1992; Yuan et al., 1996). This involves 600-700 bp in most Angiosperms, consisting of two spacers (ITS1 and ITS2) and the 5.8S gene. In most taxa, it appears to be more variable than the cp DNA or plastid regions, and for this reason has been used widely at lower taxonomic levels. In most cases, partial sequence data collected from a number of different portions of the 26S gene have been used (Hamby and Zimmer, 1988, 1992; Zimmer et al., 1989; Bult and Zimmer, 1993; Soltis and Soltis, 1998). Using data from the 26S rDNA, Ro et al. (1997) identified relationships between clades within the species *Helleboreae*, but levels of support were low for this pattern of relationships. Oxelman and Lidén (1995) used 800 bases from the 5' end of the 26S gene plus the ITS2 region in order to resolve relationships amongst some members of the Ranunculales order. However, this can be problematic in some taxa, owing to the presence of multiple copies and the possibility of sequencing fungal contaminates because of the use of universal primers (Soltis and Soltis, 1998).

Sun et al., (2001) evaluated the phylogenetic relationship within the genus *Helleborus* based on the analyses of plastid *trnL-F* and partial *matK*, and nuclear sequences for 16 currently recognised species, including several subspecies and geographical variants (Fig. 1). The molecular study provides strong support for the monophyly of *Helleborus*. However, both traditional divisions of the genus into two groups (*Caulescent* and *Scapigeri*) and two subgenera (*Helleborastrum* and *Helleborus*) have been refuted. All six currently recognised sections were monophyletic, four by default because they were monospecific. The section *Dicarpon* (*H. thibetanus*) was strongly supported as a sister group to the section *Helleborastrum* and could therefore be subsumed into that section.

2.3 Intra- and interspecies variation

Mathew (1989) divided the genus based on the general structure of the plant, the ability of the species to be hybridised, the morphology of pollen grains, and the characteristics of seeds, directly into six sections without using the group rank. He recognised 15 species. In the most recent study of the taxonomic subdivision within genus *Helleborus* based on genome wide DNA markers, six sections with a total of 22 species are found. The largest section Helleborastrum contains 16 species for which genetic relationships are still unclear (Meiners et al., 2011) (Table 2).

All hellebores have the same number of chromosomes, 2n = 32, which makes intercrossing between them possible (Fig. 2). Mathew (1989) notes that *H. niger* does not readily hybridise with the hellebores of the section Helleborastrum (i.e. *H. orientalis* etc.) but it can be artificially crossed with the two-stemmed species of hellebore (i.e. *H. argutifolius* and *H. lividus*). *Helleborus* hybrids obtained with interspecific crosses within section Helleborastrum and between sections are listed in Table 2.

Figure 2: ***Helleborus* × *ericsmithii* 'HGC Malory'®**
(Photo: A. Šušek)

Table 2: Classification of the genus *Helleborus* and their hybrids

Section	Species	Natural distribution	Growing type
Syncarpus	*H. vesicaricus* Aucher ex Boiss.	South Turkey, Syria	Intermediate
Griphopus	*H. foetidus* L.	Western, central and southern Europe	Caulescent
Chenopus	*H. argutifolius* Viv.	Corsica, Sardinia	Caulescent
	H. lividus Ait. f.	Majorca, Cabrera	Caulescent
Helleborus	*H. niger* L.	Northern Dolomites, Apennines, Northwestern Balkans, Southern Alps	Intermediate
Helleborastrum	*H. abruzzicus* M. Thomsen, McLewin & B. Mathew	Abruzzo (Italy)	Acaulescent
	H. atrorubens Waldst. & Kit.	Slovenia, North Croatia	Acaulescent
	H. bocconei Ten.	Sicily, Calabria	Acaulescent
	H. croaticus Martinis	Northeast Croatia	Acaulescent
	H. cyclophyllus Boiss.	Albania, Greece, Bulgaria	Acaulescent
	H. dumetorum Waldst. & Kit.	Austria, Slovenia, Hungary, Romania, Croatia	Acaulescent
	H. hercegovinus Martinis	Montenegro, Hercegovina	Acaulescent
	H. istriacus	Northwest Croatia, Northeast	Acaulescent
	H. liguricus	Liguria, Tuscany, Emilia Romagna (Italy)	Acaulescent
	H. multifidus Vis.	Croatia, Herzegovina, Albania	Acaulescent
	H. occidentalis Reut.	Western Europe	Acaulescent
	H. odorus Waldst. & Kit.	Albania, Hungary, Slovenia, Italy, Romania, Bosnia	Acaulescent
	H. orientalis Lam.	Turkey, Caucasus, Ukraine	Acaulescent
	H. purpurascens Waldst. & Kit.	Romania, Hungary, Czech Republic, Slovakia, Poland, Ukraine	Acaulescent
	H. torquatus Archer-Hind	Croatia, Serbia, Bosnia	Acaulescent
	H. viridis Boiss.	Central Europe, Maritime Alps	Acaulescent
Dicarpon	*H. thibethanus* Franch.	Western China	Acaulescent

Hybrids	**Parents**	
H. × *ballardiae*	*H. niger* × *H. lividus*	Caulescent
H. × *belcherii*	*H. niger* × *H. thibetanus*	Caulescent
H. × *ericsmithii*	*H. niger* × *H.*× *sternii*	Caulescent
H. × *glandorfensis*	*H.*× *ericsmithii* × *H.*× *hybridus*	Caulescent
H. × *iburgensis*	*H.*× *ballardiae* × *H.*× *hybridus*	Caulescent
H. × *jourdanii*	*H. foetidus* × *H. viridis*	Caulescent
H. × *nigercors*	*H. niger* × *H. argutifolius*	Caulescent
H. × *sahinii*	*H. niger* × *H. foetidus*	Caulescent
H. × *sternii*	*H. argutifolius* × *H. lividus*	Caulescent

2.4 Classification of *Helleborus niger*

The variability within the taxon *Helleborus niger* has been known for a long time. One of the first reports was prepared by Hayne (1829), who mentioned the form *H. altifolius*. Freyer (1938) describes the flowering plants of *H. altifolius* growing between Turjak and Škocjan in Dolenjska (southern part of Slovenia), which differ from *H. niger* in the following characteristics: leaves and flowers are developing at the same time; with age, flowers become more reddish; leaves have 9–11 narrow and long leaf segments deeply serrated towards the apex; leaf petiole and peduncle are reddish spotted, while common types of *H. niger* start to flower before the formation of leaves. Flowers are white and pink. Leaves which start to grow after flowering are leathery and have 7 wide leaf segments. The petiole is single-coloured.

Freyn (1881) describes plants from the Val Malenga valley (Lombardy) as a new variety *Helleborus niger* L. var. *macranthus* with the following morphological traits: flowers are 8 cm in diameter; nectaries are 8 mm long and have a 2.5 mm wide opening; yellow-white coloured carpels are covered with stamens at the beginning; the styles have the same length as the ovaries and are deep red on the base and white on the top; leaves are usually bluish-green and sometimes spotty. According to the same author, *Helleborus niger* L. s. str. have smaller flowers and their diameter is 5–6.5 cm. Olive-green nectaries are 5 mm long and their openings are 1.25–1.5 mm wide. The stamens are as long as carpels and the filaments are green. The sepals are widely elliptic and green-white on the adaxial side (from the bottom to the middle part) and green-reddish on the abaxial side.

In his new description of *H. altifolius*, Kerner (1884) believes that it is an autonomous species and that it differs from *H. niger*. It is widely distributed in the southern part of the Alps. In Lombardy and South Tyrol etc., it is a substitution for the species *H. niger*, which is widely distributed in North-East Alps from North Tyrol to the Schneeberg, (highest mountain in Lower Austria, a massif in the Northern Limestone Alps). it differs significantly and has larger flowers, longer peduncles and leaf petioles, larger seeds, leaf segments are bluish-green and have prickly and sticking out teeth at the margin. In his opinion, *H. niger* plants have club-shaped leaf segments, which extend in the upper part, and on the edges they have soft teeth oriented toward the tip.

One of the best descriptions and systematics was made by Schiffner (1890a) and was called "Monographia Helleborорum". He described all member of the genus *Helleborus*, which were known at that time, and recognised only 3 groups: *H. niger*, *H. niger* var. *altifolius* (Hayne) Schiffner, and *H. niger* subsp. *macranthus* (Freyn) Schiffner. In his opinion, the traits – such as size and colour of flowers, height of peduncle and length and colour of carpel style – could not be used for the division within the species due to a high variability of these traits. He believed that Kerner (1844) made a mistake when identifying subsp. *macranthus* (Freyn) Schiffner with the Hayne's taxon *H. altifolius*. The Hayne's taxon *H. altifolius* is, according to Schiffner, only a form of the species *H. niger* due to the same geographic region, while *H. niger* subsp. *machranthu*s (Freyn) Schiffner is distributed in another region and for this reason can be considered as an autonomous subspecies.

Schiffner (1890a) describes the subsp. *machranthus* (Freyn) with the following characteristics: leaf segments are broadly lanceolate and more narrow comparing to *H. niger*; the most extended part of the leaf segment is near the middle; the teeth along the margins of the leaf segments are protruding and prickly; leaves are bluish green and lustreless, sepals are less red coloured compared to *H. niger*, they are more narrow and they are overlapped at the base or to the middle; styles of carpels are longer than the structure formed by the anthers.

In the classification by Hegi (1911), the taxon *Helleborus niger* is divided into two subspecies *H. niger* subsp. *niger* and *H. niger* subsp. *macranthus* (Freyn) Schiffner. He believes that the first subspecies is highly variable with several varieties: var. *oblongifolius* Beck, var. *altifolius* (Hayne) Rchb., var. *stenopetalus* Beck, and var. *laciniatus* Gusmus.

Considering *H. niger* in its natural state, it does seem that, broadly speaking, only two variants can be recognised, and these, having some geographical significance, can be regarded as subspecies (this type of classification was followed more recently by many authors, such as Tutin et al., 1964; Ravnik, 1969; Hegi, 1975; Mathew, 1989). These are:

(1) *Helleborus niger* subsp. *niger* (leaf segments are oblong-cuneate, dark green, serrate towards the apex; flowers are up to 8 cm in diameter; it is distributed from Switzerland and Germany to Austria, Italy, Slovenia, Croatia, Bosnia, and Serbia and Montenegro) and
(2) *Helleborus niger* subsp. *macranthus* (Freyn) Schiffner (leaf segments are broadly lanceolate, bluish-green or grey-green, spinulose-serrate; flowers are 8–11 cm in diameter; it is distributed only in Italy, Slovenia and Croatia)

Ravnik (1969) analysed the preserved herbarium collections as well as living plants from several phytogeographical regions in Slovenia (Alpine, Pre-Alpine, Dinaric, Pre-Dinaric and Sub-Mediterranean) and from different locations in Austria (East Alpine, Karavanken Mountains, Dachstein, North-East Alpine) and concluded that there was only one subspecies (i.e. *Helleborus niger* subsp. *niger*), which was highly variable. Some of the variations observed amongst individuals within the Slovenian naturally growing population (Fig. 3) are presented in Figs. 4 and 5.

Figure 3: A Slovenian wild population growing in woodland
(Photo: A. Šušek)

Figure 4: Flower shapes within a wild population in Slovenia (Slovenske Konjice) showing their variation
(Photo: A. Šušek)

Figure 5: Morphological variation of terminal leaflets
(Photo: A. Šušek)

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3 Ecology and biology of *Helleborus niger*

3.1 Ecology

Origin and distribution

The hellebores are evergreen and deciduous perennials that are dispersed across Central and Southern Europe (including the Mediterranean) to the Caucasus and China (Mathew, 1989; Beckett, 1990; Jelitto and Schacht, 1995). The Christmas rose grows wild in the southern, and occasionally the northern Dolomites, Apennines, north-western Balkans, and in mountain forests of the Southern Alps (Ravnik, 1969; Hegi, 1975; Mathew, 1989; Jelitto and Schacht, 1995).

Environmental requirements

Christmas roses can grow in different geographical areas, as well as in diverse climates, reliefs, and bedrocks. In Slovenia, it grows in the sub-Mediterranean, mountainous and moderate continental climates. It grows at various altitudes between 240 m a.s.l. (e.g. Rimske Toplice – central part) and 2300 m a.s.l. (e.g. Vršič – north-western part). The average annual precipitation in regions inhabited by the Christmas rose range from 1120 mm (e.g. Celje – central part) to 2634 mm (e.g.

Bovec – north-western part), and the absolute minimum air temperature of the coldest place is –24.7 °C (Celje). It prefers a deep, well-drained soil, rich in organic matter but with no shortage of moisture.

Slovenia has at least six distinctive phytogeographical regions (Fig. 6) (Wraber, 1969), and the Christmas rose is widely distributed throughout all of them (Fig. 7). Its habitats, however, appear to be less frequent within the sub-Pannonian and sub-Mediterranean regions.

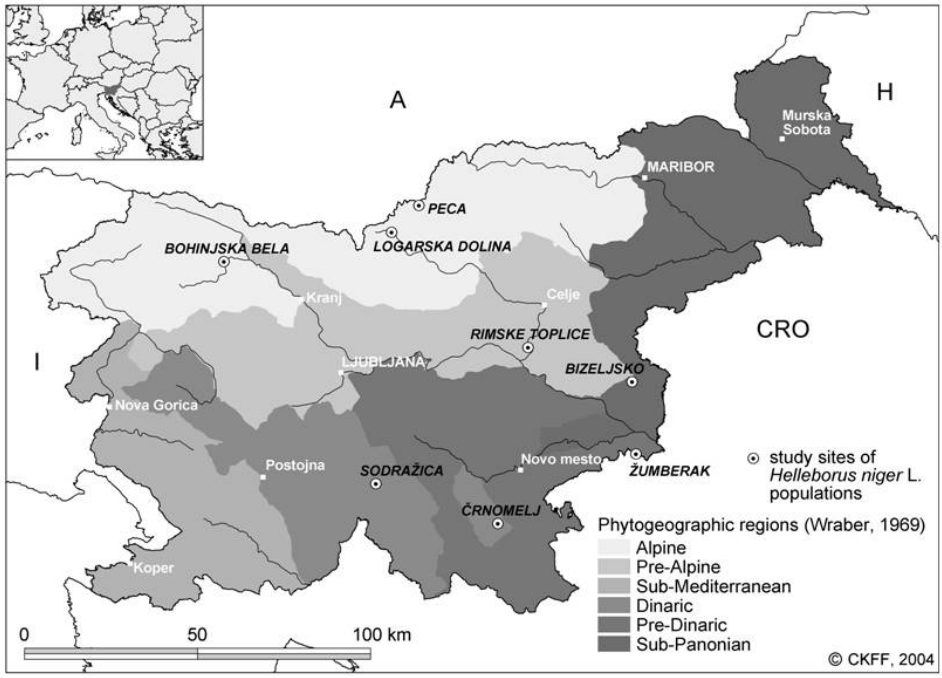

Figure 6: **Phytogeographical division of Slovenia (after Wraber, 1969), and the locations of study sites**.

The geographical map was adapted with the permission of the CKFF (the Slovenian Centre for Cartography of Fauna and Flora)

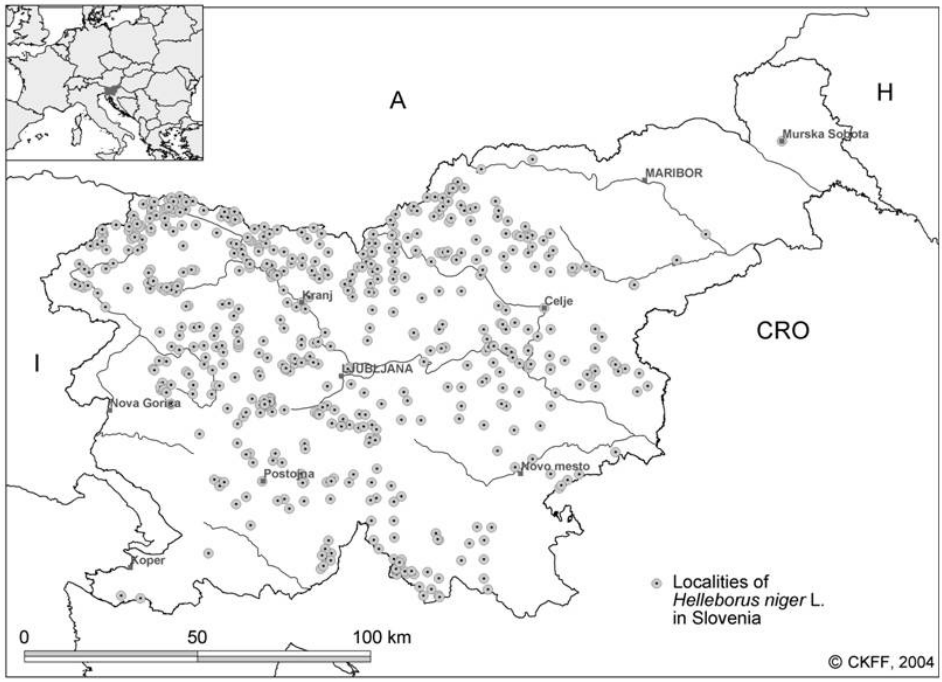

Figure 7: Localities of *Helleborus niger* in Slovenia (Jogan, 2001).
The geographical map was adapted with the permission of the CKFF (the Slovenian Centre for Cartography of Fauna and Flora)

It grows in a variety of habitats, mostly in woodlands, woodland clearings, and along woodland edges in open scrub. It grows beneath spruce (*Abies* spp.), birch (*Pinus* spp.), oak (*Quercus* spp.), beech (*Fagus* spp.), and hornbeam (*Carpinus* spp.) trees, and in association with such other plants as *Anemone nemorosa* L., *Asarum europaeum* L., *Campanula sibirica* L., *Cardamine kitaibelii* Bech., *Clematis recta* L., *Coronilla emerus* L., *Cyclamen purpurascens* Mill., *Daphne mezereum* L., *Epimedium alpinum* L., *Erica carnea* L., *Euphorbia nicaeensis* All., *Gentiana asclepiadea* L., *Gentiana clusii* Perr. & Song., *Gladiolus imbricatus* L., *Hacquetia epipactis* DC., *Helleborus atrorubens* Waldst. & Kit., *Inula ensifolia* L., *Lilium bulbiferum* L., *Omphalodes verna* Moench., and *Primula vulgaris* Hill. (Rice and Strangman, 1999).

