Biological flora of Britain and Ireland: Neottia nidus-avis

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Abstract

1. This account provides information on all aspects of the biology of Neottia nidus-avis (L.) Rich. (Bird’s-nest Orchid) that are relevant to understanding its ecological characteristics and behaviour. The main topics are presented within the standard framework of the Biological Flora of Britain and Ireland: distribution, habitat, communities, responses to biotic factors, responses to environment, structure and physiology, phenology, reproductive characteristics, herbivory, history and conservation.

2. Neottia nidus-avis is a native mycoheterotrophic orchid; it is found most frequently in the deep humus of densely shaded beech woodlands, on limestone or chalky soils in the British Isles. The species extends throughout temperate Eurasia.

3. Neottia nidus-avis is a perennial, mostly monocarpic herb with mixed mode of reproduction and many adaptations to autogamy. The perennating organ is a short rhizome; vegetative reproduction by rhizome branching or root-sprouting is rather infrequent. Because of its achorophyllous nature, N. nidus-avis is entirely dependent on its mycorrhizal basidiomycetes from the family Sebacinaceae for nutrition.

4. Plastid genome of N. nidus-avis has been reduced as a result of the relaxation of functional constraints on photosynthesis as in other mycoheterotrophic plants. Despite this, the plant has retained all enzymes to produce chlorophyll as well as some photoprotective metabolites, such as zeaxanthin and tocopherol, whose function in the mycoheterotrophic plant remains unresolved.

5. The species flowers from May to June, depending on latitude and altitude. The flowers emit a sweet scent and produce little nectar. They may be pollinated by flies, crawling thrips and ants but self-pollination is presumed to be the main mode of reproduction. Between 75% and 97% of open flowers set fruits.

6. Neottia nidus-avis is classified as Vulnerable in Great Britain. The species is scattered throughout much of the British Isles, but it is very scarce except in...
Bird’s-nest Orchid. Orchidaceae, subfamily Epidendroideae, tribe Neottieae. Neottia nidus-avis is a perennial, non-photosynthetic, mycoheterotrophic herb, with short subterranean rhizomes.

Rhizome up to 5–6 cm long, 2–5 mm in diameter, developing one rapidly decaying scale-leaf with axillary bud each year. Rhizome densely covered by numerous (20)–50–80(−120) fleshy adventitious roots. Adventitious roots 1.5–4 cm long and 1–4 mm in diameter, all of similar limited length forming a tangled mass reminiscent of a bird’s nest. From this it derives both its generic and specific Latin names (from neotteia, nest in Greek, and nidus-avis, nest of a bird in Latin) as well as names in many other languages (see 10).

Flowering shoot (15)20–40(60) cm high, 3–7 mm wide at the base, solitary or occasionally with two or more shoots from the same rhizome, erect, fairly robust, yellowish-brown and slightly glandular-hairy towards the tip. Green leaves are absent, but the lower part of the shoot is sheathed by 3–5 scale-like, yellowish-brown leaves; scale-leaves lanceolate, the upper ones longer and blunter, surrounding the shoot bud and probably protecting the flowering shoot during subsequent growth.

Inflorescence a spike, c. 10–20 cm, cylindrical and dense-flowered above, less so below, with up to 100 flowers in large plants. Bracts 4–8 mm, lanceolate, tapering to an acute apex, papery and roughly as long as the ovary and pedicel together. Flower 15 mm, yellowish-brown, with a honey-like scent. Sepals and petals 4–6 mm long and 3–4 mm wide, oval-spatulate, forming a loose fan-shaped hood over the column. Labellum 10–12 mm, brown, directed forward, hollowed into a nectar-containing cup at the base and divided distally into two broad, spreading lateral lobes, also sometimes with a subtle point or tooth on either side of the lip halfway towards the base. Spur absent. Column cylindrical, pale brownish-white, with an oblong, papillose anther on the outside. Rostellum leaf-like, concave on the upper side, forming a clinandrium in which the pollinia rest after they fall out of the anther at anthesis. Stigma positioned in front of the column, appearing V-shaped, running either side of the rostellum. Pollinia two, pale yellow, linear-oblong, each consisting of two halves containing loosely connected powdery pollen grains united in tetrads, projecting conspicuously from beneath the anther cap. Ovary ovate, 10 mm, subtly six-ribbed with glandular hairs and held on a twisted stalk that is about half the length of the ovary.

Neottia comprises 63 autotrophic and mycoheterotrophic terrestrial species distributed throughout the northern hemisphere, from temperate to arctic habitats (Pridgeon et al., 2005). Formerly, the genus included only non-photosynthetic taxa, but the long-noted similarity between species belonging to Neottia and Listera, based on generative structures (Dressler, 1990), has been confirmed by DNA-based phylogenetic classification (Chase et al., 2003; Chase et al., 2015; Zhou & Jin, 2018), leading to the recent expansion of the genus Neottia to include photosynthetic species of Listera.

Neottia nidus-avis displays several morphological variations, although with little taxonomical value (Prochazka, 1980). Plants with numerous glandular hairs on the inflorescence axis, ovary and pedicels were described as var. glandulosa Beck 1890, while the nominate variety (var. nidus-avis, or var. typica Beck) is glabrous; f. dilatata Zapał 1906 has smaller flowers and outer perianth segments only 4–5 mm long; f. brunnea Weiss (f. macrostelis Peterm.) has large outer perianth segments 5–7 mm long; f. brachystellis Peterm. is has the stylar part of column shorter than anther. Colour variation led to a description of var. pallida Wirtg. with a yellow-whitish stem and flowers and white pollinia, var. nivea Magnus ex Schulze for white plants (that lack the traces of chlorophyll and carotenoid pigments normally present, see 6.5) and var. sulphurea Ascherson and Graebner for sulphur-yellow plants (see Lewis, 2018).

Neottia nidus-avis is a native mycoheterotrophic orchid, typically inhabiting the dense shade of old, broadleaved (usually beech) woodlands. Although still widespread and locally abundant in the British Isles, it has suffered a considerable decline with shrinking habitat availability and quality throughout the 20th century, especially in south-eastern and central England.

1 | GEOGRAPHICAL AND ALTITUDINAL DISTRIBUTION

Neottia nidus-avis was recorded from 928 10–km squares (hectads) in the British Isles (about 23.8% of the total, with 874 hectares in Great Britain and 154 hectares in Ireland; Figure 1; Table 1). The species is widely scattered throughout much of the British Isles. It is locally common in mature woodland in parts of southern England (a belt from the northern parts of East Anglia through Lincolnshire and the north Midlands to northern and western Wales) but scarce in the rest of the British Isles. Although recorded from more than half of Scottish vice-counties, it is a very rare plant there and is very scattered in lowlands. Similarly, in the Republic of Ireland, although recorded from most counties, each has only a handful of populations. It is absent from the Isle of Man, Shetland, Orkney and the Inner and Outer Hebrides, except for the islands Skye and Mull (Foley & Clarke, 2005; Harrap & Harrap, 2005). In Northern Ireland,
the species is widespread in the southwest and scattered through the rest of the country.

Globally, *N. nidus-avis* has a Eurasian distribution ([Figure 2](#)). It grows from the British Isles throughout most of central Europe ([Bartha et al., 2015; Presser, 2000; Procházka & Velíšek, 1983; Vlčko et al., 2003]) with an eastern limit in western Siberia ([Vakhrameeva et al., 2008, map 25]). It is found as far north as c. 65° in Scandinavia ([Anderberg & Anderberg, 2016]) and south to northern Portugal ([Tyteca & Bernardos, 2003]), Spain ([Anthos, 2012]), Corsica, the Balearic Islands, Sardinia, Sicily, the Balkans (e.g., Romania; [Săvulescu & Pop, 1972]), Bulgaria ([Assyov et al., 2002]), former Jugoslavia states ([Beck-Mannagetta, 1903; Josifovic, 1976]), forests of central and north continental mountains of Greece ([Tsiftsis et al., 2008]), Black Sea region of Turkey ([Kreutz, 1998]), Crimea ([Kubcova, 1972]) and Caucasus ([Grossgejm, 1949]). It is absent from Crete and Cyprus ([Aliberti, 1998; Kreutz, 2004]). The species is largely confined to mountains in the south of the range and is absent from the Mediterranean lowlands. It grows in the forest zones of Armenia, Azerbaijan, Belarus, the Baltic states, Georgia, Kazakhstan, Moldova, Ukraine and the European part of Russia except in its northern regions ([Nevski, 1935; Vakhrameeva et al., 2008]). In Siberia, the species occurs in the regions Tomsk, Altai, Omsk, Kemerovo, Tyumen and Novosibirsk ([Malyschev & Peschkova, 1987, map 197]). The reported occurrence in the Far East and Japan is considered incorrect ([Foley & Clarke, 2005; Harrap & Harrap, 2005]). In Japan, there is a plant with similar ecology (woods in mountain areas) but with a
glandular stem, named *N. nidus-avis* var. *papilligera* (Ti et al., 1965), but its affiliation to *N. nidus-avis* is debated (Yagame et al., 2016) and requires further molecular analysis.

