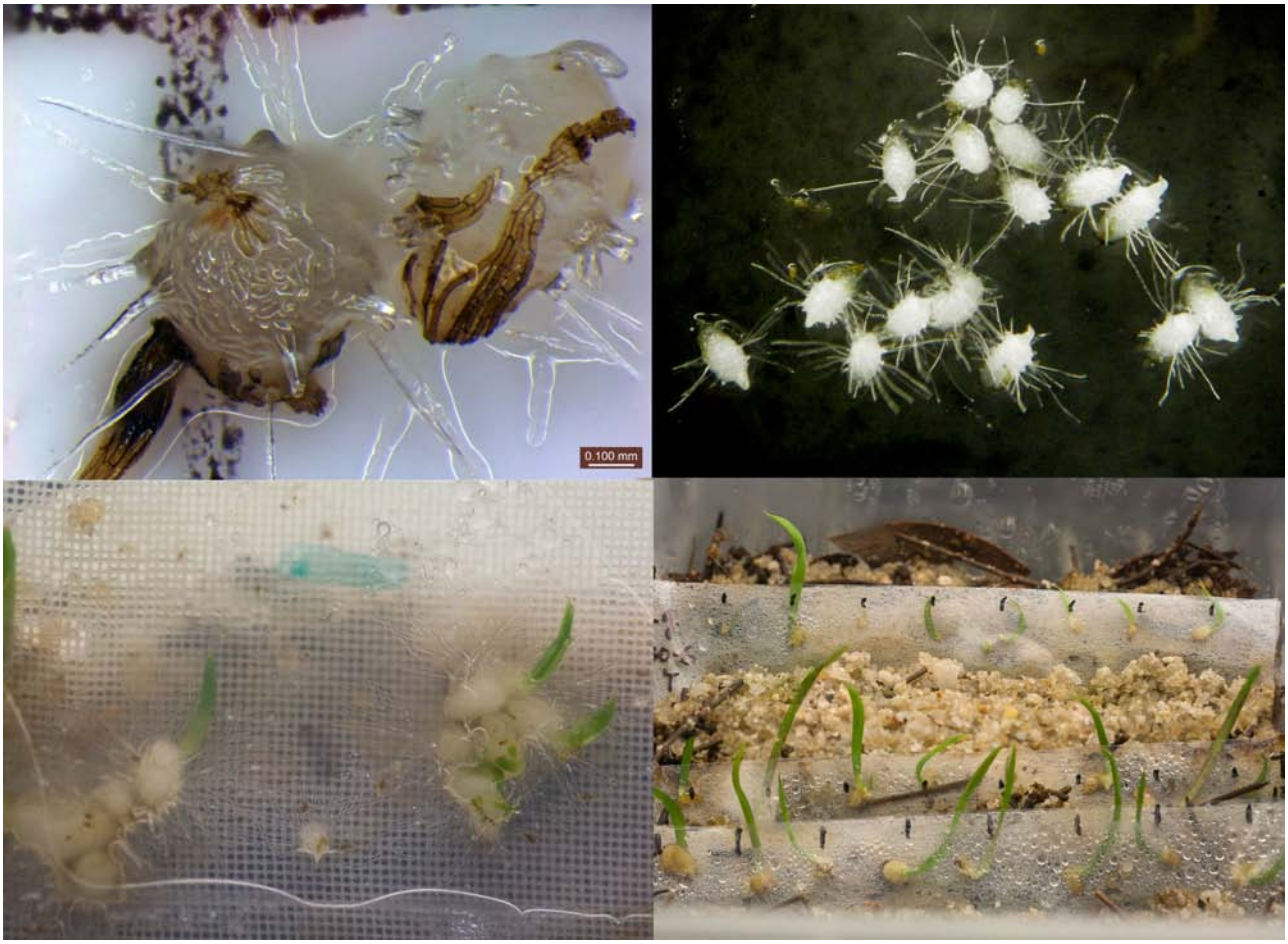
Wheatbelt Orchid Rescue Project

Final Report 7

Seed Collecting, Soil Baiting and Propagation of Orchids

Mark Brundrett & Emily Ager



Wheatbelt Orchid Rescue

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W.A. Native Orchid Study & Conservation Group

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Wheatbelt Orchid Rescue Project Final Reports

Brundrett M. 2011a. Wheatbelt Orchid Rescue Project Final Report 1. Objectives, Outcomes and Overall Conclusions. Wheatbelt Orchid Rescue Project, University of Western Australia. *Link 1*