3.2 Morphology

Under natural conditions, the Christmas rose is a rhizomatous, evergreen perennial, 15–30 cm high (Hegi, 1975), admired for its very early flowers and attractive leaves.

Its underground part is a short, branched rhizome, usually deep-rooted. The rhizome constitutes a specialised, horizontally-growing stem (de Hertogh and Le Nard, 1993). The Christmas rose has the pachymorph type of rhizome. The rhizome is knotted, blackish on the outside, white within, and sends out numerous long, simple, dependent fibres. It appears as a many-branched clump made up of short individual sections – crowns. It is determinate, meaning that each clump terminates in a flowering stalk. Young roots are pale brown, while older ones are black-brown (from which the plant receives the specific name 'niger') (Fig 8).

Petioles are stalked basal, reddish spots from 5.5 to 37 cm long. Leaves are evergreen and deeply divided into 5 to 11 segments (Ravnik, 1969), which are usually entire and sometimes serrated towards the tip. The teeth along the margins of leaf segments can be protruding and prickly. Leaf segments are oblong-cuneate or broadly lanceolate, dark green, bluish green or greyish green. The leaves that start to grow after flowering are almost leathery and can be lustreless or with lustre.

Peduncles are non-leafy flower stems, usually green at the base with more or less reddish spots in the middle and upper parts, and are from 7.5 to 37 cm long. Inflorescences are composed of 2 to 3 large, nodding flowers. Sometimes there are solitary flowers, borne on 3–5 cm long pedicels. The bracts are undivided and without teeth.

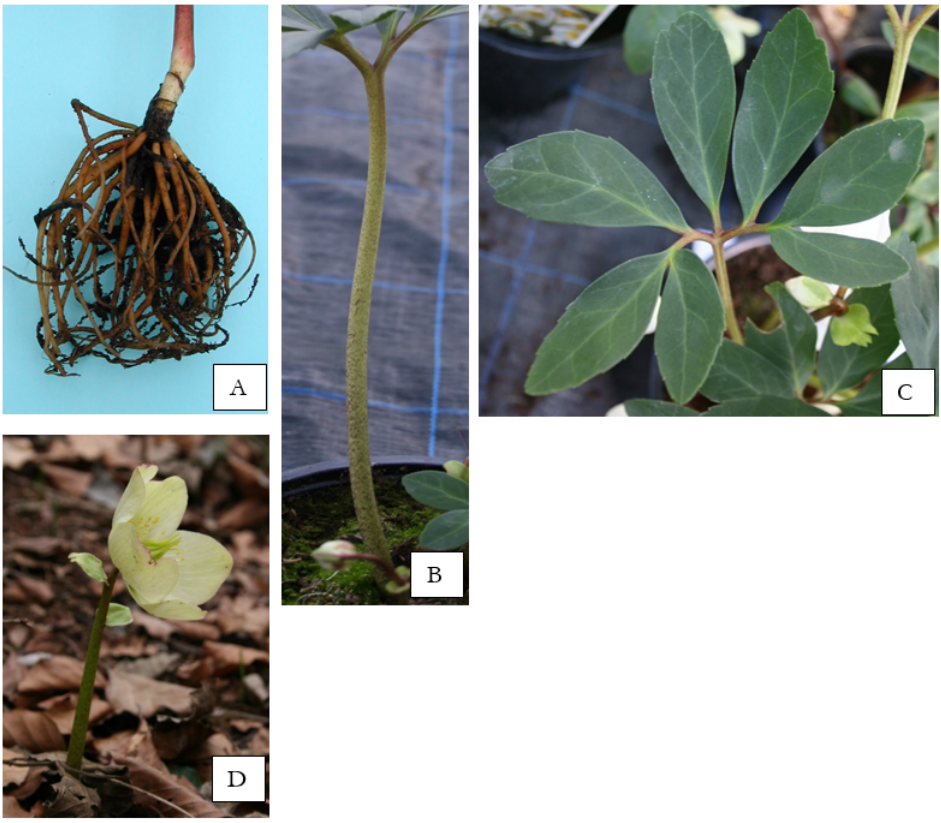

Figure 8: Parts of the plant: A – roots from a 12-month-old specimen; B – petiole; C – leaf; D – peduncle with flower
(Photo: A. Šušek)

Flowers, usually one per stem, are relatively large and flat, and their diameter, according to Ravnik (1969), ranges from 6 to 11 cm. The perianth consists of two similar whorls. The outer whorl consists of 5 large, usually overlapping, perianth segments (sepals) (Fig. 9A). The inner whorl consists of numerous stamens arranged in a spiral and small, green tubular-shaped, short stalked nectaries (modified petals, up to 32) on a cone-shaped receptacle (Mathew, 1989). Five to ten separate carpels form the central whorl of the floral structure (Trinjastič et al., 1967; Frankel and Galun, 1977). Although usually white with a green 'eye', the flowers may be pink on the reverse side, or turn pink on both sides as they age.

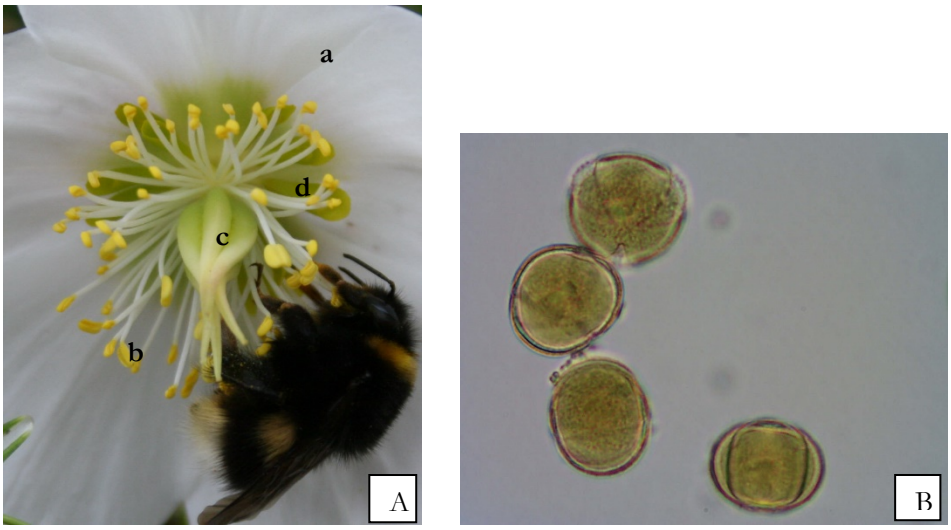

Figure 9: A – flower parts (a – sepals, b – stamen, c – pistil, d – nectary gland); B – pollen (Photo: A. Šušek)

The *Helleborus* pollen is tricolpate and has a finely or coarsely reticulate tectum (Fig 9B); it is easily distinguished from that of other genera of the Ranunculaceae family (Nowicke and Skvarla, 1983; Sun et al., 2001). The outer surface is colliculate (like a cobbled road) with a finely to coarsely reticulate pattern of depressions. In *H. niger*, there are only a few of these holes, so there is no obvious reticulate pattern, the surface appearing almost unbroken (apart from the 3 long colpi) (Mathew, 1989).

The fruit is a follicle, united at the base, at maturity dehiscent. The seeds are angular, winged or ridged, with abundant endosperm. The embryo is small and linear with two cotyledons. It is often hard to locate the embryo because of its size and similarity to the endosperm (Lockhart, 1982).

3.3 Growth and development

When the flowering season is over, the old leaves generally turn yellow or brown, and die (they have been functioning for only one year), and new ones begin to grow. At the same time, the rhizome starts to expand by elongation of the growing points produced at the terminal end and on the lateral branches. The growth is also associated with the expansion of the intercalary meristems in the lower part of the

internodes. As the plant continues to grow and the older part dies, the branches arising from one plant may eventually become separated to form individual units belonging to the same clone (Hartmann et al., 1997).

Rhizomes exhibit consecutive vegetative and reproductive stages. In a pachymorph rhizome, the growth cycle begins with the initiation and growth of lateral branches on the flowering stalk. After flowering, the flowering stalk dies, but the new lateral branches produce leaves and grow vegetatively during the remainder of the season. The continued growth of the underground stem, storage of food, and the production of the flower bud at the end of the vegetative period are strongly dependent upon photosynthesis. Consequently, foliage should not be removed during this period. The next flowering stalk will be produced in the following autumn (Hartmann et al., 1997).

During the period from May to June, new roots appear on the crown. During summer, new leaves and new vegetative buds develop. At the end of summer, the formation of floral buds is completed, and the rhizogenesis slows down. Each rhizome forms floral buds after the appearance of two new leaves at the base of the second leaf, regardless of daylight. The initiation of floral buds probably depends more on temperature than on daylight (Werner and Ebel, 1994). Without a period of low temperatures, flowers cannot develop normally. Low temperatures (above 0 °C) and shorter daylight hours have a considerable influence on the elongations of peduncles and the development of floral shape and colour. During the development of the flower, at the stem's base, a new rhizome also grows up and perpetuates the cycle (Lemper, 1984).

In its native habitats, the Christmas rose flowers from November to April. The flowering period depends on several factors, such as genotype, age, presence of pests and diseases, soil fertility, and climatic conditions. Amongst these, the most important appears to be the climatic factor. Snow and low temperatures may postpone the onset of flowering for two or more months. The examples include the autumn-winter periods in 2000/2001 and 2002/2003. The autumn-winter period in 2000/2001 was warm, and flowering in Slovenia began in the fourth week of November, whereas during 2002/2003 it was much colder, with a lot of snow, and flowering began in mid-February. The duration of flowering depends mainly on

ambient air temperatures. When average daily temperatures are 3–5 °C, flowering ends within 2–3 weeks.

The Christmas rose differs from most other hellebores in the manner in which the flower stem emerges from the soil (Fig. 10). After a period of low temperatures, the floral stems (peduncles) start to elongate. This process is in many ways similar to that in French beans (Ahlburg, 1989).

The upper end of the peduncle, just below the base of the flower, is thinner than the lower part and sharply bent like a hairpin. The sepals are folded together like an umbrella about to be put into its cover and are also furled like an umbrella, thus forming an acute tip. The flower is not protected by being enclosed within bracts. The wrinkled neck of the peduncle pushes through the soil surface and then straightens, pulling the flower out of the soil (Ahlburg, 1989).

Following fertilisation by February or March, the fruit starts to develop and is mature by May or June (depending on ambient temperatures). Simultaneously, the sepals, which are white or pink at anthesis, persist until the seeds are ripe and become intensely green (in shaded plants) or dark red (in sun-exposed plants) during that period (Salopek-Sondi et al., 2000, 2002).

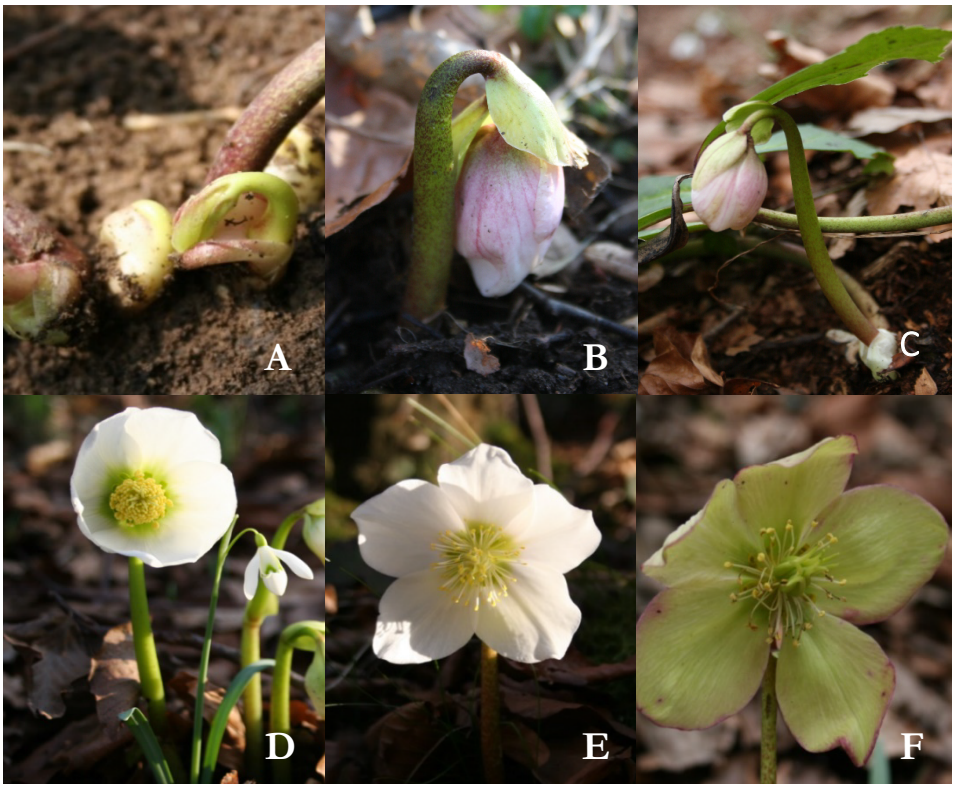

Figure 10: Different development stages of the Christmas rose flower: (A) a bud, (B) an open bud out of soil, (C) a closed flower with large stalk, (D) opening sepals, (E) an open flower, (F) an old flower
(Photo: A. Šušek)

University of Maribor Press

4 Study of pollination mechanisms and the hybridisation of Christmas rose

4.1 Pollination dynamics and ecology

Two dispersal units exist in higher plants: the pollen grain, which is a microspore with a resistant outer cover, and the seed, constituting an arrested stage of a young sporophyte. In contrast to animals and lower plants, dispersal units of higher plants are immobile, so they need external agents for dispersion. Resistance to dry conditions is required to ensure the survival of pollen and seed of terrestrial plants during this phase of dispersion.

The biological function of pollen and seed as dispersal units depends on germination and growth, and a proper ecological niche. This niche is relatively broad for seeds, but for pollen – its function depends on a much more restricted and specific ecological niche (the compatible and receptive stigma). The transfer of pollen requires extreme precision. Longevity of pollen viability is less important than the precision of this transfer. The breeding behaviour of plants depends on the pollination syndrome. Stebbins (1970) discussed the evolutionary aspects of these syndromes and concluded that "the diverse floral structures and pollination

mechanisms in angiosperms represent a series of adaptive radiations to different pollen vectors and different ways of becoming adapted to the same vector".

The adaptation to different pollen vectors is expressed by structural, spectral, and other floral specificities. The functional relation of the diverse characters to the vectors can be attributed to the type of vector – whether involuntary, abiotic and unspecialised vectors, or voluntary, i.e. specialised animal vectors.

The adaptation to voluntary specialised vectors is based on a multi-formulation of stimuli. These kinds of vectors are seen on plants as two kinds of attractants or stimuli. Primary attractants are based on food. Food comes mainly in the form of pollen and nectar. The attraction could also be sexual or because the flowers may serve as a refuge or a breeding site for insect pollinators. Secondary attractants are stimuli, based on odour and visual attraction (because of colour, shape, texture, locations, and movements of the flowers) and temperature (Frankel and Galun, 1977; Faegri and Pijl, 1979).

Adaptation to abiotic and involuntary, non-specialised, vectors is based mainly on the physical characteristics of pollen, the exposure of pollen to the dispersal agent, the pollen-catching capability of the stigmatic surface, and a high ratio of male to female gamete production.

Pollination, in most cases, is not carried out exclusively by one single agent. The process of evolutionary floral modification may be retarded by the presence of secondary dispersal agents (Stebbins, 1970). However, normally we find one of the vectors predominant. Gymnosperms feature primarily abiotic pollination, and biotic pollination is a derived condition. On the other hand, in angiosperms biotic pollination is more common, and abiotic pollination is a derived condition (Frankel and Galunm, 1977).

Biotic pollination

Biotic pollination can be performed by a large number of insects and some small vertebrates. However, only some of them are effective pollinators. Anthophilous animals of pollination syndromes relate to specific ecological conditions providing for an optimal energy budget. Insects are the predominant biotic pollen-dispersion agents; primitive insects such as beetles developed before flowering plants.

Specialised pollinators, such as bees, evolved concurrently with flowering plants and have developed a growing ability to perceive and discriminate between floral specificities. Flower discrimination is important; efficient pollination depends on successive flower visits and the flower type constancy of the pollinator. The amount of reward per flower must be sufficient to justify a visit, but not be so extensive as to limit successive flower visits.

Efficient insect pollination is importance during fruit or seed production of some crops such as clover, crucifer, cucurbit, and self-incompatible ones. Insect pollination at the appropriate time is very often a prerequisite for agricultural success, so that entomophily in cultivated plants has been the subject of extensive research activity.

Entomophilous crops are characterised by large or grouped flowers, and conspicuous perianths:

- petals are colourful and with nectar guide marks
- nectaries and scent are often present
- pollen grains are large (75–150 microns), sticky and oily, and often with an ornamented surface

Although the flower constancies of insects would be encouraged by hermaphrodite flowers, some entomophilous crops are monoecious or even dioecious (Frankel and Galunm, 1977).

Bees, in general, are the most important pollinators for cultivated plants. They are the more specialised pollen vectors and depend almost entirely on the pollen and nectar of flowers. A honeybee may visit 100 flowers per trip and carry 5 million pollen grains (20 mg). The same bee makes 5 to 10 trips a day going to flowers of different species, and could make around 4 million trips a year, which is more or less 2 kg of pollen. The problem with bees is that they don't fly if the wind speed is higher than 11 km/h, and if climatic conditions are unsatisfactory.

Bumblebees may be even more efficient pollinators than honeybees just because they work faster, for a longer time, and can carry greater amounts of pollen each trip, so we can assume much more transported pollen per year.

It is known that ordinary flies possess the power of discrimination between some colour groups (at least yellow and blue), and that they show a positive preference for yellow. Also, nectar guides have a positive effect on attracting flies. Flies are not busy collectors and their pollinating activities are irregular and unreliable. Flies, however, may be important under certain climatic conditions because they are present at all times of the year.

Butterflies are diurnal insects and have shown preferences for various colours, but this depends on the species. Their colour vision, at any rate in some species, seems to include pure red.

In regards to **wasps**, the instinctive apparatus for building up a systematic utilisation of one or very few suitable blossoms is not particularly well-developed in these insects. Whereas bees can distinguish between 2 or 3 colours at the same time, wasps can only distinguish one. Some of them can distinguish between odours and visit open blossoms so they can carry out pollination.