Isolated occurrences have been discovered on Mount Babor, part of the Tell Atlas Mountain chain in north Algeria (De Smet & Bouanza, 1984) and in the oak forests of the Kroumirie, in the north-western Tunisia (El Mokni et al., 2010).

### 2 HABITAT

#### 2.1 Climatic and topographical limitation

Preston and Hill (1997) assign *N. nidus-avis* to the Eurosiberian temperate element of the British flora. It includes species whose main distribution reaches their easterly limit between 60° E and 120° E. In the British Isles, they have a similar distribution to those of the European temperate element, although there is a more marked gradient between the south-eastern England, where the greatest concentration is found, and the north-western Scotland. *N. nidus-avis* occupies the warmer and drier regions: the mean January and mean July temperatures, and mean annual precipitation in the 10-km squares occupied by *N. nidus-avis* in Britain are 3.5°C, 15.2°C and 938 mm, respectively (Figure 3; Hill et al., 2004).

*Neottia nidus-avis* is mainly a lowland plant in the British Isles. Its altitudinal range reaches a maximum 250 m in Cumbria (Harrap & Harrap, 2005). Elsewhere in Europe it grows from sea level to 2000 m in France (Bournérias & Prat, 2005), up to 1800 m in Austria (Presser, 2000), up to 1700 in the mountains of southern Tyrol (Ziegenspeck, 1936), up to 1310 m in Tatra Mountains in Slovak Republic (Procházka & Velísek, 1983), up to 1800 m in Rila Mountains in Bulgaria (Jordanov, 1964), from 190 to 2000 m in Switzerland (www.ageo.ch), from 400 to 1650 m in eastern Macedonia (Tsiftsis et al., 2008), up to 2000 m in the mountains of Greece (Petrou & Giannakoulias, 2011), from 400 to 1200 m in Portugal (Tyteca & Bernardos, 2003) and from 200 up to 2000 m in Turkey (Kreutz, 1998). In Algeria and Tunisia, the species occupies forest zones of 1500 to 2000 m a.s.l. (De Smet & Bouanza, 1984; El Mokni et al., 2010) with high rainfall (up to 1500 mm per year) so that in both cases, the local climatic conditions resemble those in the main distribution area.

#### 2.2 Substratum

*Neottia nidus-avis* is found in moist soils with deep humus and leaf-litter layers. Ellenberg's indicator values for edaphic characteristics at the sites where *N. nidus-avis* is found (indicator values modified for UK; Hill et al., 2004) are 7 for soil reaction (the species favours sites with weakly acid to weakly basic pH and never occurs on strongly acidic soils), 4 for moisture (soil moderately moist), 5 for nutrients (moderately rich sites, rarely on nutrient-poor or nutrient-rich soils) and 0 for salinity (absent from saline habitats). In the British Isles, the species grows mostly not only on chalk and limestone soils but also on clays and sands that have a chalky or limestone component, such as boulder clay (Harrap & Harrap, 2005). Elsewhere in Europe, *N. nidus-avis* also prefers base-rich soils; Sundermann (1970) reported a pH of 7.2–8.1 for European sites based on 3 measurements of soil around *N. nidus-avis* roots and 6.4–8.5 for 21 measurements in the vicinity of *N. nidus-avis* plants. Procházka and Velísek (1983) mentioned pH values 6.1–8.5 in Czech and Slovak republics, while Tsiftsis et al. (2008) reported values from north-eastern Macedonia ranging from 4.14 to 7.74, with a
median of 5.47. The latter study, which sampled soil at rooting depth (5–15 cm), also found organic-matter content ranging from 0.97 to 35.7% (median 7.3%) and available phosphorus concentration ranging from 0.89 to 10.2 mg/100 g (median 3.3 mg/100 g). A major requirement for the orchid is the presence of its mycorrhizal associates, fungi from the family Sebacinae whose ecological preferences, beyond the presence of trees that are their carbon sources (see 6.2), remain unknown (Brzosko et al., 2017; Weiß et al., 2016).

3 | COMMUNITIES

In Britain, Neottia nidus-avis occurs predominantly in heavy shade of mature beech woodlands on free-draining, base rich and calcareous soils of W12 Fagus sylvatica – Mercurialis perennis community found in the south-eastern lowlands of Britain, where it is generally limited to the steeper drift-free faces of chalk escarpments (Rodwell, 1992). The species is particularly common in the Sanicula europaea subcommunity that develops on well-drained shallower grey mull-like rendzinas, rich in free calcium carbonate and with a surface pH generally within the range of 7–8. The dominant tree species is Fagus sylvatica, accompanied by Acer pseudoplatanus, Quercus robur, Cornus sanguinea, Viburnum lantana, Sambucus nigra and Ilex aquifolium. Tree height ranges between 8 to 15 m with 90% tree cover (Rodwell, 1992). The shrub layer is very poorly developed (10% shrub cover) with presence of Corylus avellana, Crataegus monogyna, Taxus baccata and Hedera helix. Herb cover is usually sparse but can range from 10% to a dense

FIGURE 3 Climate space graphs for Neottia nidus-avis in Great Britain (i.e., excluding Ireland, Isle of Man and Channel Islands). Climatic values for plants were calculated as the mean climate statistics for each 10-km squares (hectad) published in Hill et al. (2004). The baseline summaries were constructed by interpolation of daily weather measurements from individual met stations averaged over the 30-year period 1961–1990. Data are provided by K. J. Walker from the Botanical Society of Britain and Ireland.
The most abundant herb is *Sanicula europaea*, accompanied by *Mycelis muralis*, *Melica uniflora*, *Fragaria vesca*, *Viola reichenbachiana* and *Brachypodium sylvaticum*. At a few sites the vegetation is rich in orchids such as *Cephalanthera damasonium*, *C. longifolia*, *C. rubra* and *Epipactis helleborine*, as well as the mycoheterotrophic herb *Hypopitys monotropa*. On deep calcareous loams with few chalk particles and a high siliceous fraction on the chalk scarp of Kent, beech is rare and entirely confined to the forest margins (Ratcliffe, 1977). *Neottia nidus-avis* grows there in mixed coppice of *Fraxinus excelsior* and *Quercus robur*, with some *Carpinus betulus*, *Corylus avellana* and *Acer campestre*. The habitat supports a rich orchid flora together with *Paris quadrifolia*, *Mercurialis perennis*, *Sanicula europaea* and *Galium odoratum*. It occurs less commonly in mixed deciduous woodland and overgrown hazel coppice, and sometimes under shady old hedges, shelterbelts or planted conifers (*Pinus silvestris* and *Larix* species), especially if residual deciduous trees are present.

