Abstract

Initially mycoheterotrophic plants have recently been declining in the wild, even without apparent causes. They are affected by a number of biotic and abiotic factors. The aim of this work is to investigate how selected factors, such as nitrate or symbiotic fungi, may influence their distribution in nature. In particular, the work applies *in vitro* experiments, molecular determination of fungal symbionts and stable isotope analyses. It describes both the effects of abiotic factor, specifically nitrate, and biotic interactions of initially mycoheterotrophic plants with fungal symbionts. The inhibition of germination by extremely low concentrations of nitrate in asymbiotic *in vitro* cultures was observed in several orchid species. The degree of sensitivity of each species to nitrate corresponds with the nitrate content of the soil and the nutrient availability requirements of the species according to Ellenberg indicator values. The inhibitory effect of nitrate on orchid germination was also observed in symbiotic *in vitro* cultures. Out of five tested fungal strains, only one *Ceratobasidium* was capable of eliminating the inhibitory effect of nitrate. Furthermore, the work reveals that green mixotrophic orchids use photosynthates to nourish the aboveground parts, whereas the belowground parts are nourished almost exclusively by symbiotic fungi. Unlike autotrophs, roots of mixotrophic species are usually occupied by wide range of fungi. However, during the evolution to mycoheterotrophy, the specialisation towards narrow fungal lineage has been noted. Such a trend has been observed in both mixotrophic pyroloids with reduced leaves and their albinotic forms, as well as in mixotrophic orchids with reduced leaves and mycoheterotrophic orchids. Albino variants, with their low fitness, are less likely to be an evolutionary intermediate to mycoheterotrophic species than mixotrophic individuals with reduced leaves. Thus, the survival of initially mycoheterotrophic plants and their fungal symbionts in nature is dependent on many interrelated factors.