Ants are such small insects that they can sneak in and out of many blossoms, without even touching the anthers or stigma. Their bodies are hard and apparently unadapted for pollen transport. In unadapted species, all blossoms are punctured. In other species, the ants are attracted by the extra-floral nectaries at the bases of blossoms. It is obvious that ants crawling around flowers and inflorescences may cause geitonogamy, and that this will occur wherever they discover an available source of nectar or pollen.

Other pollinators can be **beetles.** Beetle flowers are frequently overlooked, and the visits of beetles in blossoms considered as accidental. Typical beetle pollination is rare in the European extra-tropical flora. Beetles constitute one of the oldest groups of insects. They were already numerous at the time when the first higher plants came into existence, just when *Hymenopters* and *Lepidopters,* so important during pollination today, had not yet developed. Nowadays, whilst some beetles are more or less accidental visitors to blossoms, others are habitual visitors and have developed adaptations for blossom visits.

Very few cultivated plants are pollinated primarily by **vertebrate vectors**, although vertebrates, in particular flying vertebrates such as birds and bats, are the primary pollinators for about 20% of the tropical flora.

A large number of **agricultural pests** serve as involuntary biotic pollen vectors and often become important during pollination; some of them are thrips, aphis, moths or even squirrels, rats or other mammals.

Biotic pollination becomes a function of the effective population size of the vector, the mass of the plant species, the foraging area size of the vector, the distribution of the vector, and the vector preference for alternative species and shorter foraging trips. Competition during the spore-dispersal phase may be followed by competition between genotypically different pollen grains after the deposition on the receptive surface of the ovule. Such competition could depend on differential longevity, germination or the growth of dissimilar pollen varieties, and on selective fertilisation (Frankel and Galun, 1977; Faegri and Pijl, 1979).

Abiotic pollination

There are three agents for pollen transfer classified as abiotic. All these abiotic pollination mechanisms are random in their natures, but structural adaptations of flowers, pollen, and of plant populations have evolved for increasing the efficiencies of the random pollen transfer processes (Frankel and Galun, 1977; Faegri and Pijl, 1979).

Gravity: The transfer is confined to only one dimension and hence ordinarily results in autogamy.

Air movement (*anemophily*): Known as "wind pollination" that makes the pollen dispersal movement in three dimensions. Anemophily has indiscriminate and inefficient dispersal mechanisms and, as a result, requires a large amount of pollen to ensure pollination. The prerequisite for pollen dispersion by the wind is good conditions for pollen transport over appropriate distances. Consequently, anemophily is common in deciduous forests, on prairies, and on savannahs. The mechanism is independent of the occurrences and behaviour of biotic vectors, and thus may have been established as a response to the absence of biotic pollination agents. A large amount of pollen is lost during transport by wind, so anemophilous plants are very prolific pollen producers. However, gametic waste as compensation for inefficiency of the pollination mechanism is an undesirable feature in terms of evolution. Several types of adjustments regarding pollen-releasing and -collecting structures account for the increased potency of wind-pollination. Enlarged, ornate, featherlike and numerous ovules per stigma is one of a few. In contrast to the sticky, ornamented pollen of entomophilous species, the pollen of anemophiles has a smooth, dry surface. Thus, grains are dispersed singly and not in groups. Pollen's ability to float also depends on its weight and size. This kind of pollen ranges in size between 2.5 and 250 microns, and is usually smaller than that carried by insects.

Water (*hydrophily*): This is pollination by water movement. It could be two-dimensional when taking place on water surface or three-dimensional when mediated by volumes of water or rain drops. This kind of pollination is relative rare, and pollen transported by volumes of water (submerged hydrophily) is less common.

4.1 Artificial pollination of Christmas rose

The Christmas rose flowers during winter and early spring. There are not many pollinators during this time. Observations indicate that the Christmas rose is probably entomophilous (the most important pollinators are insects, primarily bees and flies) and is predominantly an allogamous species. Its entomophilous nature is closely related to the botanical properties of the flower. Its flowers are hermaphroditic (they have male and female sexual organs) and protogynous (development of female organs before male organs in order to avoid self-fertilisation) (Salopek-Sondi et al., 2002). Self-fertilisation is possible, but primarily in later stages of flowering (Mathew, 1989; Armstrong, 2002). Flowers can also be pollinated by wind.

The analysis of Christmas rose plant–pollinator relationships was undertaken in the natural population near Doblatina (Fig. 11). The population is medium-sized and spreads over 600 m² of land. It is located at an altitude of 600 m a.s.l. The town of Doblatina (coordinates 46°11'4.78"N, 15°16'13.72"E) is located in the municipality of Laško and in the temperate continental climate of central Slovenia.

Figure 11: The town of Doblatina, where the experiment was performed, marked on the map
(Source: http://zemljevid.najdi.si/search_maps.jsp?q=Doblatina&tab=maps)

Methodology

The analysis included 50 plant specimens. Specimens were selected at random; however, very young, very old, and non-flowering specimens were not included. The randomly selected specimens were numbered and isolated (Fig. 12). To isolate the plants, we made domes out of wire and foil from polypropylene fibres (Covertan).

In the first year, we analysed the self-pollination rate. The selected plants were isolated at the flower initiation stage. In the early flowering stage, the plants were self-pollinated.

Figure 12: Isolated and free-flowering plants
(Photo: A. Šušek)

Self-pollination was carried out by squeezing or gently rolling the stamina and the stigma of the pistil using fingers. Pollination was carried out four times, in the period from 10 February to 20 February 2006. This way, we facilitated the transfer of pollen grains to the stigma of the pistil, thus causing fertilisation of the flower. In order not to transfer pollen grains from one isolated flower to another flower, we disinfected our hands using 70% ethanol before each new self-pollination procedure.

Seed collection was carried out when mature follicles were about to open. Flowers with follicles containing many seeds were cut with the flower stalk, stored in paper bags, and left in an aerated space for two weeks. Afterwards, the seeds that fell down were cleaned and evaluated. Before sowing, the seeds were stratified in a moist substrate and sown after stratification. The seeds were sown in plastic containers with holes for each studied plant, so that we could later evaluate the germination of sown seeds.

As in the first year, in the second year we examined the same plants; however, in the second year, the plants were pollinated with randomly collected Christmas rose pollen, which we applied to the stigma of the pistil with a brush. Plants were emasculated before pollen application, as this prevented self-pollination. Since we examined the same specimens in the first and second year, we were able to compare the weight and number of seeds produced as the fruit of self-pollinated plants and hybridised plants.

Analysis of plant–pollinator relationships

The analysis of plant–pollinator relationships aimed to determine the possibility of self-pollination of Christmas rose and seed production of plants based on the method of pollination (self-pollination and hybridisation). The analysis included 41 plants, which we observed to determine the number of collected seeds, total seed weight, average seed weight, number of germinated seeds and germination percentage.

The analysis of plant self-pollination found that plants are capable of self-pollination, because they grew seeds. Table 3 shows the statistical values: arithmetic mean, standard deviation, maximum and minimum value, first and second quartiles, and mean.

Table 3: Statistical parameters of studied properties in self-pollinated plants

Studied properties	Arithmetic mean (N = 41)	Standard deviation	Min.	Max.	First quartile	Mean	Third quartile
Number of gathered seeds	46.49	24.38	2	105	30	48	65
Total seed weight (mg)	554.72	290.11	36.1	1254.3	321.35	556.9	726.5
Average seed weight (mg)	12.26	3.11	6.96	19.82	9.97	11.43	14.99
Number of germinated seeds	4.39	4.65	0	14	0	3	7.5
Germination percentage	9.17	9.38	0	37.84	0	7.31	14.63

Min. – minimum number of seeds

Max. – maximum number of seeds

Maximum and minimum average seed weight were 19.8 mg and 6.9 mg, respectively. The first quartile was 9.9 mg, the third quartile 14.9 mg, and the mean 11.4 mg. The minimum total seed weight per plant was 36.1 mg and the maximum 1254.3 mg. The first quartile was 321.4 mg, the third quartile 726.5 mg, and the mean 556.9 mg.

The number of seeds collected per plant ranged from 2 to 105. The first quartile was 30, the third quartile 65, and the mean 48. Figure 12 shows that 25% of the plants had less than 30 harvested seeds, while 75% of the plants had over 65 harvested seeds per plant.

The number of germinated seeds ranged from 0 to 14 (Fig. 13). The first quartile was 0 and the third quartile 14, and the mean value between the first and third quartile equalled 3 germinated seeds. The highest and lowest germination percentage were 37.8% and 0%, respectively.

Figure 13: Germinated seeds of self-pollinated Christmas rose plants
(Photo: Š. Golec)

Comparison of seed production of self-pollinated and hybridised plants

Comparison of seed production was tested with a paired sample t-test. This is a parametric statistical test used when we have two dependent samples. It is used to test the null hypothesis, which states that there are no statistical differences between the arithmetic mean of the population.

At a 1% risk level, it can be argued that there is a statistically significant difference between the average seed weight of self-pollinated plants and the average seed weight of hybridised plants (Table 4).

Based on the t-test, we can state that the average seed weight of hybridised plants ($\bar{x}$ = 14.2) is statistically higher than that of self-pollinated plants ($\bar{x}$ = 11.3).

Different average seed weights were recorded for each plant according to the pollination method (Fig. 14). The maximum number of seeds per plant for self-pollinated plants was 105, and 137 for hybridised plants. The minimum number of seeds per plant for self-pollinated plants was 8, and 15 for hybridised plants.

In self-pollinated plants, 7 plants formed a higher average weight than hybridised plants, specifically plants 4, 10, 11, 13, 18, 21 and 23, while other plants had a lower average seed weight than hybridised plants.

The maximum average seed weights per plant in self-pollinated and hybridised plants were 19.8 mg and 21.1 mg, respectively. The minimum average seed weights per plant in self-pollinated and hybridised plants were 1.2 mg and 9.2 mg, respectively.

Table 4: Number, total weight and average seed weight per plant, based on pollination method

Plant	Self-pollinated plants (2006)			Hybridised plants (2007)		
	Number of seeds	Total seed weight (mg)	Average seed weight (mg)	Number of seeds	Total seed weight (mg)	Average seed weight (mg)
1	54	638.7	11.8	56	995.0	17.8
2	37	445.1	12.0	114	174.5	15.3
3	49	556,9	11.4	88	131.3	14.9
4	56	717.0	12.1	18	188.0	10.4
5	8	59.7	7.0	81	919.0	11.3
6	105	1254.3	12.0	14	272.0	19.4
7	34	246.5	7.3	59	653.0	11.1
8	17	186.4	11.0	29	502.0	17.3
9	11	103.7	9.4	29	321.0	11.1
10	14	183.2	13.1	18	218.0	12.1
11	34	674.0	19.8	16	160.0	10.0
12	66	658.1	10.0	15	215.0	14.3
13	44	693.6	15.8	21	213.0	10.1
14	85	100.1	1.2	116	130.5	11.2
15	54	821.0	15.2	14	264.0	18.9
16	64	552.6	8.6	45	949.0	21.1
17	67	944.8	14.1	47	865.0	18.4
18	16	242.7	15.2	35	321.0	9.1
19	52	562.0	10.8	45	558.0	12.4
20	44	396.2	9.0	53	763.0	14.4
21	85	971.6	11.4	57	584.0	10.2
22	75	736.0	9.8	137	222.0	16.2
23	14	223.2	15.9	68	103.1	15.2
24	53	517.0	9.8	63	1214.0	19.3
25	68	681.3	10.1	74	856.0	11.6
26	96	956.3	10.0	72	118.8	13.1
27	53	597.6	11.3	55	953.0	17.3
$\bar{x}$ [1]			11.3[a]			14.2[b]

[1] average value

[a, b] – values marked with different letters differ with statistical significance

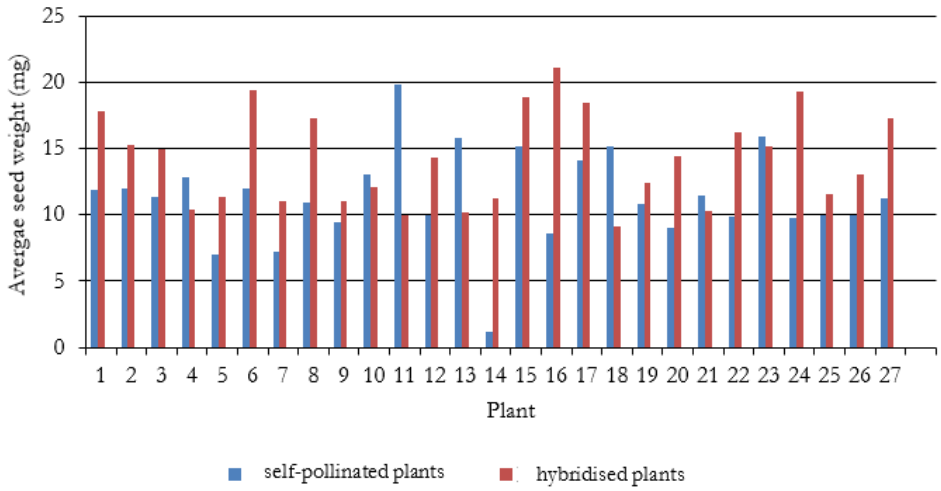

Figure 14: Average seed weight of self-pollinated and hybridised plants

Conclusions

When studying its method of pollination, we determined that the Christmas rose is a self-pollinating plant. Self-pollinated plants grew germinating seeds. Thus, we confirmed the claims made by Mathew (1989) and Armstrong (2002) that self-fertilisation is possible, but occurs mainly in the later stages of flowering. In studying seed production of self-pollinated and hybridised plants, we found that the average seed weight of hybridised plants was higher than the average seed weight of self-pollinated plants. Based on these findings, we can say that hybridised plants have higher seed yield than self-pollinated plants.

4.2 Analysis of the influences of different flower colours on the visits and behaviour of pollinating species

The pollination studies were based on recordings of visits to the flowers by various insect species. Pollinating species assemblages may vary by time of day, time of season, or location (Heinrich, 1976; Herrera, 1988; Traveset et al., 1998), and for this reason the observations took place at different times of day, throughout the flowering season.

Plants in pots were placed in the Maribor University Botanic Garden. They were protected against frost by the leaves falling off from the neighbouring trees. Based on previous observations, we concentrated on 5 groups of insect pollinators: (a) bees, (b) bumblebees, (c) large flies, (d) small dipterous flies and (e) pollinators of minor importance, including various species of wasps, ants, beetles and *Thysanoptera*. In order to analyse the colour preferences of the studied pollinators, the flowers were painted (1) Green, (2) Blue, (3) Red, (4) Violet, (5) Yellow, (6) White (not coloured flowers) (Fig. 15).

Figure 15: Artificially coloured flowers of *Helleorus niger* L. **in order to study the number of visits and behaviour of pollinating insects: (1) Green, (2) Blue, (3) Red, (4) Violet, (5) Yellow, (6) White (not coloured flowers)**
(Photo: A. Šušek)

The observations lasted for several days and involved different periods of the day:

- First day. Monitoring started at 9 a.m. and ended at 4 p.m. The weather was cloudy most of the time, but there was a period from noon to 2 p.m. when the sun shone. The temperature in the morning was 4 °C, at noon it was 10 °C, and in the evening it was 8 °C.
- Second day. Monitoring started at 9 a.m. and ended at 3 p.m. The weather turned from sunny in the morning to cloudy in the afternoon. In the afternoon it became windy. The temperatures were: 4 °C in the morning, 10 °C at noon, and 8 °C in the evening.
- Third day. The observations started at 9 a.m. and finished at 5 p.m. This day the sky was clear. The highest temperature (8 °C) was at 1 p.m.
- Fourth day. Monitoring started at 9 a.m. and ended at 3 p.m. This day was very sunny. The temperature was very low in the morning (2 °C), rising to 9 °C at 1 p.m.

The recordings were done by one experienced person, who was responsible for monitoring 60 flowers (6 different colours, 10 flowers were painted with the same colour). The plants painted with the same colour were randomly distributed over an area of approximately 8m^2. Each plant was labelled with a small tag with a number on it (Fig. 16).

Figure 16: Artificially coloured flowers for monitoring at the Maribor University Botanic Garden
(Photo: A. Šušek)

Analysis of the frequencies of pollinators visiting Christmas rose

The pollination study of insects' activities showed that there were obvious differences regarding the frequencies of visits amongst the five investigated insect groups (Fig. 17).

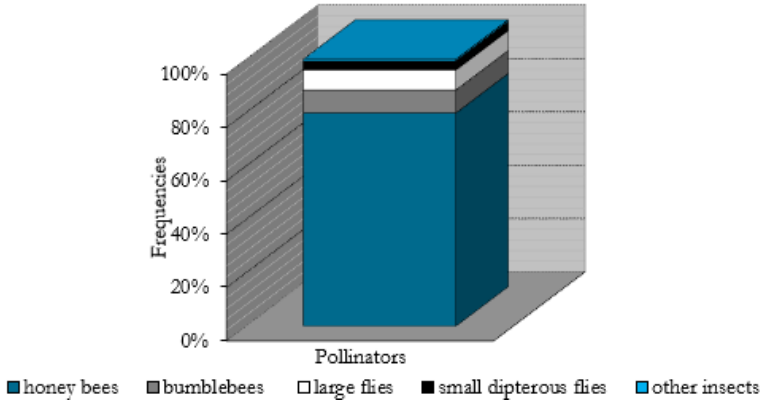

Figure 17: Frequencies of pollinators visiting *Helleborus niger* during four days of observations at the Maribor University Botanic Garden

Bees were the most active between 11 a.m. and 2 p.m. (Table 5 and Fig. 18). They were more attracted by the natural colours of the flowers (white) than other artificially coloured flowers. The total number of visits during the observation period was 109. The highest number of visits was at 12 a.m., (they visited 42 flowers in one hour) at an average of 4.2 visits per flower and 10 visits to the same flower in one day.