In northern England, Scotland and North Ireland, late frosts, low summer temperatures and heavier rainfall hinder beech dominance (Rodwell, 1992); thus *N. nidus-avis* grows there more frequently in oak woodlands and ravine ashwoods on limestone soil. The canopy of old sessile oak woods (community W11, *Quercus petraea - Betula pubescens - Oxalis acetosella* woodland) is formed by mixtures of oak (*Quercus robur*, *Q. petraea* and hybrids) and birch (*Betula pendula*, *B. pubescens* and hybrids). The well-developed understorey includes occasional *Corylus avellana*, *Ilex aquifolium*, *Sorbus aucuparia* and *Juniperus communis*. *Rubus fruticosus* agg., *Vaccinium myrtillus*, *Lonicera periclymenum* and *Blechnum spicant* make up the field layer, and the dense ground flora is dominated by extensive carpets of *Luzula sylvatica* with scattered *Oxalis acetosella*. The canopy of ashwoods growing on slopes, screes and ravines (communities W8 *Fraxinus excelsior - Acer campestre - Mercurialis perennis* woodland, and W9 *Fraxinus excelsior - Sorbus aucuparia - Mercurialis perennis* woodland) is generally dominated by *Fraxinus excelsior*, with *Corylus avellana* and occasional *Crataegus monogyna*, *Sorbus aucuparia* and *Ilex aquifolium* in the understorey. The ground flora is species-rich and is often dominated by *Filipendula ulmaria* and *Oxalis acetosella*, with species such as *Primula vulgaris*, *Potentilla sterilis*, *Geranium robertianum*, *Lathraea squamaria*, *Allium ursinum* and *Viola riviniana*. Bryophytes form a dense carpet over soils and exposed rock faces and boulders (JNCC, 2020). Elsewhere in Europe, *N. nidus-avis* grows predominantly in hornbeam and beech forests on chalk (Figure 4c; Jersáková & Kindlmann, 2004; Sundermann, 1970; AHO Sachsen-Anhalt, 2011). In the Czech Republic, the species was recorded in 572 phytosociological relevés (Chytrý & Rafajová, 2003); these can be classified into the following vegetation communities: *Carpinion* (53% of relevés), *Fagion* (27%), *Tilio-Acerion* (7%), *Quercion pubescenti-petraeae* (7%), *Genisto germanicae-Quercion* (3%) and *Ulmenion* (2%). Average cover of the tree layer of the relevés was 80%, herb layer averaged 60% with values ranging from 1% to 100%, whereas the moss layer was generally below 10%.

Occasionally, the species grows in spruce forests (*Picea abies*) with scattered beech trees on places of former beech forests.

In Northern Europe, the species typically inhabits beech woodlands of community *Fagion sylvaticae* (Diersen, 1996), but it can be also found in rich basiphilous pine forests (*Pinus silvestris*) on very shallow calcareous soils (Bjørndalen, 2015). In northern parts of Scandinavia, the thermophilous deciduous forests are replaced by birch forests or mixed deciduous forests, with more boreal species such as *Betula pubescens*, *Alnus incana*, *Populus tremula* and *Salix caprea*. Here, besides *N. nidus-avis*, other orchids such as *Cypripedium calceolus*, *Epipactis helleborine* and *Orchis mascula* may also occur in rich boreal deciduous forests (Bjørndalen, 2015). In the European-Mediterranean region, the species is found in shady beech woodlands and sclerophyllous oak forests, dominated by *Quercus ilex* or...
Q. suber, and pinewoods with various native and planted subspecies of Pinus nigra (Pignatti, 1982; Tison et al., 2014).

In the eastern part of its distribution area, N. nidus-avis grows in different types of forest (Querco-Fagetea) on rich substrata with a sparse grass cover (Didukh, 2009). For example, the species occurs in mountain pine forest in Crimea (community Erico–Pinetea: Brachypodio–Pinion pallasianae) and oak–hornbeam woodlands of community Carpinion orientalis–Quercion pubescentis, mountain beech and valley broad-leaved forests in the Caucasus, small-leaved birch or aspen forests, as well as in mixed coniferous forest in Moscow Province (Didukh, 2009; Vakhrameeva et al., 2008). For the Russian territory, Mirkin and Naumova (2012) mention N. nidus-avis as a diagnostic species of the communities Carpinion betuli and Querco roboris–Tilion cordatae.

In the only two African sites, N. nidus-avis grows in quite different forest types: under Abies numidica and Cedrus atlantica in Algeria (Kabylia; De Smet & Bouanza, 1984; Quézel & Santa, 1962) but always close to shoots of Quercus canariensis (Madoui, 2019), and under Q. canariensis and Q. suber in Tunisia (Khroumi; El Mokni et al., 2010).

All the communities described above share the presence of tree species hosting fungal species from the family Sebacinaeae that are the strictly required mycorrhizal associates of N. nidus-avis (see 6.2).

4 | RESPONSE TO BIOTIC FACTORS

Neottia nidus-avis typically grows in habitats with a sparse herb layer (AHO Sachsen-Anhalt, 2011), probably because shaded conditions limit photosynthesis, and thus it does not suffer from the competition of surrounding vegetation, unless the site is disturbed. Seed germination is dependent on the presence of suitable mycorrhizal fungi (see 6.2).

5 | RESPONSES TO THE ENVIRONMENT

5.1 | Gregariousness

Neottia nidus-avis grows singly or in sparse groups, and rarely in populations numbering up to 100 plants. However, woodland complexes can host many sparsely distributed individuals, as shown by the detailed survey of 4 km² in Darłubskia Forest (Poland), where 650 individuals were counted, of which almost 50% were solitary plants (A. Eichmann, unpubl. data). Protracted subterranean development (see 6.3) and the existence of vegetative dormancy (M.-A. Selosse; pers. obs. of dormant rhizomes with a resting bud in soil) do not allow assessment of the exact number of individuals in a population. Observations on a population growing in Polish Province showed annual dynamics from 69 to 88 plants over 4 years (Vakhrameeva et al., 2008). A 6-year observation study in a permanent 1 x 1 m² plot in Estonia showed that of the initial 11 plants only one was present in years 2, 3 and 5 and their distribution in the plot may suggest that they were not the same individuals (Kull & Tuulik, 1994). Similar observation of a fluctuating number of flowering shoots (35, 41, 6, 2 in successive years) was made during four-year observations in a 20 x 10 m² permanent plot in Poland. However, previously unobserved shoots appeared outside the permanent plot in the final year of the study and outnumbered those observed in the first 2 years on the permanent plot, indicating high spatio-temporal population dynamics (J. Minasiewicz, unpubl. data).

5.2 | Performance in various habitats

Neottia nidus-avis occupies a variety of habitats on a vast geographic area which may result in some regional habitat variability in plant performance. However, the present data are too sparse and anecdotal to allow any pattern recognition.

5.3 | Effect of frost, drought, etc.

Populations in the British Isles appear intolerant of minimum average January temperatures below 0°C (Figure 2). However, both the Siberian and montane localities indicate that it is most likely frost tolerant.

To our best knowledge there are no data on drought response of N. nidus-avis, but reduction in the number of flowering shoots may be one response, as seen in other temperate mycoheterotrophic orchids. This may be a result of reduced fungal host availability due to drought-induced reduction in fungal biomass (McCormick et al., 2009; Verrier, 2017).

As a non-photosynthetic species Neottia nidus-avis can grow in shady places, tolerating the heaviest of shade. It is one of only four species in the British and Irish flora with an Ellenberg indicator value of 2 for light (i.e., it grows in shade to deep shade with less than 5% of relative illumination; Hill et al., 2004).

6 | STRUCTURE AND PHYSIOLOGY

6.1 | Morphology and anatomy

Neottia nidus-avis is a brownish, heterotrophic plant (Figure 4a) with short rhizomes bearing numerous, tightly packed, thick, glabrous and short adventitious roots (Figure 5a). The lower part of the shoot is wrapped in 3–5 brownish scales. Leaf scales have very few vascular bundles, which are located at the leaf margin and centre, but water storage and mechanical support of adjacent tissues can be assisted by tracheid cells (Aybeke, 2012). Its mesophyll is composed of eight or nine layers of thin-walled cells with small intercellular spaces, rarely with oxalate raphides (Stern, 2014). The stomata on scale leaves are sparsely distributed on the abaxial surface, having stoma
tal pores (Figure 5b) that are smaller than those of photosynthesizing orchid taxa (Aybeke, 2012; Ziegenspeck, 1936). This is contrary to Stern (2014) who reported absence of stomata from both surfaces of scale leaves. N. nidus-avis inflorescence is densely multi-flowered
for historical review), until their detection by molecular methods was possible. Then, the symbionts were shown to be highly specific, from the Sebacinaeae (McKendrick et al., 2002; Selosse et al., 2002), a family within the Sebacinales (Basidiomycota). Unlike its sister family Serendipitaceae, from which many mycorrhizal fungi of green orchids are recruited, Sebacinaeae are as-yet unculturable (Weiß et al., 2016). This identification is congruent with ultrastructural hyphal traits revealed by electron microscopy (Barmicheva, 1990).

