Mycorrhizal fungi of *Prasophyllum*

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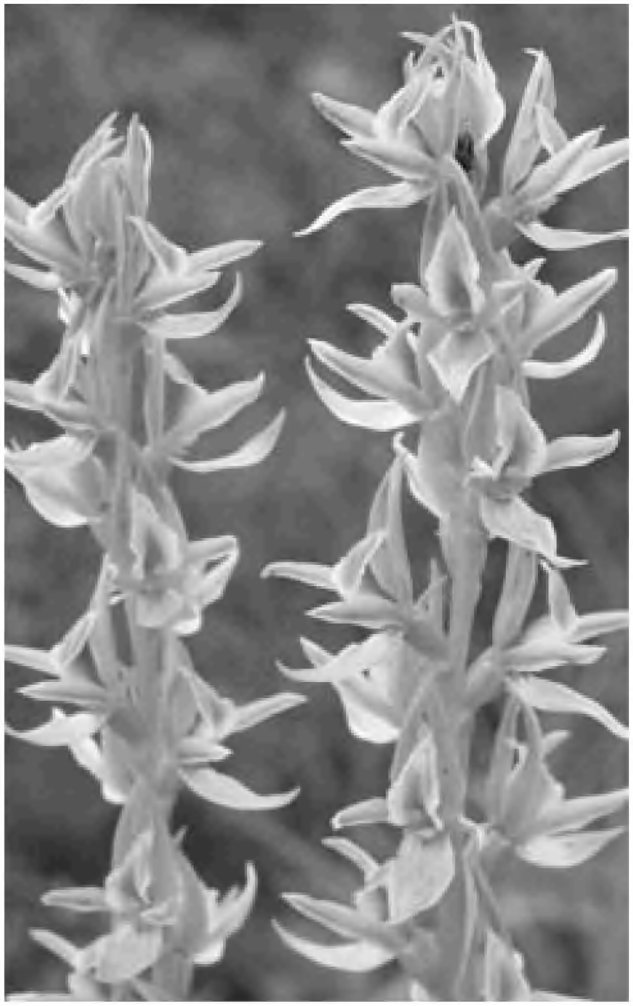
There are more than 380 orchid taxa in Victoria, at least half of which are threatened. The potential extinction of many of these orchids is largely due to habitat destruction caused by degradation from agriculture, industrial development and urbanisation. Effective conservation ultimately depends on reintroduction to field sites so as to reinforce depleted populations. For terrestrial orchids, seed germination is the preferred method of propagation as it allows genetic variability to be maintained (Batty *et. al.* 2006).

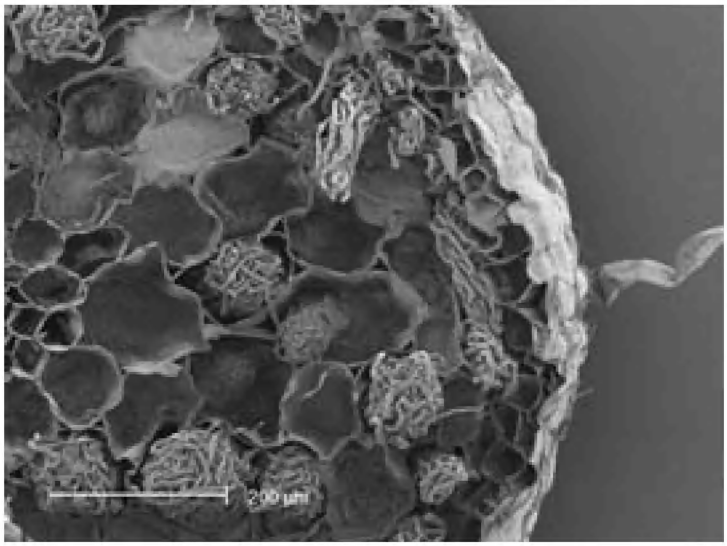
The Genus *Prasophyllum*

The genus *Prasophyllum* currently consists of approximately 80 recognised species in Australia and four species in New Zealand (Jones, 1998). Within Australia there are two centres of diversity for the genus, south-western Australia with 25 species (23 endemic) and south-eastern Australia with 50 species. Within south-eastern Australia 30 species occur in Victoria. Most are threatened and restricted in distribution. Overall, it is one of the most poorly known native orchid genera (Bishop, 1996).