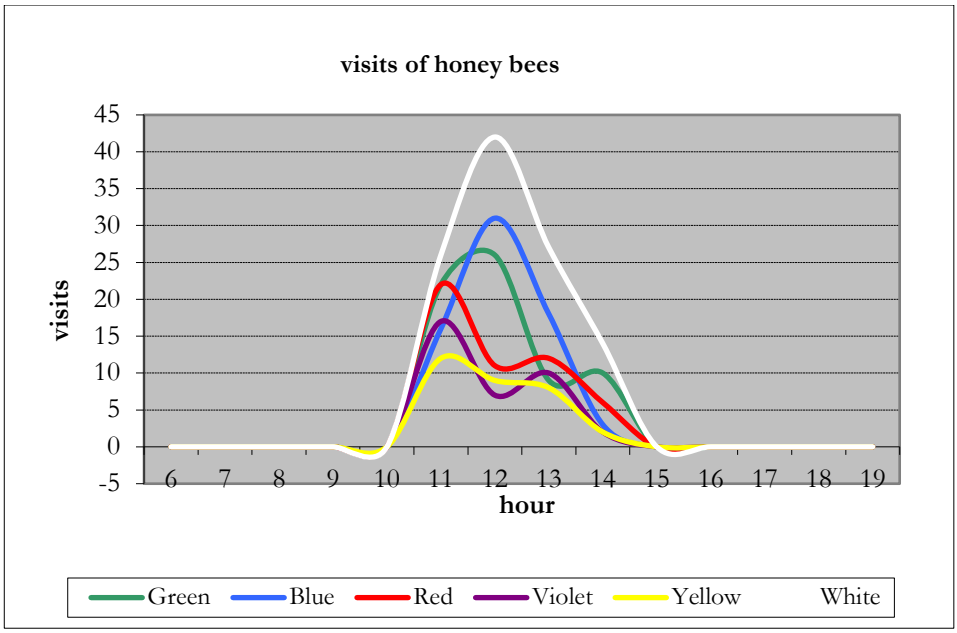

Figure 18: Relationships between different colours of flowers and visits of bees

The second more attractive artificially-coloured flowers were blue and green, with total numbers of 68 and 67 visits, respectively. The highest number of visits to blue-coloured flowers was at 12 a.m., with 31 visits per hour (3.1 visits per flower and 9 visits to one flower in one day). The green-coloured flowers were visited 22 times (2.2 per flower), with a maximum of 7 visits to the same plant. Violet- and yellow-coloured flowers were less attractive to bees.

Table 5: Visits to Christmas rose flowers by bees at the Maribor University Botanic Garden during 4 days of observations

Hours	6	7	8	9	10	11	12	13	14	15	16	17	18	Total visits
Colour (1) Green														
Mean	0	0	0	0	0	2.2	2.6	0.9	1	0	0	0	0	
Max.	0	0	0	0	0	7	7	3	2	0	0	0	0	
Sum	0	0	0	0	0	22	26	9	10	0	0	0	0	67
Colour (2) Blue														
Mean	0	0	0	0	0	1.6	3.1	1.8	0.3	0	0	0	0	
Max.	0	0	0	0	0	4	9	5	1	0	0	0	0	
Sum	0	0	0	0	0	16	31	18	3	0	0	0	0	68
Colour (3) Red														
Mean	0	0	0	0	0	2.2	1.1	1.2	0.6	0	0	0	0	
Max.	0	0	0	0	0	5	6	6	1	0	0	0	0	
Sum	0	0	0	0	0	22	11	12	6	0	0	0	0	51
Colour (4) Violet														
Mean	0	0	0	0	0	1.7	0.2	0.4	0.1	0	0	0	0	
Max.	0	0	0	0	0	6	2	4	1	0	0	0	0	
Sum	0	0	0	0	0	17	7	10	2	0	0	0	0	36
Colour (5) Yellow														
Mean	0	0	0	0	0	1.2	0.9	0.8	0.2	0	0	0	0	
Max.	0	0	0	0	0	4	3	3	1	0	0	0	0	
Sum	0	0	0	0	0	12	9	8	2	0	0	0	0	31
Colour (6) White														
Mean	0	0	0	0	0	2.6	4.2	2.7	1.4	0	0	0	0	
Max.	0	0	0	0	0	6	10	5	7	0	0	0	0	
Sum	0	0	0	0	0	26	42	27	14	0	0	0	0	109

The second more important group of visitors were **bumblebees**, however, their frequency was much lower compared to bees (Table 6). Their activity occurred between 10 a.m. and 1 p.m. at two intervals. The first interval was at 10 a.m. and the second started at 12 noon, and finished at 1 p.m. (Fig 19). The most attractive colour of flower was white. Bumblebees, in total, made 39 visits to white flowers during the observation time. The highest number of visits was between 12 noon and 1 p.m. (they visited 6 flowers in one hour), when they made an average of 0.6 visits per flower and had a maximum of 2 visits to the same flower in one day. Artificially

coloured violet flowers were not visited by bumblebees during the observation times.

Table 6: Visits to Christmas rose flowers by bumblebees at the Maribor University Botanic Garden during 4 days of observations

Hours	6	7	8	9	10	11	12	13	14	15	16	17	18	Total visits
Colour (1) Green														
Mean	0	0	0	0	0.1	0	0.1	0.2	0	0	0	0	0	
Max.	0	0	0	0	1	0	1	1	0	0	0	0	0	
Sum	0	0	0	0	1	0	1	2	0	0	0	0	0	4
Colour (2) Blue														
Mean	0	0	0	0	0.2	0	0.2	0.3	0	0	0	0	0	
Max.	0	0	0	0	1	0	1	2	0	0	0	0	0	
Sum	0	0	0	0	2	0	2	3	0	0	0	0	0	7
Colour (3) Red														
Mean	0	0	0	0	0.1	0	0.3	0.2	0	0	0	0	0	
Max.	0	0	0	0	1	0	2	1	0	0	0	0	0	
Sum	0	0	0	0	1	0	3	2	0	0	0	0	0	6
Colour (4) Violet														
Mean	0	0	0	0	0	0	0	0	0	0	0	0	0	
Max.	0	0	0	0	0	0	0	0	0	0	0	0	0	
Sum	0	0	0	0	0	0	0	0	0	0	0	0	0	
Colour (5) Yellow														
Mean	0	0	0	0	0.3	0.1	0	0.3	0	0	0	0	0	
Max.	0	0	0	0	1	1	0	2	0	0	0	0	0	
Sum	0	0	0	0	3	1	0	3	0	0	0	0	0	7
Colour (6) White														
Mean	0	0	0	0	0.3	0	0.6	0.6	0	0	0	0	0	
Max.	0	0	0	0	2	0	2	2	0	0	0	0	0	
Sum	0	0	0	0	3	0	6	6	0	0	0	0	0	15

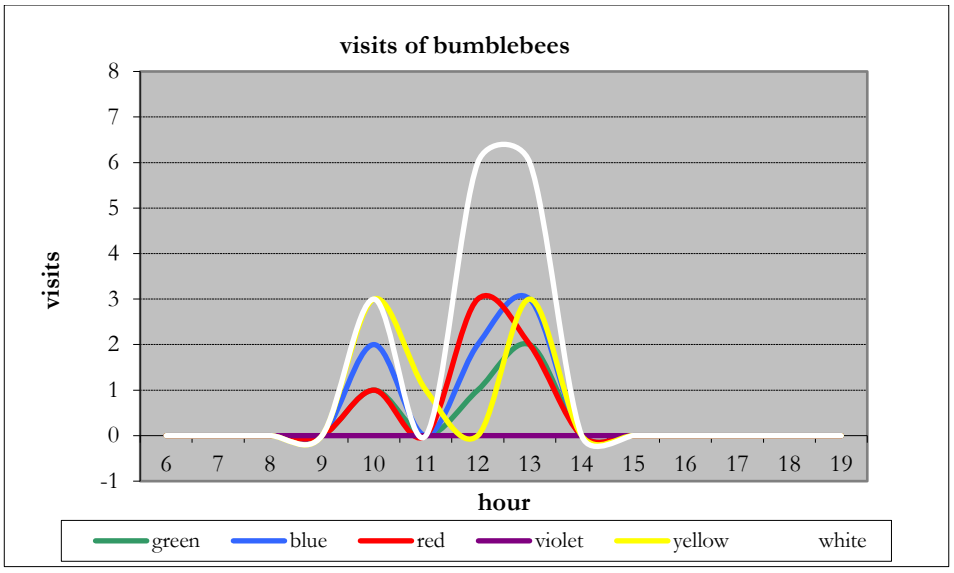

Figure 19: Relationships between different colours of flowers and visits of bumblebees

Large flies represent 7.51% of all visits of studied pollinators. Their activity occurred at 9 a.m. and ended at 2 p.m., depending on the weather conditions (Table 7, Fig. 20).

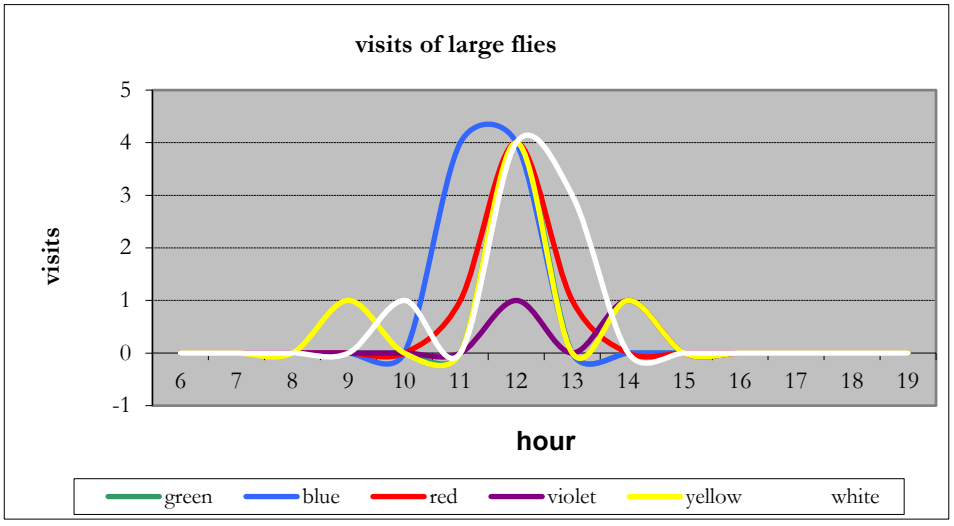

Figure 20: Relationships between different colours of flowers and visits of large flies

Table 7: Visits to Christmas rose flowers by large flies at the Maribor University Botanic Garden during 4 days of observations

Hours	6	7	8	9	10	11	12	13	14	15	16	17	18	Total visits
Colour (1) Green														
Mean	0	0	0	0	0	0	0.4	0	0	0	0	0	0	
Max.	0	0	0	0	0	0	1	0	0	0	0	0	0	
Sum	0	0	0	0	0	0	4	0	0	0	0	0	0	4
Colour (2) Blue														
Mean	0	0	0	0	0	0.4	0.4	0	0	0	0	0	0	
Max.	0	0	0	0	0	1	3	0	0	0	0	0	0	
Sum	0	0	0	0	0	4	4	0	0	0	0	0	0	8
Colour (3) Red														
Mean	0	0	0	0	0	0.1	0.4	0.1	0	0	0	0	0	
Max.	0	0	0	0	0	1	1	1	0	0	0	0	0	
Sum	0	0	0	0	0	1	4	1	0	0	0	0	0	6
Colour (4) Violet														
Mean	0	0	0	0	0	0	0.1	0	0.1	0	0	0	0	
Max.	0	0	0	0	0	0	1	0	1	0	0	0	0	
Sum	0	0	0	0	0	0	1	0	1	0	0	0	0	2
Colour (5) Yellow														
Mean	0	0	0	0.1	0	0	0.4	0	0.1	0	0	0	0	
Max.	0	0	0	1	0	0	2	0	1	0	0	0	0	
Sum	0	0	0	1	0	0	4	0	1	0	0	0	0	6
Colour (6) White														
Mean	0	0	0	0	0.1	0	0.4	0.3	0	0	0	0	0	
Max.	0	0	0	0	1	0	2	1	0	0	0	0	0	
Sum	0	0	0	0	1	0	4	3	0	0	0	0	0	8

The most attractive flowers for large flies were naturally white and artificially coloured blue flowers (Fig. 20). Large flies made 8 visits to each coloured flower during the observation time. They started visiting white flowers at 10 a.m. The highest number of visits was at 12 a.m., when they made an average of 0.4 visits per flower and 2 visits to the same flower in one day (Table 7). The period of visits to artificially coloured blue flowers was only two hours long, from 11 a.m. to 12 a.m.

During observation of visiting pollinators, we could also notice the presence of **small dipterous flies**. They represent less than 5% of all visits. They could be seen between 9 a.m. and 2 p.m., and they were most active from 12 a.m. to 2 p.m. The most attractive floral colour for small dipterous flies was natural white. In total, they made 5 visits to white flowers during the observation period, and their activity occurred between 9 a.m. and 1 p.m. in two intervals. The first interval was at 9 a.m., when they visited 3 flowers in one hour. The second started at 12 .a.m. and finished at 1 p.m., with only one visit per hour (Fig. 21). Artificially coloured green flowers were not visited by small dipterous flies during the observation period.

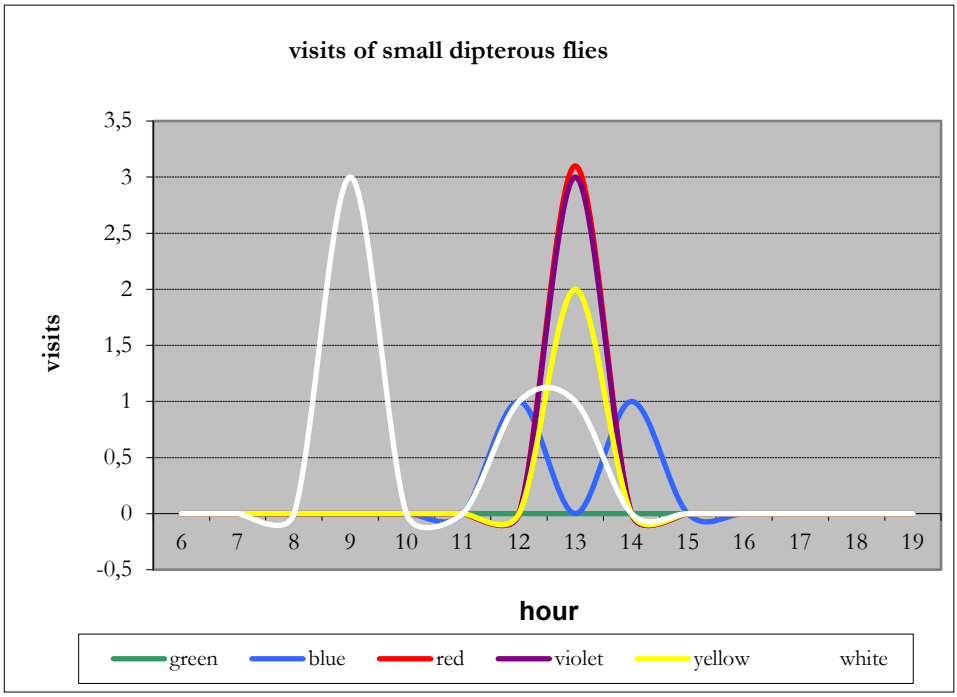

Figure 21: Relationships between different colours of flowers and visits of small dipterous flies

The group of **minor pollinators** represented less than 1% of total visits during monitoring; therefore, they were not considered important pollinators. They visited only naturally pigmented (white) flowers and artificially coloured yellow flowers. Of these two colours, they preferred white flowers (Fig. 22). White flowers were visited two times (the first at 11 a.m. and the second at 2 p.m.) by a mosquito, so this means 0.1 visits per flower.

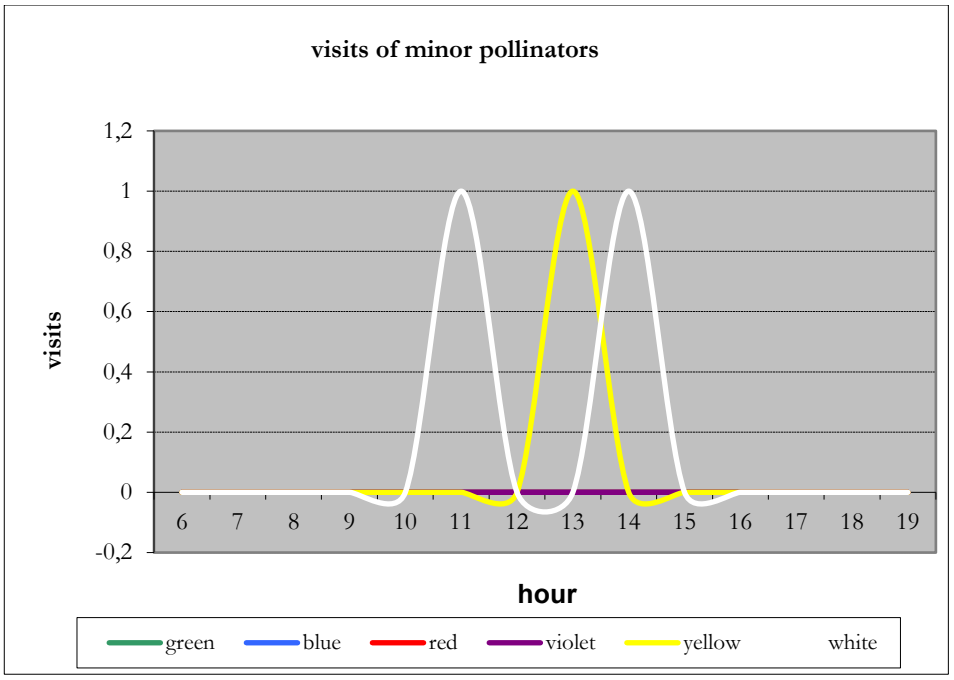

Figure 22: Relationships between different colours of flowers and visits of minor pollinators

Conclusions

According to our observations, we can confirm that the Christmas rose appears to be a predominantly entomophilous and cross-pollinating species. The activity of pollinators depends on several factors, such as insect species, time of day, and colour of flowers. The visitors of Christmas rose flowers were bees, bumblebees, large flies, small dipterous flies and other insects. The most important pollinators were bees and bumblebees. Their activity occurred from 9 a.m. (bees from 10 a.m.) to 2 p.m. They preferred the natural colour of flowers (white). The most attractive artificially coloured flowers were blue and red.

4.3 Pollination in Slovenian naturally occurring populations

The pollination studies took place at different times of day, throughout the flowering season and at three different locations belonging to different geographical regions: (1) the valley of Bohinjska Bela (500 m a.s.l., north-western Slovenia), (2) the Peca Mountains (750 m a.s.l., north-eastern Slovenia) (3) population in Žiče by Slovenske Konjice (326 m a.s.l, eastern Slovenia) (Fig. 23).