These Sebacinaeae also form mycorrhizas of the ectomycorrhizal type on surrounding tree roots (Selosse et al., 2002), through which they are inferred to extract the carbon resources supporting the whole system, as in other mycoheterotrophic plants from the temperate zone (Merckx, 2013). Indeed, whenever Sebacinaeae DNA polymorphism was observed from one orchid root to another, similar DNA variants colonised the very nearby tree roots (Selosse et al., 2002), providing evidence of a mycelial link with surrounding trees.

6.2 | Mycorrhiza

6.2.1 | Mycorrhizal diversity

Identity of fungal associates remained elusive over the 20th century, despite intensive attempts to cultivate them (see Rasmussen, 1995 for detailed description of species morphology in the beginning of this account). The plant often produces several flowering shoots on one rhizome, with up to nine on the most vigorous individuals (Vakhrameeva et al., 2008).

6.2.2 | Mycorrhizal colonisation

Mycorrhizal colonisation intensity (the percentage of root length with mycorrhizal fungi) is high in the roots and the rhizome (80%–90%, Vakhrameeva et al., 2008) in every growing season (M.-A. Selosse, pers. obs.). The infection appears in roots when they are 0.3–0.5 cm long, yet the fungal entrance point has never been observed on the roots, which are surrounded by a double layer of thin uninfected cells (Figure 5c). Selosse et al. (2002) speculate that colonisation occurs from soil to rhizome, and then to roots. The species is strongly mycoheterotrophic in all parts of its distribution range and the intensity of mycorrhizal colonisation in the rhizome increases with the plant age. Thus, mycorrhization reaches 20% in juvenile plants, but 75%–85% in adults (Tatarenko, 1996). As in all orchids, older pelotons formed in living cells (Figure 5f) undergo lysis (Figure 5d,e) before new hyphae eventually recolonise the cells (Rasmussen & Rasmussen, 2009). The same fungus is also involved at the stage of mycoheterotrophic germination, as seen from analysis of germinations recovered after in situ seed sowing (McKendrick et al., 2002; see 8.3).

6.3 | Perennation: Reproduction

Sexual reproduction by seed prevail in the species and is described in detail in Section 8.3. Neottia nidus-avis has a rather slow development from seeds. Because it is believed that during the early years the previous rhizome segment disappears and is replaced by a new but similar rhizome segment, the initial stages are virtually indistinguishable from each other in the soil, making any estimate of duration of the developmental stages unreliable. It is estimated that it takes 9–10 years from the protocorm stage (see 8.5) to the generative phase (Bernard, 1909; Rasmussen, 1995; Vakhrameeva et al., 2008; Ziegenspeck, 1936). However, Tatarenko (2002)
reported much shorter ontogenesis from Moscow Province, where it lasted for 3–5 years. When a flowering shoot develops, it remains alive until the fruits ripen, and then often dies completely together with the rhizome, suggesting a monocarpic life history (Rasmussen, 1995). However, this view of the rhizome as monopodial (i.e. growing from its tip bud exclusively) is challenged by the observations (Champagnat, 1963; Prillieux, 1856; Ziegenspeck, 1936; M.-A Selosse pers. obs.) that lateral buds, although most often dormant, allow sympodial growth so that the individual can persist. Champagnat, (1963) estimates that only 10%–60% of rhizomes die after flowering. Indeed, sympodial growth explains why some rhizomes are branched and even bear several flowering shoots (Prillieux, 1856). A growing bud is sometimes observed at the base of the current year’s shoot or, sometimes, of dead shoots of a previous year (Irmsich, 1853; M.-A Selosse pers. obs.). Vakhrameeva et al. (2008) reported numerous cases in the Caucasian and Crimean populations, where one shoot died, while a rhizome continued its growth in a sympodial mode for 2–4 more years.

Vegetative reproduction may occur through branching of a rhizome from resting axillary buds positioned on a basal part of the rhizome (Rasmussen, 1995; Tatarenko, 2002; Vakhrameeva et al., 2008). Rhizome branching is rare in immature individuals and is more typical of adult plants. Frequency of this mode of vegetative reproduction is rather low; Tatarenko (2002) noted it only in just one plant in a population of 130 individuals.

The second, more frequent vegetative reproduction mode occurs through a transformation of an adventitious root into a new rhizome (Selosse, 2003; Figure 6c). This unusual transition is favoured when roots become separated from the rhizome accidentally or by decay of their proximal part (Champagnat, 1963); this transition may result from a rhizome-to-root hormonal signalling, since Vanilla planifolia roots can be induced to form shoots upon auxin treatment in vitro (Philip & Nainar, 1988). The tip of the root thins and an outgrowth starts to proliferate from the root cap, with continuity of vascular bundle that soon shifts from the typical root organisation (xylem alternating with phloem) to that of a shoot (xylem internal to the phloem, with a central medulla; Champagnat, 1971). The resulting whitish bud (Figure 6c) is soon colonized by the symbiotic fungus and proliferates, first forming irregular outgrowths and then producing scaly leaf sheaths, which assemble around a vegetative meristem (Champagnat, 1971; Ziegenspeck, 1936). Later, the root bearing the bud gradually decays while new adventitious roots are formed (Figure 6c), generating the form of a juvenile plant. The initial outgrowth at the root tip is sometimes viewed as a developmental reiteration of a protocorm (Champagnat, 1963, 1971), yet the presence of vascular tissues, which are absent in a protocorm, makes it different. Vakhrameeva et al. (2008) noted such asexual propagation in juvenile and flowering individuals, although they were less frequent in non-flowering individuals. Such a propagation is variable in frequency: in the Urals, it was found in 25%–30% of individuals (Knyasev & Knyaseva, 1988), while in observations from Caucasus, the Crimea and Moscow Province, it did not exceed 3% (Tatarenko, 2002). Reasons for this variability among plants and among populations remain unclear. Asexual propagation through underground organs is also known from other mycoheterotrophic plants, exemplifying convergent evolution (Klimešová, 2007; Roy et al., 2009). It may be favoured by the local presence of the vital mycorrhizal fungus, which often displays patchy distribution, making vegetative propagation an essential adaptive trait for these strictly fungal-dependent plants (Roy et al., 2009). The fact that seeds germinate better around adults (see 8.4) supports this patchiness.

Whether asexual propagation adapts somehow to fungal availability or physiological status remains unclear.

6.4 | Chromosomes

The chromosome number reported for Neottia nidus-avis is 2n = 36 (Capineri & Rossi, 1987; Kliphuis, 1963; Rice et al., 2015; Ruiz, 1995). A detailed karyological study by Bartolo et al. (2010) found the karyotype of N. nidus-avis to be composed of 11 metacentric, 1 submetacentric, 2 subteloentric and 4 telocentric pairs. Chromosome pair 1 is long; pairs 2 to 18 are progressively shorter; pair 11 possesses a small satellite on the short arm. All chromosomes have centromeric C-bands. Pair 2 shows a terminal C-band in the long arm. Pairs 3, 5 and 18 have heterochromatic short arms (Bartolo et al., 2010). The authors draw attention to a large number of large telocentric
chromosome pairs in comparison with the genus *Epipactis* (also tribe Neottieae) and frequent associations among telomeric chromosomes in metaphase.