Prasophyllum species are obligate mycotrophic plants, which rely on fungi for seed germination. The fungi are also thought to provide nutrients to the adult plants. Current conservation protocols for terrestrial orchids in Australia require propagation with symbiotic mycorrhizal fungi. Unfortunately there is a paucity of knowledge regarding the mycosymbiont of *Prasophyllum*, hampering conservation and re-introduction efforts. Anecdotal evidence has shown that often the mycorrhizal



Left: Prasophyllum *sp.* aff. validum. *Right: The endangered orchid* Prasophyllum diversiflorum.
Photos: Department of Sustainability and Environment Victoria.



Pelotons (balls of fungal hyphae) visible in a section of Prasophyllum *root. Photo: Emily McQualter*

fungi isolated from adult plants do not germinate seed collected from the same plant. Seed germination trials conducted by the Victorian Department of Sustainability and Environment have had no success in germinating *Prasophyllum* seeds in a range of species. One possibility for this lack of success is that the fungi may have been collected at the wrong time of year. Therefore before recovery plans can be implemented for *Prasophyllum,* basic biological information is required regarding the nature of the mycorrhizal relationship.

This study focuses on two threatened *Prasophyllum* species: *P.* sp. aff. *validum* and *P. diversiflorum*, both from south-western Victoria. *Prasophyllum* sp. aff. *validum* grows in low open grassy heathlands and *Prasophyllum diversiflorum* (Gorae Leek Orchid) grows along open watercourses and around swamps on heavy black loams.

Area of Fungal Colonisation in *Prasophyllum*

Following the use of Scanning Electron Microscopy (SEM) it has been found that the area of fungal colonisation in both species of *Prasophyllum* during early leaf development occurs in the roots, particularly in the upper sections of the root. The colonisation primarily occurs in the cortical cells, the fungi entering the orchid through the epidermis and forming balls of hyphae known as 'pelotons' inside the plant cells. The areas that fungi colonise in orchids differ between genera. In *Caladenia* (Spider Orchids) pelotons are primarily found in the stem-collar region of the plants, while in *Pterostylis* pelotons are found in the underground stem (Ramsay *et. a*l. 1986). The morphology of the fungi in both species of *Prasophyllum* is similar but the number of cells colonised appears to be unpredictable. According to Warcup (1981), the main fungus associated with *Prasophyllum* is *Ceratobasidum cornigerum,* although others occur less commonly.

Ex situ orchid seed baiting trials are currently being conducted to determine whether the fungus that is required to germinate the seed is located in soil from sites where the orchids occur naturally. After three weeks the orchid seeds have already reached stage two germination (seeds have swollen, rhizoids developed and meristem is forming), indicating that the compatible fungus is present. However, the fungi isolated from the adult plants have, after two months of trialling, not yet germinated seed. These preliminary results suggest that for both species, the fungi that germinates seed is different to that found in the tissue of adult plants.

Still to Come

Mycorrhizal fungi will be isolated from adult plants at three more times throughout the year: during the period of flower bud growth (winter), while flowering (spring) and as the fruit develops (spring) to determine whether fungal colonisation and type of mycorrhizal fungi changes throughout the different growth periods. The ability for the isolated fungi to germinate seed will be tested with seed from the Millennium Seed Bank at Royal Botanic Gardens Melbourne. As most mycorrhizal fungi from Australian terrestrial orchids do not sporulate in culture and therefore cannot be identified by normal taxonomic means, DNA from fungal isolates will be ITS-sequenced and closest GenBank matches will be determined. The information gained in this study will provide the basis for further re-introduction and conservation studies.

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