The first location was not far from a rural area, whilst the second and the third were in an isolated area, completely in the wild. Based on previous observations, we concentrated on 5 groups of insects: (a) bees, (b) bumblebees, (c) large flies, (d) small dipterous flies and (e) pollinators of minor importance, including various species of wasps, ants, beetles, and *Thysanoptera*. The observations lasted for several days; however, only 3 days were suitable for 12-hour-long recordings in Bohinjska Bela and 2 days on Peca Mountains. These observations started early in the morning (at 6 a.m.) and ended in the evening (at 6 p.m.) at the populations in Bohinjska Bela and the Peca Mountains. The recording was done by two experienced persons and each of them was responsible for monitoring 30 flowers in close vicinity. In the population in Žiče by Slovenske Konjice, we observed pollination of 42 specimens. We started the observation in the morning (at 8 a.m.) and ended in the afternoon (at 5 p.m.). The observation was carried out by one experienced person. Each of the monitored flowers was marked with a small label.

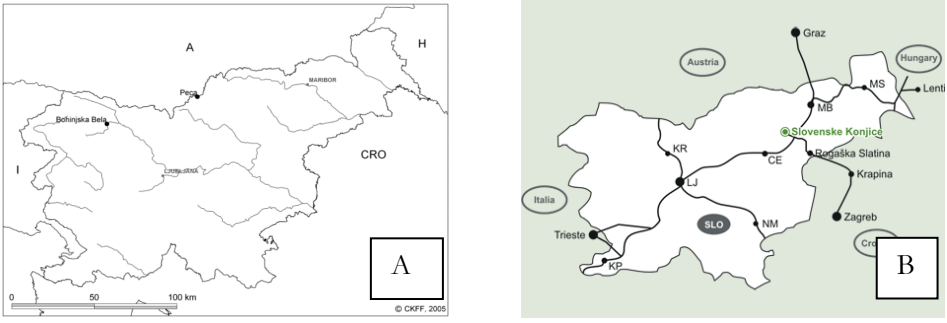

Figure 23: Studied locations of *Helleborus niger* in Slovenia A – Peca and Bohinjska Bela (●); B – Slovenske Konjice (Source: A – The map was adapted with the permission of the CKFF (the Slovenian Centre for Cartography of Fauna and Flora); B – http://zemljevid.najdi.si/search_maps.jsp?q=Doblatina&tab=maps)

Analysis of pollinator activity in Žice by Slovenske Konjice

When analysing the activity of pollinators in Žiče by Slovenske Konjice, we found obvious differences in the frequency of individual groups of insects observed (Table 8). During the study period, large flies were the most important pollinators. They were most active in the morning, between 8 and 9 a.m. (on average, there were 0.26 visits per plant in two days). The second most common pollinators were small dipterous flies, which were most active between 1 p.m. and 2 p.m. (0.21 visits per plant). Bees were also important pollinators, most active between 3 p.m. and 4 p.m. (on average, they made 0.36 visits per plant).

Table 8: Pollinator visits in the natural population in Žice by Slovenske Konjice

Time	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17
					Bees					
Mena	0	0	0	0	0.02	0.21	0.07	0.02	0.36	0
SD	0	0	0	0	0.15	0.52	0.26	0.15	0.58	0
Max.	0	0	0	0	1	2	1	1	2	0
Sum	0	0	0	0	1	9	3	1	15	0
					Bumblebees/wasps					
Mena	0	0	0.02	0	0.02	0.09	0.05	0.02	0.02	
SD	0	0	0.15	0	0.15	0.3	0.21	0.15	0.15	
Max.	0	0	1	0	1	1	1	1	1	
Sum	0	0	1	0	1	4	2	1	1	
					Large dipterous insects (flies)					
Mena	0	0.26	0.12	0.12	0.05	0.12	0.05	0.1	0.12	
SD	0	1.4	0,33	0.5	0.21	0.4	0.21	0.3	0.33	
Max.	0	9	1	3	1	2	1	1	1	
Sum	0	11	5	5	2	5	2	4	5	
					Small flies					
Mena	0	0.02	0.09	0.07	0.14	0.05	0.21	0.21	0.12	0.05
SD	0	0.15	0.3	0.26	0.35	0.31	0.31	0.41	0.33	0.21
Max.	0	1	1	1	1	2	2	1	1	1
Sum	0	1	4	3	6	2	2	9	5	2
					Other					
Mena	0	0	0.02	0	0.02	0.02	0	0.05	0.05	
SD	0	0	0.15	0	0.15	0.15	0	0.21	0.21	
Max.	0	0	1	0	1	1	0	1	1	
Sum	0	0	1	0	1	1	0	2	2	

During the observation, we recorded a different number of visits to the observed flowers (Fig. 24). The maximum number of visits per flower was 9. Five flowers were not visited by pollinators during the period of observation.

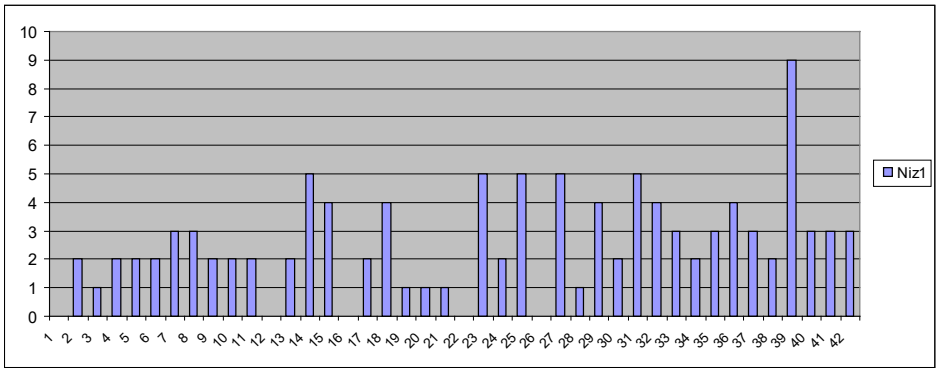

Figure 24: Number of pollinators by individual observed flower between 7 and 8 March 2009

Based on the analysis of pollinators, it was possible to determine the movement or activity of pollinators as a function of temperature (Table 9; Fig. 25). Bees were most active as pollinators when the air temperature rose above 7 °C. Bees were also active when the air temperature rose above 10 °C.

On the first day of the study, a very small number of pollinators were observed in predominantly cloudy weather and relatively low temperatures. For example, we observed no bees, butterflies, mosquitoes or other insects. There were only a few flies, bumblebees and wasps. Insects from the group of small flies were the most active.

On the second day of the study, in clear weather and slightly higher temperatures, we observed higher pollinator activity. The frequency of all five groups of insects increased, especially when the daily temperature was highest. Interestingly, the most common pollinators were large dipterous insects (flies) and small flies.

Table 9: Influence of temperature on the visitation of studied pollinators

Temp. range[1]	Bees	LDI[2]	Bumblebees and wasps	Small flies	Other[3]
3 to 5 °C	0	3	0	1	0
5 to 7 °C	0	5	2	7	1
7 to 10 °C	15	12	4	5	2
over 10 °C	14	11	4	19	4

[1] Temp. range – temperature range; [2] VDI – large dipterous insects; [3] Other – various species of wasps, ants, beetles and *Thysanoptera*

Large dipterous insects were present at all times, regardless of temperature conditions. With increasing temperature, the frequency of this group of pollinators also increased. When the temperature ranged between 3 and 5 °C, we observed 3 pollinators. Most pollinators were observed when the daily temperature was between 7 and 10 °C. During the warmest part of the day, when the temperature was above 10 °C, we observed 11 large dipterous insects.

The activity of bumblebees and wasps also depended on the daily temperature. Before noon, when the temperature was between 3 and 5 °C, we did not observe any pollinators from this group. When the temperature rose and ranged between 5 and 7 °C, we observed 2 pollinators; when the temperature ranged between 7 and 10 °C, we observed 4 pollinators. We also observed 4 pollinators when the temperature rose above 10 °C.

Insects of the group of minor pollinators were most common pollinators. In the early morning hours, when the temperature ranged between 3 and 5 °C, we observed one pollinator of this group; when the temperature ranged between 5 and 7 °C, we observed 7 pollinators, and when the temperature ranged between 7 and 10 °C, we observed 5 pollinators. Most pollinators, i.e. 19, were observed when the temperature rose above 10 °C.

The fifth group of pollinators consisted of other insects not specifically defined – we called this group 'other pollinators'. We observed that when the temperature ranged between 3 and 5 °C, no pollinator was present; at temperatures between 5 and 7 °C, we observed only one pollinator, and at temperatures between 7 and 10 °C, we observed 2 pollinators of this group of insects. When the temperature rose above 10 °C, we observed 4 pollinators.

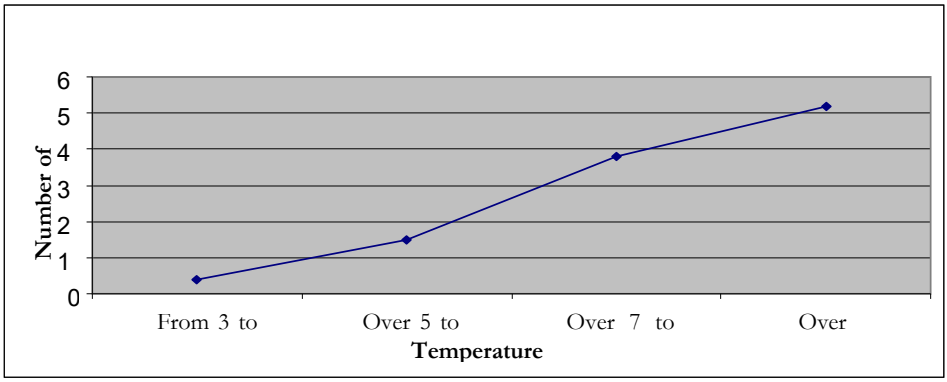

Figure 25: Number of pollinators by temperature

Analysis of pollinator activity in the population of Bohinjska Bela and Peca Mountains

The analyses of insect activity showed that there were obvious differences in the frequency of visits amongst the insect groups investigated, their locations, and times of day (Table 10, Fig. 26).

Bees were the most important pollinators within the population of Bohinjska Bela. Most of these probably came from apiaries in nearby villages. The highest activity occurred between 10 a.m. and 11 a.m. (on average 2.067 visits per plant over two days of observation). The second more common visitors in this population were small dipterous flies belonging to the families *Drosophilidae* and *Sciaridae*, which were most active between 12 noon and 1 p.m., and between 2 p.m. and 3 p.m. (on average 0.333 visits per plant). The visits of the remaining groups of pollinators, such as bumblebees, wasps, ants, beetles, and *Thysanoptera*, were insignificant (less than 2.3% of visits).

In the isolated populations on the Peca Mountains, the most frequent visitors were small dipterous flies, with the highest activity between 10 a.m. and 1 p.m. and between 4 p.m. and 5 p.m. (on average 0.033 visits per plant). Similar frequencies of visits were also found for larger species of flies belonging to the family *Syrphidae* (these were most active between 8 a.m. and 10 a.m., and between 11 a.m. and 1 p.m.), bees (these were most active between 9 a.m. and 10 a.m.) and the remaining

group, including wasps, ants, beetles and *Thysanoptera,* (these were most active between 12 noon and 1 p.m.).

Table 10: Visits to flowers by insect pollinators of two naturally occurring populations in Bohinjska Bela and the Peca Mountains (20–22 and 29–30 March 2003)

Hours	6	7	8	9	10	11	12	13	14	15	16	17	18
Bohinjska Bela: bees													
Mean	0	0.1	0.117	1.267	2.067	1.667	0.75	0.4	0.516	0.033	0.033	0.067	0
SD	0	0.399	0.372	1.696	1.998	1.874	0.950	0.668	0.873	0.181	0.181	0.311	0
Max.	0	2	2	6	8	9	4	3	4	1	1	2	0
Sum	0	6	7	76	124	100	45	24	31	2	2	4	0
Peca: bees													
Mean	0	0	0	0.033	0.033	0	0.016	0	0	0.016	0	0.016	0
SD	0	0	0	0.181	0.181	0	0.129	0	0	0.129	0	0.129	0
Max.	0	0	0	1	1	0	1	0	0	1	0	1	0
Sum	0	0	0	2	2	0	1	0	0	1	0	1	0
Bohinjska Bela: bumblebees													
Mean	0	0	0	0	0.05	0.017	0.017	0.05	0.016	0	0	0	0
SD	0	0	0	0	0.219	0.129	0.129	0.387	0.129	0	0	0	0
Max.	0	0	0	0	1	1	1	3	1	0	0	0	0
Sum	0	0	0	0	3	1	1	3	1	0	0	0	0
Peca: bumblebees													
None													
Bohinjska Bela: large flies (belonging to the families *Muscidae* and *Syrphidae*)													
Mean	0	0	0.067	0.267	0.133	0.217	0.05	0.016	0.083	0	0.05	0	0
SD	0	0	0.311	0.733	0.430	0.783	0.219	0.129	0.334	0	0.219	0	0
Max.	0	0	2	4	2	4	1	1	2	0	1	0	0
Sum	0	0	4	16	8	13	3	1	5	0	3	0	0
Peca: large flies (belonging to the family *Syrphidae*)													
Mean	0	0	0.033	0.033	0.05	0.033	0.033	0	0	0	0	0	0
SD	0	0	0.181	0.181	0.219	0.181	0.181	0	0	0	0	0	0
Max.	0	0	1	1	1	1	1	0	0	0	0	0	0
Sum	0	0	2	2	3	2	2	0	0	0	0	0	0
Bohinjska Bela: small dipterous flies (belonging to the families *Drosophilidae* and *Sciaridae*)													
Mean	0	0.083	0.133	0.183	0.15	0.183	0.333	0.233	0.333	0.067	0.117	0.267	0
SD	0	0.334	0.342	0.390	0.404	0.431	0.705	0.592	0.773	0.311	0.372	0.482	0
Max.	0	2	1	1	2	2	3	3	4	0	2	2	0
Sum	0	5	8	11	9	11	20	14	20	4	7	16	0
Peca: small dipterous flies (belonging to the families *Drosophilidae* and *Sciaridae*)													
Mean	0	0	0.016	0.05	0.033	0.033	0.033	0.016	0.05	0.067	0.033	0	0
SD	0	0	0.129	0.286	0.181	0.181	0.181	0.129	0.286	0.362	0.258	0	0
Max.	0	0	1	2	1	1	1	1	2	2	2	0	0
Sum	0	0	1	3	2	2	2	1	3	4	2	0	0
Bohinjska Bela: other insects (various species of wasps, ants, beetles and *Thysanoptera*)													
Mean	0	0	0	0.05	0.016	0.033	0.05	0.033	0	0.017	0.033	0	0
SD	0	0	0	0.219	0.129	0.181	0.286	0.181	0	0.129	0.181	0	0
Max.	0	0	0	1	1	1	2	1	0	1	1	0	0
Sum	0	0	0	3	1	2	3	2	0	1	2	0	0

Peca: other insects (various species of wasps, ants, beetles and *Thysanoptera*)													
Mean	0	0	0	0.016	0	0	0.033	0	0	0	0	0	0
SD	0	0	0	0.129	0	0	0.181	0	0	0	0	0	0
Max.	0	0	0	1	0	0	1	0	0	0	0	0	0
Sum	0	0	0	1	0	0	2	0	0	0	0	0	0

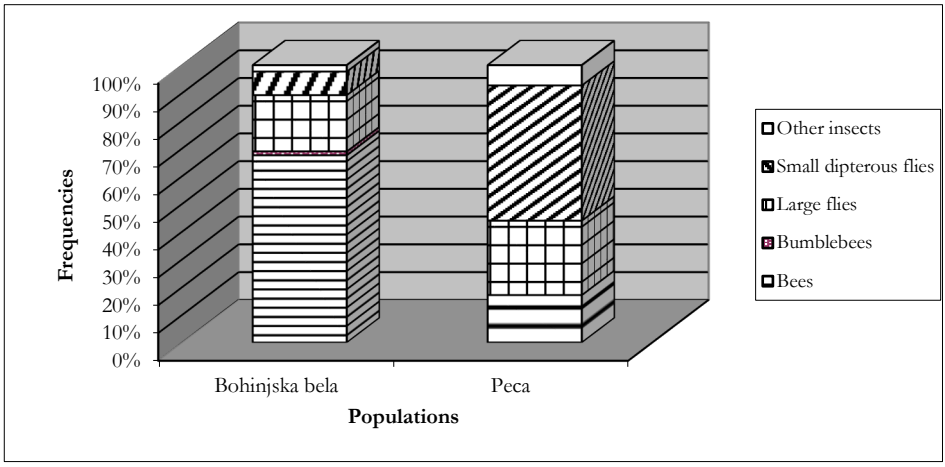

Figure 26: Frequencies of pollinators visiting *Helleborus niger* during two days of observations in Bohinjska Bela and Peca

Conclusions

In literature, the Christmas rose is considered to be a predominantly cross-fertilising species. Flowers are hermaphrodite (having functional male and female sexual organs) and protogynous (Salopek-Sondi et al., 2002). Our observations of the protogynous nature of the species and the visits of pollinating insects suggest that it could be an entomophilous and a predominantly cross-pollinating species. The main attractants for insects are the shapes and colours of flowers, and the presence of pollen and nectar (Bronstein, 1994; Kearns, 1997). Odour is not generally very strong and, according to our observations, does not appear to be attractive for the main pollinators (bees), although it appears to be attractive for flies. Hazel nuts were much more attractive for bees at that time (*Corylus* spp.), as were various *Primula* species and especially the male individuals of willows (*Salix* spp.). Here, it is important to mention that Christmas rose flowers produce relatively large quantities of light and dry pollen that can easily be dispersed by wind.

A relatively low number of insects visited the Christmas rose flowers on the Peca Mountains (some of the insect visitors were probably not pollinators), and numerous well-developed seeds suggested that the wind could also have been one of the pollinating agents. The indicators of predominant entomophily were as follows: large flowers (the largest flowers having a diameter of up to 13 cm); stigmas generally above anthers (a mechanism that helps prevent self-pollination); presence of odour during flowering; and protected sexual organs (they were not exposed as in typical anemophilous plants, e.g. in hazel nuts, European walnuts, and maize). Flowering Christmas rose plants are usually interspersed amid other plants, such as small trees, shrubs, dry ferns, and grasses, which are usually taller than Christmas roses, and in this way reduce wind velocity, and consequently the efficiency of wind pollination.

According to our observations, the Christmas rose appears to be a predominantly entomophilous and a cross-pollinating species. The most important pollinators are insects such as bees and flies. Their activity depends on several factors such as insect species, location, and time of day. They prefer the natural colour of flowers (white). The most attractive artificially coloured flowers were blue and red. Some of the visiting insects were probably not pollinating agents. Relatively low frequencies of insect visitors on the Peca Mountains and the presence of numerous well-developed seeds suggest that wind could also be one of the pollinating agents. Self-pollination is probably rare, due to the protogynous nature of the flowers and specific floral structures.