### 6.5 Physiological data

The species is mycoheterotrophic from germination to adulthood and thus obtains all mineral and carbon resources from its mycorrhizal fungi (see 6.2) that are shared with surrounding trees. Although *N. nidus-avis* has a little chlorophyll *a*, many carotenoids (Reznik, 1958; Reznik et al., 1969; Seybold & Egle, 1937) and even traces of chlorophyll *b* (Haspelova Horvatovicova & Holubkova, 1980; Pfeifhofer, 1989), their amounts and ratios (a/b ≥ 40), which is 3- to 10-fold higher than in photosynthetic plants, do not allow photosynthesis (Pfeifhofer, 1989; Reznik, 1958). Moreover, Cameron et al. (2009) reported fluorescence values indicative for ineffective photosynthesis. Beyond pigments, the absence of photosynthesis was directly confirmed by the lack of carbon fixation (Cameron et al., 2009; Hudák et al., 1997; Montfort & Küsters, 1940) and the absence of O₂ evolution in light (Menke & Schmid, 1976).

The plastid genome is correspondingly much reduced (92 kb, i.e., 35% smaller than in photosynthetic orchids; Logacheva et al., 2011). Although gene loss is less pronounced than in some other mycoheterotrophic orchids (e.g. Asiatic species from the same genus; Feng et al., 2016), all genes encoding photosynthetic proteins have been lost, as well as some RNA polymerase subunits, but most genes for the translational apparatus and some housekeeping genes (*accD, clpP, ycf1*, *ycf2*) have been retained (Logacheva et al., 2011). The loss of photosynthesis coincides with the pseudogenisation of the large subunit of RuBisCO enzyme, *rbcL*. In *N. nidus-avis*, at least three distinct *rbcL* sequences likely located in the plastid genome have been described. Different plastomes, each containing a different pseudogene, can be present in the same plant – a condition called heteroplasy (Cafasso & Chinalli, 2012).

The mechanism by which organic resources are extracted from the fungus remains unclear. They may be transferred either through the living interface between the partners, the intracellular peloton (Figure 5d) or during the final degradation of old pelotons (Figure 5e; Dör & Kollmann, 1969). Contradictory evidence has been published on this in mycoheterotrophic orchids (reviewed by Selosse, 2014), but the idea that they simply exploit biotrophic nitrogen transfer in the form of amino acids which are used as a source of carbon by mycoheterotrophic plants is gaining momentum (e.g. Lallemand et al., 2019). This may be the reason for the high N content of mycoheterotrophic plants (Merckx, 2013; Stöckel et al., 2014). As expected for roots of a mycoheterotrophic plant, expression levels of genes related to plant-pathogen and symbiont interactions were high, along with enzymatic activities connected to substrate degradation (i.e. proteasome and glycosaminoglycan degradations) as well as of the transportome (e.g., ABC transporters and solute carriers; Jąkalski et al., 2021).

The use of fungal resources gained from surrounding trees, which has been demonstrated for some mycoheterotrophs (*Hypopitys monotropa, Corallorhiza trifida* and *Rhizantella gardneri*) by labelling of tree photosynthates (Hynson et al., 2013), has not yet been directly demonstrated for *N. nidus-avis*. This species, nevertheless, shares with these other mycoheterotrophs the features indicating such functioning. First, the plant is spontaneously enriched in ¹¹N and ¹³C isotopes (Stöckel et al., 2014; Suetsugu et al., 2020), a feature that reflects the ¹¹N and ¹³C abundance in fungi ectomycorrhizal on trees but differs from that in saprotrophic fungi (Hynson et al., 2013). Thus, the classical view of *N. nidus-avis* as a ‘saprotrophic’ plant (Hudák et al., 1997) is incorrect, even considering only the biology of its fungal associates. The second piece of evidence for a link to surrounding trees as a carbon source comes from radiocarbon (¹⁴C) measurements that evaluate the time elapsed since photosynthetic carbon fixation: the short transfer time observed in *N. nidus-avis*, with a radiocarbon age similar to that of autotrophic plants (Hatté et al., 2020; Suetsugu et al., 2020), strongly differs from the older radiocarbon age of mycoheterotrophic plants associated with saprotrophic fungi and can be explained by rapid carbon transfer through mycelial links. Finally, *N. nidus-avis* is the only mycoheterotrophic species linked to trees for which there is evidence for sharing not only the same fungal species but also the same individual fungal mycelia (Selosse et al., 2002).

### 6.6 Biochemical data

The plant is often viewed as a chlorophyllous, which is not entirely true (see 6.5). One anecdotal trait demonstrates this: when put into solvent, heated water, or close to a flame, the inflorescence colour shifts from brownish to greenish (Figure 5g; Mangenot & Mangenot, 1966; Reznik, 1958). Chlorophyll is most likely associated with the brown coloration of *N. nidus-avis*, as chlorophyll is absent in white var. *nivea* (Figure 5f; Reznik, 1958). The explanation for the brown colour and the experimental greening is either from a second pigment masking chlorophyll (Reznik, 1958) or, our preferred explanation, a link to thermosensitive chlorophylls and/or carotenoids-protein complexes that modify the light absorbance spectrum (Menke & Schmid, 1976), as found in brown algae (Menke, 1940). Indeed, recent transcriptome analyses confirmed that *N. nidus-avis*, unlike other mycoheterotrophic plants – *Epipogium aphyllum* and *Gastrodia elata* – expresses the full range of genes required for the synthesis of chlorophyll as well as some chlorophyll a/b binding proteins that are mostly activated in flowers (Jakalski et al., 2021). These pigment-protein complexes are stored in plastids, whose altered structure reflects a loss of photosynthetic function. These are spindle-like with numerous, isolated (not grana-forming) thylakoids, arranged in parallel or coiled as globules (or droplets), but are devoid of starch in accordance with the absence of photosynthesis (Hudák et al., 1997; Mangenot & Mangenot, 1966; Menke & Schmid, 1976). These plastids are found in shoots, even in the phloem (Danilova & Barmicheva, 1990). The
role of these pigments is, thus, linked to aerial conditions, possibly to mimicry of surrounding dead leaves. This adaptation is important for plants that have lost chlorophyll as shown for Monotropis odorata, where the presence of dried bracts resembling leaf litter effectively protects plants from herbivory (Klooster et al., 2009). The pigments may also have some other role directly related to light. Menke and Schmid (1976) offered evidence for cyclic photophosphorylation, for instance, with ATP production by cyclic functioning of Photosystem I. The possibility of this pathway generating energy with neither O₂ evolution nor CO₂ fixation deserves closer inspection; however, absence of most plastid and nuclear genes coding for Photosystem I and cytochrome b6/f does not support this hypothesis (Jąkalski et al., 2021).

The unusually high zeaxanthin content in shoots (Haspelova Horvatovicova & Holubkova, 1980; Pfeifhofer, 1989) is suggestive of a xanthophyll cycle, which plays an important role in the protection against oxidative stress. On the other hand, the absence of conversion of violaxanthin to zeaxanthin on illumination (Haspelova Horvatovicova & Holubkova, 1980), confirmed by the lack of the crucial enzyme of the cycle, points to alternative reasons for zeaxanthin accumulation (Jąkalski et al., 2021). Finally, the reason for high concentrations of tocopherol in N. nidus-avis (Reznik et al., 1969), another metabolite connected to photoprotection, deserves further investigation. A clear understanding of the role of all these metabolites in N. nidus-avis remains pending.

7 | PHENOLOGY

Seeds of N. nidus-avis germinate in spring, in humid substrates after contact with a suitable mycorrhizal fungus (Rasmussen, 1995). The experiment conducted by McKendrick et al. (2002) involving successive sampling of previously buried seed samples showed a slow progression of underground seedling growth, measured as the volume of each germinated seedling. After 9 months, the volume of protocorms was 0.05–0.13 mm³. It successively increased to 0.14–0.37 mm³ after 11 months; 0.38–0.99 mm³ after 18 months; 1.00–2.72 mm³ after 23 months; and 2.73–54.59 mm³ after 30 months in soil. Between 18 and 23 months of growth, pear-shaped protocorms begin to branch. All seedlings with a volume greater than 1.64 mm³ are branched. At this stage, rootlets develop from the protocorm and subsequently shoot-bud develops from the apical meristem (see 8.5).

Mature rhizome formation may take several years (see 6.3). Rhizome growth in vegetative individuals starts in April–June in areas with dry and hot summers and in May–August in milder climates. During this period, an individual sprouts a new annual segment of monopodial rhizome consisting of 6–7 growth units (Champagnat, 1963; Ziegenspeck, 1936). In unfavourable seasons, rhizome growth does not occur and the terminal bud remains dormant. The flowering shoot meristem is formed in the terminal bud of the underground rhizome one season before sprouting (Vakhrameeva et al., 2008). Flowering is from May to June. The shoot persists until fruit maturation in August-September, and then dies and dries completely, often but not always together with rhizome (see 6.3).

8 | FLORAL AND SEED CHARACTERS

8.1 | Floral biology

A variable number of flowers is borne on the flowering shoots, usually 25–35. Flowers open acropetally and age sequentially. The sepals and petals form a loose hood arching over the lip that secretes nectar in the basal shallow cavity (Figure 4d; Claessens & Kleynen, 2011). Sometimes only a few drops are produced, depending on the air humidity (Müller, 1883). In younger flowers, nectar can be found in large quantities. The flowers produce a sweet scent, perceived as honey-like or mouldy (Summerhayes, 1951; Ziegenspeck, 1936). The anther opens before anthesis, then quickly dries out and falls up. At the same time, the rostellum margins curl up exposing the mealy pollinia in the groove of the rostellum (Figure 4b). Stigmatic lobes run either side of the rostellum. On the crest of the rostellum are minute rough points, which are particularly sensitive to touch, causing the expulsion of the viscid matter that glues pollinia to a pollinator; this so-called touch-sensitive rostellum is a unique, shared feature within the genus Neottia (Claessens & Kleynen, 2011; Darwin, 1862). After that, the rostellum bends towards the stigma, preventing freshly removed pollinia from being deposited in the same flower. After 2 days, the rostellum recovers its original position (Claessens & Kleynen, 2011). The rostellum then shrinks and the stigma appears swollen. At this time, pollinia hanging over the rostellar edge can touch the stigma, causing self-pollination regardless of whether the mechanism was triggered or not.

Several authors have observed flies, crawling thrips and ants as regular visitors (Figure 4d; Burns-Balogh et al., 1987; Müller, 1883; Prochážka & Velísek, 1983; Reinhard et al., 1991; van der Cingel, 2001). Both Darwin (1862) and Müller (1883) observed self-pollination and described the adaptation of flowers to this obviously predominant pollination mode. Their findings are supported by observations of Claessens and Kleynen (2011), who inspected many flowers of N. nidus-avis over various years and found that pollinia were rarely removed from the plants. In many plants, the rostellum spontaneously expelled the sticky fluid, dried out and became non-functional. The pollinia soaked with stigmatic fluid disintegrated and self-pollination took place. Although the exact proportion of self- to cross-pollination needs to be empirically tested, the preliminary results of population genetic studies based on 14 populations and the diversity of eight nuclear microsatellite markers (J. Minasiwicz, unpubl. data) seem to confirm prevalence of autogamy in the species, as indicated by the high average value of the fixation index (Fᵢₛ = 0.798). A majority of populations was genetically diverse, indicating that there is at least some allogamous pollination by insects to generate genetic diversity.
Individuals of *N. nidus-avis* have been observed flowering and fruiting underground (Figure 4c; Bernard, 1899; Fuchs & Ziegenspeck, 1926; Ziegenspeck, 1936), but no research has been conducted to reveal whether this is an anomaly or a frequent phenomenon.

### 8.2 Hybrids

No inter- or intrageneric hybrids are known.

### 8.3 Seed production and dispersal

The natural fruiting rates are generally high, including 75%–85% in Moscow Province (Vakhrameeva et al., 2008), 72%–97% in Germany and Switzerland (Claessens & Kleynen, 2011), determined from specimens in the herbarium database of Hungarian orchids (Molnár et al., 2011), and references therein), and 80% in Moscow Province (Vakhrameeva et al., 2008), 72%–97% in France and Germany (Claessens & Kleynen, 2011), 85% in Moscow Province (Vakhrameeva et al., 2008), 72%–97% in France and Germany (Claessens & Kleynen, 2011), and references therein. Smreciu and Currah (1989) obtained a low germination success with fungal strains identified as *Ceratobasidium cereale* (a fungal pathogen from grasses) and *Rhizoctonia (= Tulasnellia anat- icula* (isolated from *Platanthera obtusata*), where the seedlings grew to a length exceeding that of testa. However, no further development occurred, as these fungi do not form a functional mycorriza with *N. nidus-avis*.

A very low seed germination percentage of less than 28% was recorded during a 3-year experiment with in situ buried seed packets conducted on a large population of the species growing in a beech woodland in Hampshire, UK. (McKendrick et al., 2002). The authors noted that seed germination success varied within the site, with very low germination frequency in the plots located further from the adult plants, thus indicating that the vicinity of adults offers higher abundance of appropriate mycorrhizal Sebacinaeae fungi for germination (Sebacinaeae cannot be cultivated in vitro; see 6.2). Even lower germination success of *N. nidus-avis* was observed in evergreen forest dominated by *Quercus ilex* in Sardinia (Stöckel et al., 2014); germination was recorded in 2.7% of seed packets, resulting in only about 0.1% of germinating seeds within a year of seed burial.

### 8.5 Seedling morphology

The mycorrhizal seedling (protocorm) represents a weakly differentiated body typical for all orchids. Seedling ontogeny follows descriptions in Bernard (1899, 1902), Fuchs and Ziegenspeck (1926), McKendrick et al. (2002), Selosse et al. (2011), Vakhrameeva et al. (2008) and Ziegenspeck (1936). Germination starts with an increase in translucence of the embryo within the testa. After fungal penetration through the testa and in cells at the base (micro-pylar end, virtual position of the suspensor; Figure 6a.2) of the embryo, seedlings increase in size to c. 0.003 mm³. Most seedlings of size 300 × 200 μm (c. 0.0119 mm³) contain pelotons of fungal hyphae, with digestion stages in some cells (Figure 6a.3). At the chalazal end, parenchyma cells become filled with starch. After that, protocorms continue to expand until they burst through the testa as small, rhizoid-free and unbranched (Figure 6b) and eventually become pear-shaped (Figure 6a.4,b). Subsequent developments involve the production of side branches at right angles to the main axis of the protocorm, first root initiation (Figure 6a.3) and the formation of a cone-shaped apical bud, sometimes with leaf primordia. After the beginning of growth of the terminal bud on a protocorm, a juvenile shoot forms with roots on the first internode. The seedling then consists of a rhizome of 0.3–1 cm in length with 3–15 roots 0.2–0.9 mm long. Transition into the immature stage is characterised by an increase in the rhizome size (up to 2 cm long), the number of roots (20–50) and beginning of axillary bud differentiation on the rhizome. The protocorm often persists as a comma-shaped body at the extremity of the rhizome.

### 8.4 Viability of seeds: germination

Asymbiotic in vitro germination experiments were unsuccessful, as were symbiotic germination trials with fungi normally mycorrhizal with green orchids, so-called ‘rhizoctonia’ (Rasmussen, 1995 and references therein). Smreciu and Currah (1989) obtained a low germination success with fungal strains identified as *Ceratobasidium cereale* (a fungal pathogen from grasses) and *Rhizoctonia (= Tulasnellia anat-icula* (isolated from *Platanthera obtusata*), where the seedlings grew to a length exceeding that of testa. However, no further development occurred, as these fungi do not form a functional mycorriza with *N. nidus-avis*.

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(Ziegenspeck, 1936). The adult stage is reached when the rhizome is c. 3 cm long, with 80–120 roots, each 1.5–3 cm long.

9 | HERBIVORY AND DISEASE

9.1 | Animal feeders or parasites

Because the underground organs of *N. nidus-avis* contain starch, the shoot buds are often damaged or destroyed by soil invertebrates and insect larvae (Vakhrameeva et al., 2008). Unlike other orchid species, the flowering shoots are not damaged by deer or wild boar (Jersáková, pers. obs.).

9.2 | Plant parasites and diseases

Dark necrotic spots are often noticeable on the roots (Selosse, pers. obs.), but the causative pathogen remains unknown.