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5 Economic importance of *Helleborus niger*

Recent studies have indicated that the Christmas rose is becoming one of the favourite perennial garden selections that can be grown as a cut flower plant or a flowering potted plant. Owing to its lower flowering temperature requirements, in comparison with traditionally grown ornamentals, this crop has the potential for greenhouse production. The Christmas rose can also be cultivated as cut flower or potted plant, and can be forced to flower under greenhouse conditions around Christmas or New Year.

5.1 Uses

Ornamental plant

The Christmas rose is known for its early flowering, and it may be an attractive winter perennial. Its ability to bloom during the 'darkest' months of the year, when everything else is frozen, makes it highly valuable. It can be grown as a garden plant (Fig 27). In most cases, it is grown in winter and spring gardens. It is also ideal for woodland gardens or for cultivation on patches of ground in front of houses. Christmas roses can also be grown as potted plants (Fig. 28). They can be used as specimens in individual containers, or they can be used as part of winter and spring

mixed planting. By forcing, we can obtain early flowering. As with flowering potted plants, they can be sold a week before All Saints' Day (November 1) and more often during the Christmas season. Finally, the Christmas rose can be grown as a cut flower plant (Fig. 29). Its production is organised in such a way as to supply the market before the Christmas season in order to take advantage of a significantly higher price.

Figure 27: Christmas rose as an attractive winter perennial
(Photo: A. Šušek)

Figure 28: Christmas rose as a potted plant maintains its ornamental value even after flowering, when floral leaves turn green
(Photo: A. Šušek)

Figure 29: Christmas roses as cut flowers

Medicinal applications

The name hellebore has been associated with plants of medicinal interest for at least 2200 years and probably much longer, as Theophrastus (Greek philosopher, 372–287 BC) used the name as if it were already well-established in Greek history (Mathew, 1989). Very early records of the use of the plant for medicinal purposes are a matter of controversy, due to the misnaming of plant species as a result of false botanical identification. The name *Helleborus* has been used in some cases to describe other plants (Woodville 1810). However, it is enshrouded in fable and legend of great antiquity. According to Greek tradition, shepherd Melampus first realised its properties by observing its effect on his goats; later he is said to have used it successfully to cure the daughters of Proteus, King of Argus, of mental derangement, by dosing them with the milk of goats that had eaten the plant (Wood and Bache, 1839; Encyclopaedia Britannica, 1910). The plant was formerly known as Melampode after Melamphu. Pliny the Elder (23–79 AD), a natural scientist from

antiquity, wrote that physicians from 1400 BC used it to treat nervous disorders and hysteria (Chevallier, 1996). Hellebore was a respected medicinal plant with miraculous curative properties, especially for mental disorders. External treatment of lice is also amongst the ancient uses. It also shares a reputation as a classic poison with hemlock, nightshade, and aconite.

Extracts of the *Helleborus* species were used as phytopreparations with immune-stimulatory properties in Roman traditional medicine (2nd century BC to 4th century AD). During the Byzantine period (4th to 15th century AD), and especially between the 6th and 11th century AD, medicinal tradition improved with original medical thinking. Physicians of the times were aware of the therapeutic, pain-relieving or sedative qualities of many herbs and also of *H. niger* (Ramoutsaki et al., 2002). In the 18th and 19th century, *H. niger* was mentioned as a diuretic, emmenagogue and cathartic, a melanagogue recommended for female obstructions, hysteric and hypochondriac fits, melancholy, madness, epilepsy, leprosy, and inveterate quartans in the 18th century. It was also documented that its use can lead to inflammations of mucous membranes (gastric or intestinal), skin inflammation, and even vesication (Wood and Bache, 1839, Baláz et al., 2020). In Germany, *H. niger* is used in homeopathy and as adjuvant therapy in the treatment of tumour patients in anthroposophical medicine (Bussing and Schweizer, 1998). In state Pharmacopoeas of the Habsburg Empire and Austro-Hungarian Monarchy, the root of *H. niger* is presented in the list of simple drugs (Baláz et al., 2020). In folk veterinary medicine in Italy, *H. niger* was used for curing kidney disorders and also as an anaesthetic (Viegi et al., 2003). It has also been used in the treatment of dropsy, amenorrhoea, nervous disorders, and hysteria, and the root is also applied externally as a local irritant (Felter and Lloyd, 1898; Grieve, 1971). The drug in small doses increases the force of the heart's contraction, slows the pulse and increases arterial tension (Felter and Lloyd, 1898; Launert, 1981; Lust, 2001).

Chemical compounds found in *H. niger,* in addition to the cardiac glycosides helleborin, hellebrin, and helleborein, are saponosides and the ranunculoside derivative, protoanemonin. For medicinal applications, the only source is the rhizome. The root is an anthelmintic, a cardiac, cathartic, diuretic, emetic, emmenagogue, irritant, in addition to being violently narcotic and a drastic purgative (Grieve, 1971; Launert, 1981; Lust, 2001).

Hellebrigenine (the aglycone of hellebrine) has been used as a cardiotonic to complement digitalin and strophanthin. The rhizome is used for treating some skin ulcers. In addition, earlier uses of hellebore include its use as a purgative, local anaesthetic and abortifacient. These applications have been abandoned, except in veterinary treatment, where a decoction is still used to treat mange (Chevallier, 1996).

Homeopathic medicine uses a tincture (i.e. alcoholic extract; the alcohol inhibits enzymes thereby preventing the breakdown of, for example, protoanemonin) prepared from the rhizome to treat eclampsia, epilepsy and certain psychoses, as well as meningitis, encephalitis, headaches, psychic disorders, enteritis, and spasms (Launert, 1981).

Hellebore poisoning is rare. The high amounts of ranunculin or protoanemonin in the leaves, stems and flowers are responsible for skin disorders (dermatitis, following exposure to bruised root material, leaves, stems and flowers), eye (powdered root), and gastrointestinal irritations (following ingestion).

5.2 Production

The demand for Christmas rose has been increasing over recent years. The plants grown in pots can be sold throughout the year. From early spring until winter, the plants can be sold as perennials for planting outside. During the autumn–winter period, flowering plants grown in pots are the most desirable. The highest demand is generally before Christmas. As cut flowers, Christmas roses can be sold from November to April. In most instances, the highest price can be achieved in December. Its production represents a niche for producers, because the supply of quality plants on the market is still insufficient, as evidenced by numerous examples.

The Christmas rose is a well-known ornamental plant; however, it is relatively new in intensive production (Figs. 30 and 31). The main problems in intensive production are: low multiplication rate, insufficient information regarding genetic resources, poor information regarding ecology, the effects of fertilisers on plant development, and the spread of diseases (especially in monocultures).

Figure 30: Production of Christmas roses as potted plants (Photo: A. Šušek)

Figure 31: Christmas rose growing in an open greenhouse for cut flower production
(Photo: A. Šušek)

Cultivation of the Christmas rose in the future

In the future, the Christmas rose will probably become a much more important ornamental species than it is now. However, it is difficult to predict what will happen regarding its cultivation. Its popularity will depend on several factors, such as the availability of other competitive species on the market, economical cultivation, especially the expenses associated with reproduction, general prosperity of the economy, marketing approaches, and the availability of highly attractive cultivars. Multiplication of such cultivars will probably remain as one of the key factors. The multiplication coefficient based on rhizome cuttings is relatively low, and it takes a long time for newly developed cultivars to be multiplied to such a degree that they can be sold on the market. Market preferences could change during this necessary period. Efficient multiplication will therefore be essential for a successful cultivation and marketing.

Christmas roses are often grown from seedlings or rhizome cuttings cultivated within disinfected soil or inert substrata, essentially to lower the risk of contamination by pathogenic agents and to ensure controlled conditions in order to obtain a homogenous material. If it takes place under sterile conditions, it eliminates or drastically reduces the populations of most organisms that are normally present within the soil, including useful ones, such as plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi. Soil microorganisms, especially in the rhizosphere, are involved in most, if not all, plant–soil exchanges. It is likely that complex associations of species play a major role in the stability of a natural ecosystem (Atlas and Bartha, 1998). Soil microorganisms are of ecological importance, determining plant biodiversity, ecosystem variability, and productivity (Vessey, 2003). Inoculation with a single microorganism species can have positive effects on the growth of *Helleborus* plants, especially on root growth. The effects on plant growth generally depend on the type of microorganism and the microbial mixture introduced into the substrate (Dunabeitia et al., 2004**)**.

The commercial production of Christmas roses based on propagation from seedlings is probably the more efficient and the cheapest method of reproduction. However, it has been found useless for registered varieties, since the seedlings are always phenotypically variable and unreliably display varietal characteristic such as flower colour, flower size, time of flowering or crucial leaf characteristics. In vivo

vegetative propagation (via rhizome cuttings) has, for many years, played an important role in the commercial cultivation of the Christmas rose. Apart from commercial cultivation, it is also widely used in genetic breeding: for the multiplication of parental lines in order to ensure seed production. The classical method of in vivo vegetative propagation (cloning) is often too slow, too sophisticated and/or too expensive.

In the future, micropropagation will probably become a much more important technique employed in large-scale production. It enables relatively fast multiplication, and the resulting plant materials are disease free. Since the discovery that plants can be more rapidly cloned '*in vitro*' than '*in vivo*', knowledge concerning '*in vitro*' vegetative propagation has grown rapidly. However, '*in vitro'* cultivation of *Helleborus* is still considered to be difficult. The main problems are the high degree of pathogenic contamination when rhizome buds are used for initiating an aseptic culture, a high variation of offspring individuals if seedlings were used as initial explants, and a low multiplication rate of culture from meristem tips.

Christmas rose cultivars in the future

Several companies in the European Union are engaged in breeding hellebores (e.g. Heuger Gartenbaubetriebe (Glandorf, Germany), FBA Plants B.V. (the Netherlands), Nachtvlinder B.V. (the Netherlands), Het Wilgenbroek BVBA (Belgium), BVBA Helleborus (Belgium)). One of the leading companies in breeding of hellebores is Heuger Gartenbaubetriebe (Glandorf, Germany). Their Helleborus Gold Collection® is known throughout the world. On over 35 hectares (5.5-hectare greenhouse) they grow young plants, pre-finished plants and finished flowering plants for nurseries, wholesalers and for auctions, selling them all across Europe, Asia, and North America.

In the future, Christmas rose cultivars will have to satisfy the demands of the existing markets, which are highly selective, and producers will have to reduce the pollution within the environment (i.e. the use of pesticides will have to be drastically reduced). At the same time, energy for heating greenhouses will probably become very expensive.

Cultivars will have to be crucially resistant to all crucial pests and diseases, well adapted to a variety of environments (changes of humidity and temperature), whilst at the same time retaining all the crucial attractive characteristics.

The cultivars used as potted plants will have to be compact, with shorter leaf petioles, smaller blades and shorter stems, and with flowers just above the leaf blades. For cut flower cultivation, cultivars should be characterised by very long stems, several inflorescences and large flowers. The cultivars used as garden perennials should be adapted to a higher level of insolation, and the plants should be strong and vigorous, with many leaves and greater inflorescences. The flowering should last as long as possible.

Cultivars that can be classified strictly within these specific groups have yet to be created. The existing cultivars generally include characteristics from all three groups, and they can be grown as potted plants, for cut flower growth production and as garden perennials. In order to obtain cultivars with the specific characteristics of the above mentioned groups, it will be necessary to evaluate the existing germplasms and apply an adequate genetic breeding technique. Some of the traits, especially quantitated with lower heritability, could be reasonably modified by agronomic practices such as exposure to light or shade, increased fertilisation with nitrogen, keeping soil moisture at high or low levels, increasing temperature in greenhouses and the use of gibberellins.

Available genetic resources for breeding

The Christmas rose is distributed all over central Europe including northern Italy, the eastern parts of France, Switzerland, southern Germany, Austria, western Hungary, Croatia, Bosnia and Hercegovina, and Slovenia. Large germplasm collections do not exist. In several countries (e.g. United Kingdom, the Netherlands, Germany, Belgium, USA, Japan, Australia, New Zealand), there are small collections that are used as genetic sources for direct selection in order to obtain materials suitable for commercial purposes or for genetic breeding.

Slovenia is one of the centres of diversity in terms of Christmas rose. Its genetic diversity is considered to be relatively high, which has been demonstrated by morphological and partly by molecular analyses (Šušek et al., 2005; Šušek et al., 2007). In order to get more information about the existing variation in Slovenia and other countries, it will be necessary to establish systematic national and international germplasm collections, and to describe accessions according to an appropriate descriptor list. Characterisation of genetic materials has to involve highly inheritable morphological traits that are important for the selection and/or are easy for any determination. Šušek (2008) described 72 morphological traits that appear to be at least partly genetically controlled and could be used as descriptors of Christmas rose genotypes. The more important traits are listed in Table 11.

In current genetic breeding, the most urgently required resources are those for the following traits: growth vigour, dwarfism, long stems (tall plants), inflorescences with numerous flowers, large flowers, attractive floral colours, variegated floral colours, double flowers, slow and long-lasting flowering of individual flowers, continuous flowering over long periods, early flowering (in November), late flowering (from April to June), adaptability to extreme environments (dry, wet, shaded, exposed to full solar radiation), adaptability to greenhouse conditions, room conditions (when used as a potted plant), suitability for long-distance transport, tolerance of storage for long periods, resistance to all crucial diseases (black spot, false mildew, black root on rhizome, stem, roots and crowns, crown rot, fusarium wilt, bremia; sclerotinia). At present, resistance to pests and diseases is not a priority because most cultivation is under controlled conditions where pesticides are regularly used. However, in order to reduce pollution of the environment, genetic resistance will probably become much more important and will also reduce production costs.

Table 11: The most important taxonomic and morphological traits that can be used as descriptors of *Helleborus* niger genotypes (based on Šušek, 2008; Šušek et al., 2007)

Trait	Variation range
General characteristic	
Growth vigour:	poor; intermediate; high; very high; other
Growth habit:	erect; semi-erect; semi-prostrate; prostrate (spreading); other
Leaves	
Number of leaves/plant:	< 5; 5–10; 11–15; 16–20; > 20
Predominant lamina orientation:	vertical; semi-vertical; semi-horizontal; horizontal; horizontal with drooping tip; horizontal with tip pointing upwards; other
Lamina waxiness:	non-glossy (non-shining); glossy (shining); only young leaves glossy; only mature leaves glossy; other
Lamina colour:	yellow; pale green; green; dark green; light purple; dark purple, chimeras, variegations; other
Average number of leaflets:	< 5; 5–10; > 10
Shape of terminal leaflet:	elliptic; ovate; obovate; oblong-cuneate; oblanceolate; lanceolate
Terminal leaflet tip:	acute; obtuse; other
Terminal leaflet base:	attenuate; acute; cuneate; oblique; other
Terminal leaflet margin:	entire; crenate; sinuate; dentate; denticulate; serrate; serrulate; other
Leaves	
Distribution of teeth of terminal leaflet:	no teeth; upper third; upper third; on whole margin; other
Maximal width of terminal leaflet (cm)	
Terminal leaflet length (cm)	
Terminal leaflet width-to-length ratio	
Pigmentation of main leaflet veins (colour chart)	
Terminal leaflet stalk length (cm)	
Petiole	
Petiole junction colour (colour chart)	
Petiole basic colour (colour chart):	colour of basal third; colour of middle third; colour of top third;
Petiole length (cm)	
Peduncle[1]	
Peduncle orientation:	erect; semi-erect; curved
Peduncle length[2]	
Number of bracts:	0–1; 2–3; 4–5; > 5
Shape of bracts:	entire; divided
Peduncle orientation:	erect; non-erect; curved; other
Peduncle basic colour (colour chart):	colour of top third; colour of middle third; colour of basal third

Trait	Variation range
Inflorescence	
Number of inflorescences per plant:	< 5; 6–10; 11–20; 21–30; 31–40; 41–50; > 50
Average number of flowers per inflorescence:	solitary flower; 2; 3; 4; > 4
Flower	
Odour intensity:	absent; very weak; weak; moderate, strong
Odour type:	pleasant; not pleasant; other
Flower shape during anthesis:	flat; flattish; bell-shaped; narrow bell-shaped; other
Position of floral axis:	erect; semi-erect, horizontal; drooping
Flower diameter (cm):	< 4; 4–6; 6–8; 8–10; 10–12; > 12
Number of sepals:	< 5; 5; 6; > 6
Sepal shape:	elliptic; lanceolate; oblanceolate; oblong; obovate; ovate; oval; rhomboid
Sepal margins:	entire; crenate; sinuate; dentate; denticulate; serrate; serrulate;
Colour of sepals (adaxial side) (colour chart)	
Colour of sepals (abaxial side) (colour chart)	
Colour of sepal margins (colour chart)	
Position of sepals:	separated; slightly fused at the base; overlapping; other
Number of stamens:	1–30; 31–60; 61–90; 91–120; > 120
Position of anthers:	forming a circle around female portion; irregularly distributed; other
Anther colour (during anthesis):	light yellow (normal); dark yellow; pink; red; purple; blue or blue-purple; other
Number of carpels:	< 3; 3–6; 7–10; > 10
Colour of carpels at the beginning of anthesis:	light yellow (indicating sterility); pale green (indicating poor vigour); green; purple or purple-red; not uniform; variegated; other
Style length:	short (< 1/10 of the pistil height); intermediate (1/10–1/5 of the pistil height); elongated (> 1/5 of the pistil height)
Stigma pigmentation:	light red; red or purple-red; blue or blue-purple; stigmas on the same flower differ in colour; other
Petal shape:	flat; flat with curved margin; tubular; funnel; other
Number of petals:	1–10; 11–15; 16–20; > 20
Petal colour:	yellow; yellow-green; green; green-purple; purple; variegated; other

Trait	Variation range
Fruit	
Fruit formation:	absent; present; rarely present
Mature fruit colour:	yellowish green; green; dark green; green with red patches; red; purple; other
Number of fruits/plant	
Number of seeds/fruit:	< 5; 5–10; 11–20; > 20
Seed	
Seed colour:	light (almost white); light brown; darker brown; darker red or purple; black; colour variations within seeds from the same fruit head; other
Dry seed shape:	elongated; elliptic; oval; conical; irregular; other

[1] mature peduncle (during flowering)
[2] the distance from soil surface to the base of the flower

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6 Production technology

6.1 Propagation

The Christmas rose can be propagated from seed in relatively large numbers (40–60 seeds per flower), but the result will always provide a certain amount of variation. Classic vegetative propagation based on rhizome cuttings is relatively slow and time-consuming, but it ensures that all offspring individuals are genetically identical. On the other hand, '*in vitro*' culture shows great potential within vegetative propagation.

Seed propagation

Seed germination of the Christmas rose is very slow because of dormancy. Mayer and Poljakoff-Mayber (1975) indicated that in some *Ranunculus* species immature embryos are the main cause of dormancy. They stated that seeds having immature embryos must complete their development before germination can begin, and that the period required for such embryos to reach maturity varied from a few days to several months. Atwater (1980) stated that seed structure is remarkably similar within plant families and their close relatives. She listed a member of the *Ranunculaceae* species as having basal rudimentary embryos. This type of dormancy was also confirmed by Lockhart (1982). Bullowa et al. (1975) found that seed germination in *Ranunculaceae* is commonly delayed, since their dry dispersal units contain immature

embryos. In many species this delay can be enhanced by the presence of inhibitors in the endosperm that need to be leached or neutralised.

The germination rate can be improved by storing seeds in warm conditions (which are needed for the completion of embryo development) before treatments leading to germination are started. Seeds should be collected when they are very close to maturity. After exposure to temperatures of approximately 20 °C for 10–12 weeks, seeds must receive a cool treatment at 5 °C for 10–12 weeks (Maurer and Dickel, 1979; Horn, 1996; Seidler, 1999). After the cold period, seeds are exposed again to higher temperatures of 18–20 °C. During this 'warm–cold' treatment and during germination, we have to keep a sufficiently high level of moisture (seeds should not dry out). Germination starts approximately 20 weeks after sowing (Fig. 32). For sowing, we can use conventional seed flats with a depth of 6–7.5 cm. The sowing medium is usually the standard sowing medium (1 part – sieved *sphagnum* peat moss; 1 part – perlite; 1 part – sterilised loam). The germination is epigeal, which means that cotyledons emerge and are photosynthetic (Seidler, 1999) (Fig. 33). Hellebore germination is a lengthy process, and seeds do not germinate at the same time. As there is not much space in the flats, young seedlings must be transplanted as soon as they are large enough to be handled. In most cases, this is at the moment when the first leaves have started to develop between the cotyledons. It seems that the small plants suffer much less from disturbance at this stage than they do later on – probably because the root is not yet long, branched, and vulnerable (Ahlburg, 1989). During commercial production, seedlings are usually transplanted in 70-cell trays or in 6-cm pots. They have to be watered regularly, and a soluble fertiliser containing 20:20:20 ($N:P_2O_5:K_2O$) must be added once every two weeks. The seedlings are ready for forcing after 3–4 years.

Figure 32: Christmas rose seeds after treatment leading to germination
(Photo: A. Šušek)

Figure 33: Christmas rose seedlings
(Photo: A. Šušek)

Vegetative reproduction

The commercial production of Christmas roses based on propagation by seed is probably the most efficient and cheapest method of reproduction. However, it has been found useless for named varieties, since seedlings are always phenotypically variable and do not reliably display flower colour, flower size, time of flowering or the leaf characteristics of the parental plants.

Stem and leaf cuttings do not generally root well (Seidler, 1999). The most suitable method for the maintenance of a variety is the division of rhizomes. An established Christmas rose plant develops a congested rhizome with a mass of fibrous roots and often has many growing buds. In pachymorph rhizomes, individual crowns or culms (crown designates that are part of a plant at the surface of the ground from which new shoots are produced) are cut off at the point of attachment to the rhizome, and the pieces are transplanted to a new location. Each set (detached unit) that will be able to regenerate should have at least one growing point with attached roots, at least one healthy and fully developed leaf on the top of the rhizome, and one or two 0.5–2 mm white buds on the base of the rhizome (Fig. 34) (Lemper, 1985). The division is usually carried out at the beginning of the growth period (in March) or near the end of the growing period (in the middle of August).

In spring (March), it is very difficult to divide source plants into parts without introducing damage. For this reason, it is advisable to choose parts with well-developed, large rhizomatic buds that represent a group of buds. The consequence is a lower number of plants; however, the plants are bigger. Success is heavily dependent on the health and vigour of the source plants because the division parts do not form a new root system until mid-June. When dividing plants, it is important that we do not damage leaves and roots. The development of new leaves and rhizomes depends on assimilates stored in the reduced root system. In order to ensure fast recovery of division parts, it is advisable to place the rhizome crown 2–3 cm deep in the substrate. If the conditions are optimal, the new leaves begin to develop, and the old part of the rhizome starts to decay.

When multiplication is carried out in summer, from mid-July to the beginning of August (during the period of active root growth), it is possible to split a clump down to individual crowns from a plant that has been growing for 12 months, resulting in as many as 5 to 7 new plants, which will flower in the same year (Lemper, 1985). The new divisions have the opportunity to become established before the winter and well before any dry weather in the following summer (Table 12). If the division is carried out in spring, the young plants need careful attention during dry weather in summer. The division parts can either be planted out in open ground and left to become re-established, or they can be potted and kept in a cold frame for a while until they are well rooted and thus ready to be planted out.

'*In vivo*' vegetative propagation (via rhizome cutting) has for many years played an important role in commercial production of the Christmas rose. Apart from commercial production, it is also widely used in genetic breeding: for the multiplication of parental lines in order to ensure seed production (i.e. seed of parental lines and/or hybrid seed), for breeding purposes (to multiply hybrid progeny in order to evaluate their commercial and/or breeding value and to multiply the materials treated with mutagenic substances in order to detect useful mutants), and to maintain accessions in gene banks. This type of vegetative propagation is also very important during genetic breeding: the parental lines have to be maintained and propagated vegetatively to ensure seed production. The technique is also required for setting up gene banks. It is also useful during mutation breeding, in order to multiply the material treated with mutagenic substances.

The classical method of '*in vivo*' vegetative propagation (cloning) is often too slow, too difficult and/or too expensive. According to Rupprecht and Meissner (1985), cloning can produce about 1000 plants from one single parent plant within a period of 10–20 years. In the last 15 years, since the discovery that plants can be more rapidly cloned '*in vitro*' than '*in vivo*', knowledge concerning '*in vitro*' vegetative propagation has grown rapidly. However, '*in vitro*' cultivation of *Helleborus niger* (Fig. 35) is still considered to be difficult (Lim and Kitto, 1995; Seyring, 2002; Poupet et al., 2006; Dhooghe and Van Labeke, 2007; Beruto and Curir, 2009; Gabryszewska, 2017). Springer (1995) reported a successful attempt at '*in vitro*' propagation without giving detailed information about either the *Helleborus* species propagated or the cultivation method used. Lim and Kitto (1995) presented a method used for *H. orientalis*. The propagation of shoots was carried out '*in vitro*', and the rooting was done '*ex vitro*'. Seyring (2002) reported that he successfully propagated *H. niger* by means of '*in vitro*' cloning. Recently, Gabryszewska (2017) presented that the efficacy of hellebore micropropagation (initiation and stabilisation of culture, multiplication and rooting '*in vitro*' and acclimatisation '*ex vitro*') has been influenced by several factors, such as: type of initial explants, genotype, growth regulators, and environmental factors (temperature, sucrose, nitrogen salts, phosphorus).

Table 12: Timetable of activities associated with the conventional vegetative propagation of Christmas rose, and the developmental phases of the plants

Period	Activity and characteristics of plant development
End of July	Split of rhizomes into 5–7 units, planting in individual pots
August to end of October	Establishment of the root system and secondary growth of leaves
November	Preparation of plants for the generative phase (forcing)
Mid-December to end of January	Flowering, pollination and fertilisation
February	Seed formation
March to April	Leaf development, rhizome enlargement
May to end of October	Root growth and development of leaves

Figure 34: Vegetatively propagated plant obtained by dividing a rhizome
(Photo: A. Šušek)

Figure 35: '*In vitro*' plantlet
(Photo: A. Šušek)

6.2 Biotisation in production technology

Horticulturists generally attempt to control several crucial environmental factors that influence plant growth, such as light, temperature, moisture, and nutrients. Better control can be achieved in greenhouses. Many of the factors associated with soil are often overlooked. In most cases, emphasis is placed on the moisture and nutrients within the medium. Most researchers and growers recognise the importance of good aeration and drainage, nutrient-bearing capacity, and freedom from insects, disease, and weeds, as well as harmful chemicals. However, the latter viewpoint overlooks the role played by non-pathogenic soil microorganisms in the growth and development of plants.

Micropropagation is becoming the most widely used technique in a large-scale production of Christmas rose because classical vegetative production based on rhizome cuttings is time-consuming and cannot always guarantee success (Seyring, 2002; Dhooghe and Van Labeke, 2007).

Plant roots serve a multitude of functions in the plant, including anchorage, provision of nutrients and water, and the production of exudates with growth-regulatory properties (Bertin et al., 2003). Inadequate root development and activity are the more common causes for the failures of young vegetatively propagated plants.

Microbial activity in the plant rhizosphere has substantial effects on plant performance and productivity (Bonkowski et al., 2000; Vessey, 2003). Plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal (AM) fungi are able to colonise plant roots and stimulate plant growth when applied to seed, tubers, or roots.

These microorganisms represent an integral part of many cultivated plants and are also an essential component of soil fertility because of their roles as bio-regulator, bio-fertiliser and bio-control agents (Guillemin, 1994; Gianinazzi et al., 1995, 1996; Smith and Read, 2008). Plant biotisation represents a very promising technology for improving the qualities of plants and ensuring the most appropriate development within the context of sustainable horticulture. Recent studies indicate that improvement in plant growth and health can be achieved by either inoculating selected strains of beneficial microorganisms (separately or in combination) or by applying cultural practices that favour the development of indigenous beneficial rhizosphere microbial populations in soils, and suppressing pathogenic ones (Budi et al., 1999; Avis et al., 2008; Ronco et al., 2008).

Effect of rhizosphere bacteria and endomycorrhizal fungi on the development of Christmas rose

Microbial activity has been found to increase root branching, areas, and biomasses of several plants such as pineapple (Guillemin et al., 1992), rhododendron (Lemoine et al., 1992), *Pinus radiata* (Dunabeitia et al., 2004), pea (Hynes et al., 2008), and maize (Hameeda et al., 2008). Experiments on raspberries showed substantial influence on root growth and antagonistic effects against soil pathogens by *Agrobacterium radiobacter* (strain K1026) (Lemoine et al., 2000). The possibility of introducing beneficial microorganisms (biotisation) during the acclimatisation period of *Helleborus niger* plants propagated through rhizome cuttings and material obtained by '*in vitro*' multiplication was studied. Microbial inoculation is intended to be an

efficient and a relatively simple procedure, which should be incorporated into nursery practice.

Plant material in the first experiment was propagated from mother plants that had been growing in a greenhouse for two years. New plants were obtained by vegetative propagation based on rhizome cuttings. These cuttings were transplanted into one-litre plastic pots filled with sterile substrate (peat, perlite and compost mix, (60:20:20, v:v:v)) and inoculated or not with beneficial rhizosphere microorganisms. Young developing plants were transferred directly under greenhouse conditions (18–25 °C day-night, 70% relative humidity and natural photoperiod). During spring and summer, the plants were watered daily with distilled water mixed with fertilisers (a commercial solution Plant-Prod® 20:20:20 N:P:K), whereas during autumn and winter, watering took place once or twice a week, depending on needs.

In the second experiment, the '*in vitro*' propagated plantlets were transplanted from the agar medium into 200-ml plastic pots filled with a sterile substrate (peat, perlite and compost mix, (60:20:20, v:v:v)), inoculated or not with beneficial microorganisms. The transplanted plantlets were placed into misting tunnels (having 100% relative humidity) for four weeks before being transferred to the greenhouse under the same conditions as in the first experiment.

The '*in vivo*' and '*in vitro*' propagated plants did not belong to a clone. The experiments were conducted with commercial products of AM fungi and bacteria (M for endomycorrhizal fungi, A for *Agrobacterium radiobacter* and M+A for the mixture). A homologated commercial product of endomycorrhizal fungi (Endorize, Agrauxine) was incorporated into the disinfected substrate at the rate of 5% (v:v). The commercial *Agrobacterium radiobacter* strain K1026 (Nogall, Bio Care Technology) was applied to the substrate at the dilution of 10^6 cfu/g. This bacterial inoculum is recognised in Australia, USA, and Europe to prevent crown gall.

Endomycorrhizal infection

All inoculated '*in vitro*' and '*in vivo*' plants had a low level of infection (F % less than 5%) whether they were inoculated with *A. radiobacter* or not.

Biotisation of '*in vivo*' plants

Analyses of plant survival rates that were evaluated after 12 months of growth indicated that the highest survival rate was obtained for the mycorrhizal inoculation (52.5%). Inoculation with both microorganisms resulted in the lowest survival rate (Table 13).

Insignificant effect on plant dry weight was observed. However, *A. radiobacter* inoculation significantly increased the root/whole plant rate in comparison to that of the control plants, and to the binary mixtures containing endomycorrhizal fungi and *Agrobacterium*.

Table 13: Survival rate and whole plant/root ratio of the Christmas rose plants developed by rhizome cuttings inoculated with *Agrobacterium radiobacter* (strain K1026) and a commercial product of endomycorrhizal fungi, after 12 months of growth.

Treatment	Survival rate		Dry weight of whole plant (g)	Dry weight of roots (g)	Root/whole plant ratio
	N=40	(%)			
Control	18	45	8.93	7.47	0.83^{bc}
M+	21	52.5	9.3	8.28	0.88^{ab}
A+	20	50	12.76	11.54	0.91^{a}
MA+	17	42.5	10.95	9.09	0.81^{c}

N – number of plants
M+ – inoculated with endomycorrhizal fungi
A+ – inoculated with *Agrobacterium radiobacter*
MA+ – inoculated with mixture of endomycorrhizal fungi and *Agrobacterium radiobacter*
^{abc} – values followed by the same letter are insignificantly different ($P < 0.05$)

Biotisation of '*in vitro*' plants

Differences in the survival rate (between inoculated and non-inoculated individuals) of plants originating from the '*in vitro*' technique were observed after 4 months of growth in a sterile peat/perlite/compost substrate. *Agrobacterium radiobacter* had the highest stimulatory effect on the survival rate (the survival rate was 25% higher in comparison with the control plants) (Table 14). The mixture of both microorganisms did not have any positive effects on plant survival rate.

The effects of biotisation on the growth of '*in vitro*' propagated plants are shown in Table 8. The highest growth was obtained with *Agrobacterium radiobacter* inoculation. After the four-month period, there was insignificant effect from the bacterial and endomycorrhizal inoculations in comparison with the control treatment. However, *Agrobacterium radiobacter* significantly ($P < 0.05$) stimulated plant and root growth when compared to inoculation with the microorganism mixture.

The root/whole plant ratio was the highest for plants without microorganisms. The root/whole plant ratio of plants inoculated with *Agrobacterium radiobacter* and with both microorganisms (*A. radiobacter* and endomycorrhizal fungi), was significantly lower than that of the non-inoculated plants.

Table 14: The effects of inoculation with *Agrobacterium radiobacter* (strain K1026) and a commercial product of endomycorrhizal fungi on Christmas rose plants developed by 'in vitro' technique, after four months of growth.

Treatment	Survival rate N=20	Survival rate (%)	Dry weight of whole plant (mg)	Dry weight of roots (mg)	Root/whole plant ratio
Control	13^{bc}	65	53.15^{ab}	38.4^{ab}	0.73^{a}
M+	14^{ab}	70	54.4^{ab}	37.4^{ab}	0.69^{ab}
A+	18^{a}	90	87.2^{a}	57.1^{a}	0.65^{b}
MA+	11^{c}	55	40.3^{b}	25.65^{b}	0.64^{b}

N – number of plants
M+ – inoculated with endomycorrhizal fungi
A+ – inoculated with *Agrobacterium radiobacter*
MA+ – inoculated with a mixture of endomycorrhizal fungi and *Agrobacterium radiobacter*
^{abc} – values followed by the same letter are insignificantly different ($P < 0.05$)

Discussion and conclusions

Ornamental plants are often grown from seedlings or cuttings produced in disinfected soil or in inert substrata, mainly to lower the risk of contamination by pathogens and to ensure controlled conditions for obtaining morphologically homogeneous material. Plant micropropagation is also being increasingly used, especially for genetically improved and pathogen-free materials. The use of these techniques eliminates or drastically reduces the populations of microorganisms, which are generally present in soil, including beneficial ones (Linderman, 1986; Cordier et al., 2000).

The rhizosphere microorganisms that are known to act as a phytostimulators, or possess antagonistic activities toward plant pathogens, can be used alone or in conjunction. However, very little data is available about horticultural plant biotisation with more than one microorganism (Datnoff, 1995; Azcon-Aguilar and Barea, 1997; Cordier et al., 2000; Vestberg et al., 2004).

The results show that inoculation with a single microorganism can have positive effects on the growth of *Helleborus* plants, especially on root growth, as compared to the dual inoculation. The effects on plant growth generally depend on the type of microorganism and the microbial mixture introduced into the substrate (Dunabeitia et al., 2004**)**. In the case of *Helleborus niger*, it seemed that those microorganisms used separately were the more efficient. *Agrobacterium radiobacter* was found to be stimulating the growth and the survival rates of plants, and could be considered as an effective plant growth-promoting agent for Christmas rose plants propagated by the micropropagation technique ('*in vitro*') or by rhizome cuttings ('*in vivo*'). The survival rates of those plants originating from the '*in vitro*' technique and growing in the substrate inoculated with *Agrobacterium radiobacter* was 90% (the survival rate of control plants was 65%). In the same experiment, the dry weight of a whole plant was significantly higher when compared to inoculation with the microorganism mixture (Table 14). The biotisation of '*in vivo*' plants with *A. radiobacter* significantly stimulated plant growth when compared to the binary mixtures (Table 13).

When comparing the two methods of vegetative multiplication, the endomycorrhizal fungi were more efficient for plants obtained by '*in vivo*' propagation, where they had the highest stimulatory effect on the survival rate (52.5%) (Table 12). It is important to note that *A. radiobacter* and endomycorrhizal fungi mixtures may have negative effects on the rate of plant survival and plant growth. The survival rates of plants inoculated with both microorganisms was 55% (control 65%) for '*in vitro*' plants, and 42.5% (control 45%) for '*in vivo*' plants, and the dry weight of the whole '*in vitro*' plants was 32% lower when compared to the control plants (Tables 13 and 14). Similar effects were observed by Cordier et al. (2000) on micropropagated strawberries. Among the main reasons that could explain these results could be an imbalanced ratio between the release of organic substances by plants (mainly as root exudates that act as either signals or growth substrates) and root-associated biota (combination of two microorganisms).