10 | HISTORY

*Neottia nidus-avis* was first described by Carolus in Linnaeus (1753), as *Ophrys nidus-avis*. Subsequent authors moved the species into various genera, such as *Epipactis* (Crantz, 1769), *Listera* (Curtis, 1778), *Helleborine* (Schmidt, 1793), *Malaxis* (Bernhardi, 1800), *Serapias* (Steudel, 1821), *Neottiodium* (Schlechtendal, 1823) and *Distomaea* (Spenner, 1825). Richard (1818) named the species *Neottia nidus-avis*, and this name was retained though several synonyms followed: *N. macrostelis* (Dulac, 1867) and *N. squamosa* (Bernhardi, 1800), and *N. orobanchoidea* (Saint-Lager, 1880).

Both the English common name – Bird’s-nest Orchid and the scientific name – *N. nidus-avis* refer to the roots forming an untidy mass, which vaguely resembles a roughly built nest of a wood pigeon or rook. Similarly, other colloquial names relate to the bird-nest appearance (Irish – Magairlín neide éin, Welsh – Tegeirian nyth aderyn, Norwegian – Fuglereir, Dutch – Vogelnestje, French – Nid-d’oiseau), bird’s nest of roots (German – Vogelnestwurz) or nest of helminths (Czech – hlístník).

Perhaps the earliest British record is that of Gerard, mentioned in his *Herball* in 1597, where he referred to finding of ‘*Satyrium abortive, sive Nidus Avis*’ growing in the middle of a wood in Kent, 2 miles from Gravesend (Foley & Clarke, 2005).

The symbiotic (mycoheterotrophic) germination of orchid seeds was discovered in this species by Noël Bernard (1899), who serendipitously observed a bent shoot of *N. nidus-avis* buried in the soil under the layer of dead leaves with germinating seeds tightly covered by mycelial filaments (Selosse et al., 2017). This finding gave him the opportunity to study the first germination stages in orchids. Yet, the interpretation of mycorrhizae (i.e. the understanding that coiled structures in the cells are of fungal origin) should be credited to Reissek (1847), in the first part of the 19th century.

11 | CONSERVATION

*Neottia nidus-avis* has suffered a considerable decline throughout the 20th century, particularly between 1930 and 1970, and especially in south-eastern England (Preston et al., 2002). Although this decline appears to have slowed, localised losses have continued to occur throughout its British and Irish range over the past 20 years (K.J. Walker, pers. comm. 2022). Kull and Hutchings (2006) calculated a 54% decline in the number of hectarcd occupied by the species in the United Kingdom, between the periods 1930 and 1969 and 1987 and 1999. A much less severe decline of 11% was reported for Estonia. Harrap and Harrap (2005) estimated the proportional loss of occupied hectarcd during the period 1500–1986 as 27.5% and 29% for Britain and Ireland, respectively, with a relatively large proportion of the British losses being recent. Outside the British Isles, the Bird’s-nest Orchid is also declining (see below the ranking of the species in national red lists).

The species is very vulnerable to habitat disruption, and most losses are probably due to changes in woodland management, woodland clearance, use of heavy machinery in forestry operations, destruction of the habitat by fire and conversion of deciduous woodland into conifer plantations. In addition, this orchid is affected by urbanisation, tourism and related infrastructure expansion, as well as plant collection (Rankou et al., 2014). In recent years, the loss of old broadleaved woodland has slowed down as the conservation and cultural values of this habitat have become generally recognised. However, its especially rapid decline from apparently suitable habitats in south-eastern England and East Anglia suggests additional causes, possibly decreased spring and summer rainfall coupled with increased atmospheric deposition of nitrogen and other pollutants. Additionally in woodlands, tree seedlings continue to be damaged by increasing numbers of deer (particularly in southern England) and sheep (in the uplands) that hinder spontaneous regeneration of host species important for Sebacinaeae fungi.

While habitat conservation remains the only way of protecting *N. nidus-avis*, along with the vast majority of other mycoheterotrophic plants, attempts are also being made to develop their ex situ propagation, potentially enabling their reintroduction into suitable habitats. The most promising method for germination of mycoheterotrophic plants appears to be one that initiates an ex situ triplicate symbiosis with tree seedlings, fungi and orchids (Warcup, 1985) and although initial application of this method has failed to initiate seed germination of *N. nidus-avis*, there is still room for refinements (Hughes, 2018). Micro-propagation of *N. nidus-avis* has also been attempted, but this experiment did not develop beyond callus formation (Sheyko & Musatenko, 2011).

The species is classified as *Near Threatened* in the Red List for Great Britain (Cheffings & Farrell, 2005) and of *Least Concern* for Wales (Dines, 2008) but was recently categorised as *Vulnerable* in England, due to marked decline in area of occupancy (Stroh et al., 2014). On the Irish Red List, the species ranks of *Least Concern* category (Wyse-Jackson et al., 2016). Globally, the species is of *Least Concern* on the European Red List of Vascular Plants (Bilz et al., 2011). In the continental Atlantic zone,
the species is rare, as shown by its protection in Bretagne and Limousin regions of France, as well as in Belgium and Luxembourg (Bournérias & Prat, 2005). Regionally, the species is listed as Critically Endangered in Netherlonds (Soortenbank, 2017), Near Threatened in the Czech Republic (Grulich, 2012) and Norway (Kålås et al., 2010), Least Concern in Upper Austria (Hohl et al., 2009) and Switzerland (BAFU, 2016), but it is not listed for the whole of Austria (Nikfeld, 1999), Estonia (Red Data Book of Estonia, 2008), Germany (Korneck et al., 1996), Denmark (Wind & Pihl, 2010), France (UICN France, MNHN, FCBN & SFO, 2009), Sweden (Aronsson et al., 2010) or Ukraine (Didukh, 2009). It is not listed in the red lists and books of Slovak Republic (Feráková et al., 2007), Bulgaria (Petrova & Vladimirov, 2009), Greece (Phtios et al., 2009), Poland (Kaźmierczakowa et al., 2016), Spain (Banares et al., 2008), Finland (Rassi et al., 2001), Italy (Rossi et al., 2013), Slovenia (Skoberne, 2007) and Lithuania (Rašomavičius, 2007). The species is protected as a rare plant in Russia (in 16 regions, Vakhrameeva et al., 2008), in Romania (Oltean et al., 2009) and in Hungary (Király, 2007).

AUTHORS’ CONTRIBUTION
All authors contributed equally to writing this account. All authors contributed critically to the drafts and gave the final approval for publication.

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CONFLICT OF INTEREST
The authors have no conflicts of interest to declare.

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DATA AVAILABILITY STATEMENT
Data sharing not applicable – no new data generated.

ORCID
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REFERENCES
Alibertis, A. (1998). The orchids of Crete and Karpathos. Albertis Antonis, Anderberg, A., & Anderberg, A.-L. (2016). Den virtuella floran. Elektronisk Publikation, Naturhistoriska riksmuseet. http://linnaeus.nrm.se/flora

Anthos. (2012). Information System of the plants of Spain, Real Jardín Botánico, CSIC - Fundación Biodiversidad. Retrieved from http://www.anthos.es

Arditti, J., & Ghani, A. K. A. (2000). Tansley Review No. 110. Numerical and physical properties of orchid seeds and their biological implications. New Phytologist, 145(3), 367–421.

Aronsson, M., Edqvist, M., Andersson, U.-B., Bertilsson, A., Ericsson, S., Mattiasson, G., & Stahl, P. (2010). Käräväkar - vascular plants (Tracheophyta). In U. Gärdenfors (Ed.), The 2010 Red List of Swedish Species (pp. 201–221). ArtDatabanken.

Assyov, B., Dimitrov, D., & Vassilev, R. (2002). Conspectus of the Bulgarian vascular flora. Distribution maps and floristic elements (2nd ed.). Bulgarian–Swiss Biodiversity Conservation Programme.

Aybeke, M. (2012). Comparative anatomy of selected rhizomatous and tuberous taxa of subfamilies Orchidoideae and Epipendroideae (Orchidaceae) as an aid to identification. Plant Systematics and Evolution, 298, 1643–1658.