The stimulation of microbial development and activity could also depend on the growth substrate. Azcon-Aguilar et al. (1992) concluded that the use of 50% disinfected soil had provided better mycorrhizal effect. However, this effect may depend on the genetic structures of individual plants. The growth of micropropagated kiwi was increased by mycorrhizal infection, using only 5% sandy soil in a peat/perlite mix (Shubert et al., 1992), whereas Lovato et al. (1994) observed growth enhancement of endomycorrhizal wild cherry and common ash using potting mixture containing 20% clay-loam soil. Therefore, the application of multi-microbial substances to plants requires knowledge and understanding of the (in)compatibility systems between different beneficial microorganisms in their interactions within the mycorrhizosphere, rhizosphere, rhizoplane, and endorhizosphere.

The root/whole plant ratio could be modified by microorganisms for the plants obtained by micropropagation. This has already been reported for other inoculated plants. The ratio of inoculated plant was reduced. Inoculated plants showed a greater increase in shoot production than root production, so that micropropagated plants with beneficial microorganisms possess a more efficient underground absorbing system (Guillemin et al., 1997; Rodrigez-Romero et al., 2005; Padilla et al., 2006; Kapoor et al., 2008)

This study indicates that plant biotisation could be a promising technique for improving the qualities of plants and for ensuring an appropriate development within the context of sustainable horticulture. Different responses to microbial treatments suggest that microorganisms have to be screened before being used. Moreover, the application of multi-microbial biotisation also requires the understanding of the (in)compatibility between different beneficial microorganisms and their interactions.

6.3 Agro-environmental requirements

For sale as flowering potted plants before Christmas, young Christmas rose plants are best potted in April and May. We use 10- to 15-centimetre pots. We can also use larger pots, planting several plants together. It is very important to plant seedlings at the same depth or no more than 1 cm deeper than they grew before transplanting. Planted plants can be cultivated outdoors (with the possibility of shading), but root

development in a greenhouse and subsequent cultivation outside is more reliable. The area where we grow plants in pots must be free of weeds and we have to cover the soil with a permeable fabric. Once the plants develop roots in a pot, we spread them out at an appropriate distance (avoiding too much distance) and let them take root, as this will help them better withstand the high summer temperatures. In summer, the plants should be kept in shade, preferably with high humidity. Flowers that have developed too soon are removed, as this encourages the growth of new flowers. During forcing in protected areas, we need to be alert for possible development of diseases (*Botrytis* and *Peronospora*). Additional air ventilation is recommended.

Growth media. The Christmas rose appreciates deep, well-drained soils rich in organic matter, but with no shortage of moisture. The potting substrate should contain 60% peat, 15% perlite and 20–25% clay. The selection of growth media depends on the irrigation system and the size of the pots. It grows best at a pH around 6.0 (measured in $CaCl_2$). The growth media is fertilised using 0.5 kg/m^3 of quick-release fertiliser (14:16:18 N:P:K), 0.2 kg/m^3 of micronutrient fertiliser (e.g. Micromax), and 5 kg/m^3 of slow-release fertiliser (8–9 months). It is important that the concentration of salts in the growth media is not high. Acid soils and growth media with too much nitrogen encourage the development of *Coniothyrium* disease.

Fertilisation. In the period when the roots are inactive (from November to the end of April), the requirement for nutrients is very low. During intensive root growth (from May to October), plants require a constant, moderate supply of nutrients. Analysis of growth media during different time periods helps to determine the time when fertiliser is required. The values presented here are averages and may require adjustments for specific cultural and environmental conditions.

Several weeks after transplanting, when the plants develop new roots (June), we start using water-soluble fertilisers, such as 18:12:18, twice a month with a 0.15% (1,5 g/l) fertiliser solution, or with a 0.03–0.07% (0.3–0.7 g/l) fertiliser solution with each watering. At the beginning of the growth period, it is advisable to start with a 0.03% concentration of fertilisers (diluted in water); later the concentration can be increased to 0.05–0.07%. Nitrogen is added mainly in nitrate form. The optimal values for nitrogen, phosphorus, and potassium are 80–100 mg/l, 50–60 mg/l, and 12–140 mg/l, respectively.. Excessive fertilisation with nitrogen (more than 50 mg/l) can

cause black spot. Salt content should be 0.5–1 g/l throughout the duration of fertilisation, but never more. During forcing (in November and December), there is a significant need for nitrogen to ensure the growth of inflorescence, and therefore the addition of N (as nitrate potassium or lime nitrate) is required.

Watering and humidity. During summer, the humidity of the substrate and the atmosphere should be at a constant level without stagnation or dry periods. Plants are left to slightly take root in the soil, which has an added effect on a more even supply of moisture. In areas with less than 400 mm of precipitation during the period from May to October, irrigation is essential. It is very important that these plants grow in sufficiently high air humidity during summer (60–90% air humidity). In these conditions, black spot disease (*Coniothyrium hellebore*) usually does not cause serious damage. During the forcing period, high air humidity is not recommended, as it may lead to the spread of diseases caused by *Botrytis* spp. and *Peronospora pulveracea*.

Light. In the first part of the year, the Christmas rose requires sunlight, similar as deciduous trees and shrubs. During summer, the plants prefer partial shade. High light intensity increases the invasion of *Coniothyrium*, which can make production unprofitable. The required illumination during forcing varies between 5,000 and 15,000 lux (Lemper, 1984, 1985).

Shading. The Christmas rose originated in mountainous areas, and is therefore not adaptable to the higher air temperatures of a moderate continental climate. In order to ensure optimal growth conditions, the plants have to be shaded. Shading is important for prolongation of the peduncles, especially when the producers grow 'short type' cultivars. One can also obtain bigger flowers with clean, white colours. Full covering is required for the first 14 days of the forcing period. Plants have to be shaded using black material (polyethylene or patch), and the temperature must reach 15 °C (Lemper, 1984, 1985).

Forcing/Temperature. In early or mid-November, depending on the weather, we move the plants to a protected area, where there is no chance of frost. It is important that plants are exposed to low temperatures of 2–4 °C for a sufficient time (at least 2 weeks), as an insufficient duration of the cold phase reduces the number of flowering plants and the quality of flowers. By the beginning of December, the temperature can rise to 5–10 °C. Higher forcing temperatures, e.g. 12–16 °C, impair

flower development, adversely affecting plant quality and resistance to low temperatures after the plants are sold. In the last five days before sale, temperatures can range from 12 to 16 °C. These temperatures frequently have to be modified according to the specific requirements of cultivar(s) and existing growth conditions.

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7 Diseases and pests

There are several pests and diseases that can affect the Christmas rose during production. The damage often depends on the genetic structure of the plant material, the growth stage, and the environmental conditions. Some of these pests and diseases can create significant damage. The most effective type of pest management programme is one that prevents the occurrence of problems. Crop monitoring in conjunction with adequate production technology is essential for effective pest control; chemicals should not be relied on as the only control measure. Chemical control is associated with pollution of the environment (pollution of air, soil, underground water, and plants) and pathogen resistance. Protection (i.e. control of pests and diseases) should be based on measures that are not destructive to the environment.

7.1 Diseases

Viral disease

Hellebore black death. The symptoms are black spots or streaks on leaves, stems, and flowers. The plants become stunted and may die. This disease is mainly found in various cultivars and hybrids of *Helleborus orientalis*. The Hellebore aphid is probably the vector of the disease, which is spread during sap feeding activities.

Other, less important viruses that have been isolated from hellebore plants are HeMV (*Helleborus* mosaic virus), AMV (alfalfa mosaic virus), and CMV (cucumber mosaic virus).

There is no treatment currently known for this disease. The removal of infected plants, avoidance of infected planting material, control of aphids, and proper greenhouse sanitary procedures decrease the chances of aspermy.

Bacteria

Soft rot (*Erwinia carotovora*). This disease is characterised by the sudden wilting and collapse of the plant. A part of the rhizome may become soft and slimy, whilst the roots remain intact. Petioles and peduncles may also become soft and slimy. Hot weather facilitates rapid progress of the rot.

Proper spacing, avoiding splashing water, immediate disposal of diseased plants, and application of an appropriate chemical control will reduce the severity of this disease.

Fungi

(1) Black spot (*Coniothyrium hellebori* Cooke & Massee). This is considered to be the most serious disease of the Christmas rose. It causes large, irregular black or brown spots on both sides of the leaves, merging to create dead areas on the leaves. The spots have concentric zonations. In spring, there may be black, canker-like spots on the leaves, stems, and flower stalks. The stems shrivel at the point of infection and fall over. The leaves and unopened flower buds wilt. The flowers may also have black spots. Other parts of the plant turn yellow, and most of the leaves and flowers may be damaged. Infection with black spot is more likely when plants are in badly chosen positions, the pH of the soil is too low, or too much fertiliser containing nitrogen has been used.

Control measures involve the removal of all infected parts (leaves and flowers), which must be destroyed to prevent the spread of the disease. The remaining parts of the plant should be treated with one of the following fungicides: Dithane Ultra (mancozeb) 200 g; Polyram DF (metirame zinc) 200 g; Saprol (triforine) 150 g, Cupravit 150 g, Brestan 60 30 g.

Permanent treatments (sprays) over a period of 2–3 weeks are very important. The fungicide has to be well-spread on the surface of the affected plant material. Treatments with fungicides such as Antracol (propineb), Benlate (benomyl), Captan, Cercobin M, Daconil, Euparen (dichlofluanid), Filicidin, Dithan (mancozeb), Vinicoll and Zineb have been found to be insufficiently effective.

(2) False mildew (*Peronospora pulveraca*). This is the second more important disease after black spot. New leaves remain small and crisp, and show irregular brown or grey spots on the adaxial side. The infected plants begin to flower earlier, but the flowers have various spots of a brown-green tone.

Control measures involve the removal of all infected parts (leaves and flowers), which must be destroyed to prevent the spread of the disease. The remaining parts of the plant should be treated with fungicides as follows: Dithan Ultra (mancozeb) 200 g, Polyram Combi (metirame zinc), Previcur (propamocarb) N 150 g, Aliette 250–500 g, Fonganil 50 g. Permanent treatment over a period of 2–3 weeks can be done at the same time as spraying against black spot.

(3) Black rot of rhizome, stem, roots, and crowns (*Rhizoctonia solani*). These stem, root, and crown rots are caused by soil-borne organisms and are most destructive in wet conditions. Sometimes, the same symptoms occur when plants are infected with *Fusarium* or *Phytium*. The rot occurs on the crown above the soil surface, usually in summer. Old leaves become yellow, and small cracks appear on the bases of the peduncles. The infected plants wilt during the day, and eventually will not regain turgidity.
Fungicides can be used to stop or to eliminate the pathogen: Basitac 75 PM (mepronil) 100 g, Benlate (benomyl) 150 g, Rovral (iprodione) 150 g; Cryptonol (oxyquinoline) 200 ml, Antracol (propineb) 200 g, Orthocid 83 200 g, Ronilan (vinclozolin) 100 g, Captan 50 200 g. Proper media preparation and sanitary greenhouse practices will help to reduce the potential for infection.

(4) Crown rot, grey mould or Botrytis blight (*Botrytis cinera*). Botrytis is favoured by cool temperatures (10–16 °C) and high relative humidity, and may sporulate on dead or drying plant tissue. It can also be severe at high temperatures. The spore germination requires up to 12 hours of continuous freestanding water on the plant surface. The crown rot is a soft decay of flowers and leaves, often in the crown. The

affected parts are often covered with a downy grey mould. During forcing, wide burnt spots appear on the leaves and round brown spots on the flowers.

Ventilation is an important measure in the greenhouse because spore germination is inhibited by air movement of 0.9 m/s. In order to control the disease, environmental conditions should be improved. The more appropriate chemicals are the following: Scala (pyrimethanil) 200 g; Octave (prochloraz) 100g; Rovral 100 g, Ronilan (vinclozolin) 100 g, Euparene (dichlofluanid) 150 g.

(5) Fusarium wilt (*Fusarium oxysporum*). This fungus is soil borne and first infects the roots and then invades the vascular tissue, causing severe wilting and eventual death of the plant. This vascular disease usually begins with yellowing at the base of the leaf blade or elsewhere on the leaf. The petioles begin to dry at the base. The bases of the stems become brown and rot. The spots enlarge; discoloration may occur in roots and rhizomes, and subsequently the plants fade. Warm temperatures and high relative humidity are conducive to the development of this disease.

The best solution for combating *Fusarium* wilt is the use of cultivars that are relatively resistant, or disease-free plants. Proper media preparation and sanitary greenhouse practices will help reduce the potential for infection. Fungicidal spray applications are ineffective, as the disease is systemic, but fungicidal drenches early in the course of the disease infection can be beneficial.

(6) Bremia or (Mildew). The adaxial (upper) side of the leaves is pale, yellowish with irregular pinkish spots. On the abaxial (lower) side, a greyish mould develops. The leaves decay and dry out. Bremia is more frequent in young plants. The fungicides used for controlling this disease are as follows: Acylon Tabac (metalaxyl + manebe) 200 ml, Antrocol (propineb) 250 g, Elveiss (oxadixyl + cymoxanil) 150 g.

(7) Sclerotinia (*Sclerotinia desphinii*). A cottony mass appears on affected tissue usually on the base of the peduncles. Sclerotia (hard, black masses) may form within the stem. The fungicides used for controlling this diseases are as follows: Rovral (iprodione) 100 g, Octave (prochloraz) 100 g, Ronilan (vinclozolin) 100 g, Nustar EC (flusilazole) 20 ml.

(8) Oidium. On the leaves, white powder appears and spreads across the whole lamina, and thus slows down the growth. The fungicides used for controlling this disease are as follows: Anvil (hexaconazole) 40 ml, Systhane (myclobutanil) 70 ml, Horizon (tebuconazole) 100 ml, Saprol (triforine) 100 ml.

(9) Pythium root rot or basal stem rot (*Pythium* spp.). *Pythium* is a soil-borne organism that prefers excessively soil moisture. Spores are spread by contaminated soil, water, tools and other implements. *Pythium* is a water mould, so it is particularly severe in poorly drained media. Sterilisation of the medium will be beneficial in controlling *Pythium*, but only if serious efforts are made to maintain a sanitary condition of the growing area.

7.2 Pests

Insects

(a) The hellebore aphid (*Macrosiphum hellebori*) and some other related species could also attack the plants. They can form enormous colonies on old flowers and on the adaxial sides of newly developing leaves in spring and early summer. This causes a loss of vigour and also sometimes a distortion of the leaves, and the plants become covered with a sticky mess of honeydew. There are many insecticides on the market to control these pests.

(b) Common swift or garden swift moth (*Hepialus lupulinus*). The larvae of this butterfly eat the roots and the root crown of several plants (*Chrysanthemums*, *Dahlia*, *Anemone*). They live in the soil. Each year, they produce new eggs, and subsequently all stages of the animal evolution are present. Plants begin to grow later, and sometimes the flowers bend down. Infections can persist and increase year after year. The damage appears in autumn, in winter and at the beginning of spring. The effective control is as follows: Orthene 50 (acephate) 1.8 kg, Baythroid (cyfluthrin) 300 ml, Cerbere (cypermethrin) 300 ml, Decis (deltamethrin) 300 ml.

(c) Leaf miner (*Phytomyza hellebori*). This is considered to be a less dangerous pest. It disfigures leaves. The plants seem to have the ability to tolerate the damage. The removal of old foliage in winter stops the larvae from completing their development.

Nematodes

(a) Leaf nematodes (*Aphelenchoides fragarie* or *Aphelenchoides ritzemabosi*). They are microscopic, non-segmented round worms which are spread through the stomata by splashing water. They cause angular lesions on the leaves. The damage to the plant progresses upward. On the leaves, there are brown-black spots limited by the veins. The leaves begin to yellow and become dry, and plant growth slows down.

(b) Root nematodes (*Partylenchus*). These pests suck liquid substances from roots and cause root galls and therefore weaken the plants. They are soil borne or can be transmitted to uninfected soil through contaminated plants or soil. The plants straggle up and begin to turn yellow. The root system is highly reduced and mouldy. The growth is weak. Clean stock and pasteurised media are the best preventative measures for nematodes. The chemical products used for controlling these nematodes are Curater (carbofuran) 10 g/m^2, and Temik (aldicarb) 10 g/m^2.

Slugs and snails

They often hide in slits in the bottom of Christmas rose pots during the day. They eat root tips and young buds (in the early part of the year when these are just emerging from the soil). They can also climb the plants and eat young leaves and flowers of mature plants. If slime trails are detected in early morning hours, it is evident that snails and slugs are present and need to be brought under control. For controlling slugs and snails, various commercial products based on metaldehyde can be used (at concentrations of 1 g/m^2).

Mammals

(a) Mice and rats. These can eat seedlings, and they can also eat the buds and flowers of mature plants. They often leave distinctive, neat piles of debris at the base of the plants.

(b) Forest animals. Forest animals such as wild pigs may damage plants growing in the wild. They may damage plants while searching for food or while traversing the area inhabited by Christmas rose plants.

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HELLEBORUS NIGER: SYSTEMATICS, ECOLOGY, POLLINATION AND PRODUCTION TECHNOLOGY

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Abstract The scientific monograph covers the botanical classification of the genus *Helleborus* L. and the Christmas rose species (*Helleborus niger* L.), which has undergone numerous changes throughout its botanical history. The Christmas rose is becoming increasingly important in the ornamental plant market. Its characteristic flowering in the coldest months of the year represents a great advantage over many other species of ornamental plants. As the Christmas rose does not require high temperatures to start flowering, its cultivation can be considered affordable and environmentally friendly. The supply of attractive varieties is likely to be one of the key factors that will affect its popularity in the future. The number of genetically improved (bred) varieties is limited on the market, so selective breeding of Christmas rose will become inevitable in the future. The presented results of pollination analyses, related to insect activity, are important for breeding. Observations indicate that the Christmas rose is an entomophilous and a predominantly allogamous species. Knowledge of plant ecology and cultivation technology will also be exceptionally important. In intensive cultivation, the production technology for Christmas roses is still to a large extent incomplete. The monograph includes scientific findings on the ecology and biology of the…

Ključne besede: Christmas rose, *Helleborus niger* L., systematic, pollination, cultivation, beneficial microorganisms

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