BAFU. (2016). Rote Liste Gefäßpflanzen. Bundesamt für Umwelt BAFU.

Banares, B. A., Blanca, G., Güemes, H. J., Moreno, S. J. C., & Ortiz, S. (2008). Red list of Spanish vascular flora. Tragsatec.

Barmicheva, K. M. (1990). Ultrastructure of Neottia nidus-avis mycorrhiza. Agriculture, Ecosystems & Environment, 29(1), 23–27.

Bartha, D., Király, G., Schmidt, D., Tiborcz, V., Barina, J., Csiky, G., Jakab, B., Lesku, A., Schmotzer, R., Vidéki, V., Vojtkó, A., & Zólyomi, S. (2015). Distribution atlas of vascular plants of Hungary. Nyugat-magyarországi Egyetem Kiadó.

Bartolo, G., Brullo, C., Pulvirenti, S., Scrugli, A., Terrasi, M., & Saverio, D. (2010). Advances in chromosomal studies in Neottieae (Orchidaceae): Constitutive heterochromatin, chromosomal rearrangements and speciation. Caryologia, 63, 184–191.

Beck-Mannagetta, G. (1903). Flora Bosne. Hercegovine i novopazarskog sandžaka. Gymnospermeai i Monocotyledones., Zemaljska Štamparja.

Bernard, N. (1899). Sur la germination du Neottia nidus-avis. Comptes Rendus de l’Académie des Sciences, 128, 1253–1255.

Bernard, N. (1902). Études sur la tubérisation. Annales Des Sciences Naturelles, Botanique, Paris, 9, 1–196.

Bernhardi, J. J. (1800). Systematices Verzeichnis der Pflanzen, welche in der Gegend um Erfurt gefunden werden. Ester Theil.

Bliz, M., Kell, S. P., Maxted, N., & Lansdown, R. V. (2011). European Red List of vascular plants. Publications Office of the European Union.

Bjørndalen, J. (2015). Protection of Norwegian orchids – a review of achievements and challenges. European Journal of Environmental Sciences, 5, 121–133.

Bojnansky, V., & Fargašová, A. (2007). Atlas of seeds and fruits of Central and East-European flora: The Carpathian Mountains Region. Springer.

Bournérias, M., & Pr’at, D. (2005). Les Orchidées de France, Belgique et Luxembourg, deuxième édition. Biotope.

Brzosko, E., Ostrowiecka, B., Bolesh, J., Gromotowicz, A., Gromotowicz, M., Orzechowska, A., Orzolek, J., & Wojdalska, M. (2017). Seed dispersal in six species of terrestrial orchids in Biebrza National Park (NE Poland). Acta Societatis Botanicorum Poloniae, 84(3), 3557.

BSBI. (2021). Botanical Society of Britain and Ireland’s Distribution Database. Retrieved from https://database.bsbi.org/
Cameron, D. D., Preiss, K., Gebauer, G., & Read, D. J. (2009). The chlorophyll-orcharding carbohydrate Corallorhiza trifida derives little carbon through photosynthesis. New Phytologist, 183(2), 358–364.

Capineri, R., & Rossi, W. (1987). Numeri cromosomici per la flora Italiana: 1127–1135. Informatore Botanico Italiano, 19, 314–318.

Champagnat, M. (1963). Vers une étude expérimentale des problèmes de morphogénèse posés par Neottia nidus-avis Rich. Annales de La Faculté Des Sciences de Clermont, 26, 115–138.

Champagnat, M. (1971). Recherches sur la multiplication végétative de Neottia nidus-avis Rich. Annales de Sciences Naturelles Botanique et Biologie Végétale, 12, 209–247.

Chase, M. W., Cameron, K. M., Freudenstein, J. V., Pridgeon, A. M., Hill, M. O., Madsen, T., Salazar, G., den Berg, C., & Schuiteman, A. (2015). An updated classification of Orchidaceae. Botanical Journal of the Linnean Society, 177(2), 151–174.

Cheatings, C. M., & Farrell, L. (2005). The Vascular Plant Red Data List for Great Britain. Species Status 7. Joint Nature Conservation Committee.

Chytrý, M., & Rafajlová M. (2003). Czech National Phytosociological database: Basic statistics of the available vegetation-plot data. Preslia 75, 1–15. Retrieved from http://www.sci.muni/botany/vegsc i/dbase.php

Claessens, J., & Kleynen, J. (2011). The flower of the European Orchid. Form and function. Schrijen-Lippertz druk.

Crantz, H. J. (1769). Classis cruciforum emendata. J.P. Kraus.

Curtis, W. (1778). Flora Londinensis. William Curtis.

Danilova, M. F., & Barmicheva, E. M. (1990). Root phloem ultrastructure of the saprophytic orchid Neottia nidus-avis. Agriculture, Ecosystems & Environment, 29(1), 73–77.

Darwin, C. (1862). On the various contrivances by which British and foreign orchids are fertilised by insects. John Murray.

De Smet, K., & Bouanza, F. (1984). La structure forestière du mont Babor. Silva Gandavensis, 50, 64–84.

Dudukh, Y. P. (2009). Red Data Book of Ukraine. Vegetable Kingdom. Global Consulting.

Diersen, K. (1996). Vegetation Nord Europas. Eugen Ulmer Verlag.

Dines, T. D. (2008). A vascular plant red data list for Wales. PlantLife.

Dörr, I., & Kollmann, R. (1969). Fine structure of mycorrhiza in A vascular plant red data list for wales. Vegetation Nord Europas. Diersen, K. (1996).

Dulac, J. (1867). Flore du département des Hautes-Pyrénées. F. Savy.

El Mokri, N., Mahmoudi, M. R., & El Aouni, M. H. (2010). Neottia nidus-avis (L.) C. Rich. (Orchidaceae). Planta (Berl), 89, 372–375.

Dressler, R. L. (1990). The orchids. Natural history and classification. Harvard University Press.

Dulac, J. (1867). Flore du département Hautes-Pyrénées. F. Savy.

El Mokri, N., Mahmoudi, M. R., & El Aouni, M. H. (2010). Neottia nidus-avis (L.) C. Rich.: une nouvelle orchidée pour la flore de la Tunisie. L’ Orchidophile. Revue de La Société Française d’ Orchidophilie, 186, 181–187.

Feng, Y. L., Wicke, S., Li, J. W., Huang, W. C., & Jin, X. H. (2016). Lineage-specific reductions of plastid genomes in an orchid tribe with partially and fully mycoheterotrophic species. Genome Biology and Evolution, 8(7), 2164–2175.

Ferákova, V., Maglocký, Š., & Marhold, K. (2001). Červený zoznam paprstorastov a semenných rastlin Slovenska. In D. Baláž, K. Marhold, & P. Urban (Eds.), Červený zoznam rastlin a živočíchov Slovenska (pp. 44–77). Ochrana Přírody 20.

Foley, M., & Clarke, S. (2005). Orchids of the British Isles. Griffin Press in association with the Royal Botanic Garden.

Fuchs, A., & Ziegenspeck, H. (1926). Die Entwicklungsgeschichte der Arten der einheimischen Orchideen und ihre Physiologie und Biologie. II. Listera, Neottia, Goodyera, Botanical Archives, 16, 360–413.

Grossgejm, A. (1949). Opredelitel rastenij Kavkaza. Sovetskaya Nauka.
Yagame, T., Ogura-Tsujita, Y., Kinoshita, A., Iwase, K., & Yukawa, T. (2016). Fungal partner shifts during the evolution of mycoheterotrophy in Neottia. *American Journal of Botany*, 103, 1630–1641.

Zhou, T., & Jin, X.-H. (2018). Molecular systematics and the evolution of mycoheterotrophy of tribe Neottieae (Orchidaceae, Epidendroideae). *PhytoKeys*, 94, 39–49.

Ziegenspeck, H. (1936). *Orchidaceae. Lebensgeschichte Der Blütenpflanzen Mitteleuropas. Band 1, Abteilung 4*. Eugen Ulmer.

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