Brundrett M. 2011b. Wheatbelt Orchid Rescue Project Final Report 2. Population Size and Vital Statistics Data for the Granite Spider Orchid (*Caladenia graniticola*). Wheatbelt Orchid Rescue Project, University of Western Australia. *Link 2*

Brundrett M. 2011c. Wheatbelt Orchid Rescue Project Final Report 3. Population Size and Vital Statistics Data for the Ballerina Orchid (*Caladenia melanema*). Wheatbelt Orchid Rescue Project, University of Western Australia. *Link 3*

Brundrett M. 2011d. Wheatbelt Orchid Rescue Project Final Report 4. Population Size and Vital Statistics Data for the William's Spider Orchid (*Caladenia williamsiae*). Wheatbelt Orchid Rescue Project, University of Western Australia. *Link 4*

Brundrett M. 2011e. Wheatbelt Orchid Rescue Project Final Report 5. Population Size and Vital Statistics Data for the lonely Hammer Orchid (*Drakaea isolata*). Wheatbelt Orchid Rescue Project, University of Western Australia. *Link 5*

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Brundrett M. 2011g. Wheatbelt Orchid Rescue Project Final Report 8. Translocation of Orchids in Wheatbelt Nature Reserves. Wheatbelt Orchid Rescue Project, University of Western Australia. *Link 8*

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Brundrett M. 2011. *Wheatbelt Orchid Rescue Project: Case Studies of Collaborative Orchid Conservation in Western Australia.* University of Western Australia, Crawley, Western Australia.

1. Introduction and Objectives

The Wheatbelt Orchid Rescue (WOR) project is a Lotterywest funded collaboration between the Western Australian Native Orchid Study and Conservation Group (WANOSCG), the School of Plant Biology at the University of Western Australia (UWA), the Friends of Kings Park and the Department of Environment and Conservation (DEC). This project aims to help conserve Critically Endangered orchids in the Western Australian Wheatbelt by obtaining knowledge required for sustainable management and directly contributing to recovery actions. Please refer to WOR Report 1 for further information about objectives and outcomes of this project.

Mycorrhizas are symbiotic associations between specialised soil fungi and plants, that are primarily responsible for nutrient transfer from plant to fungus (food) and fungus-to plants (fertiliser) (see the mycorrhizas.info website). On a global scale, the majority of plants in natural ecosystems have mycorrhizal associations (Brundrett 2009). Orchid mycorrhizas differ structurally from those of other plants and involve unique fungi. They consist of coils of hyphae in cortical cells of the root, stem or protocorms (germinating seedlings) of orchids. These symbiotic associations are essential to germinate the tiny seeds of orchids and are probably also essential for the nutrition of adult terrestrial orchids (Brundrett 2007). These also differ from the mycorrhizas of other green plants, as the fungus can provide both the energy and mineral nutrients required by orchids and the fungi seem to receive very little or no benefit from the plant (Brundrett 2004). There can be no doubt that most WA orchids are extremely dependent on mycorrhizal fungi to obtain nutrients from soil, because they often have highly reduced or non-existent roots and so lack the capacity to directly gain soil minerals required for growth.

Many orchids require a particular fungus to germinate while others can form mycorrhizas with a wider diversity of them (Bonnardeaux et al. 2007). Unlike the mycorrhizal fungi of *Eucalyptus* trees, many of which are conspicuous as mushrooms in winter, orchid fungi are usually invisible in nature. In fact the easiest way to detect that presence of these fungi is by using orchid seed as bait, since it will only germinate in soil if a compatible fungus is present (see Section 2). Knowledge of orchid fungus diversity and ecology is essential for us to understand orchid biology and ecology.

The primary objective of propagation trails described in this report was to produce seedlings of orchids for translocation trials. Several different propagation methods were used with the same seed lots and both rare and common orchid species were included to allow both the performance of orchid species and different methods to be compared as a secondary objective.

1. Seed Collection

Seed of rare and co-occurring common orchids was required for seed baiting and propagation. In total, over 250 seed samples were collected. Seed collections of rare orchids are listed in Table 1.

Seed bags were placed on flowers to ensure good quality seed was collected (Fig. 1). Seed bags developed for the WOR project were made of thin interfacing material (thin non-woven Vilene TM). Squares of this fabric wrapped around developing pods and fastened at top and bottom were found to be highly effective and easy to use (Fig. 1BC). These allowed seeds to be collected several months after they reach maturity and greatly reduced loss of seed to grazing animals. Seed bags were placed on naturally pollinated or flowers with a developing capsule, or flowers were cross-pollinated by hand if natural seed set was low (Fig. 1A). Seed was cleaned by sieving and stored at 4°C in vials (Fig. 1 DF).

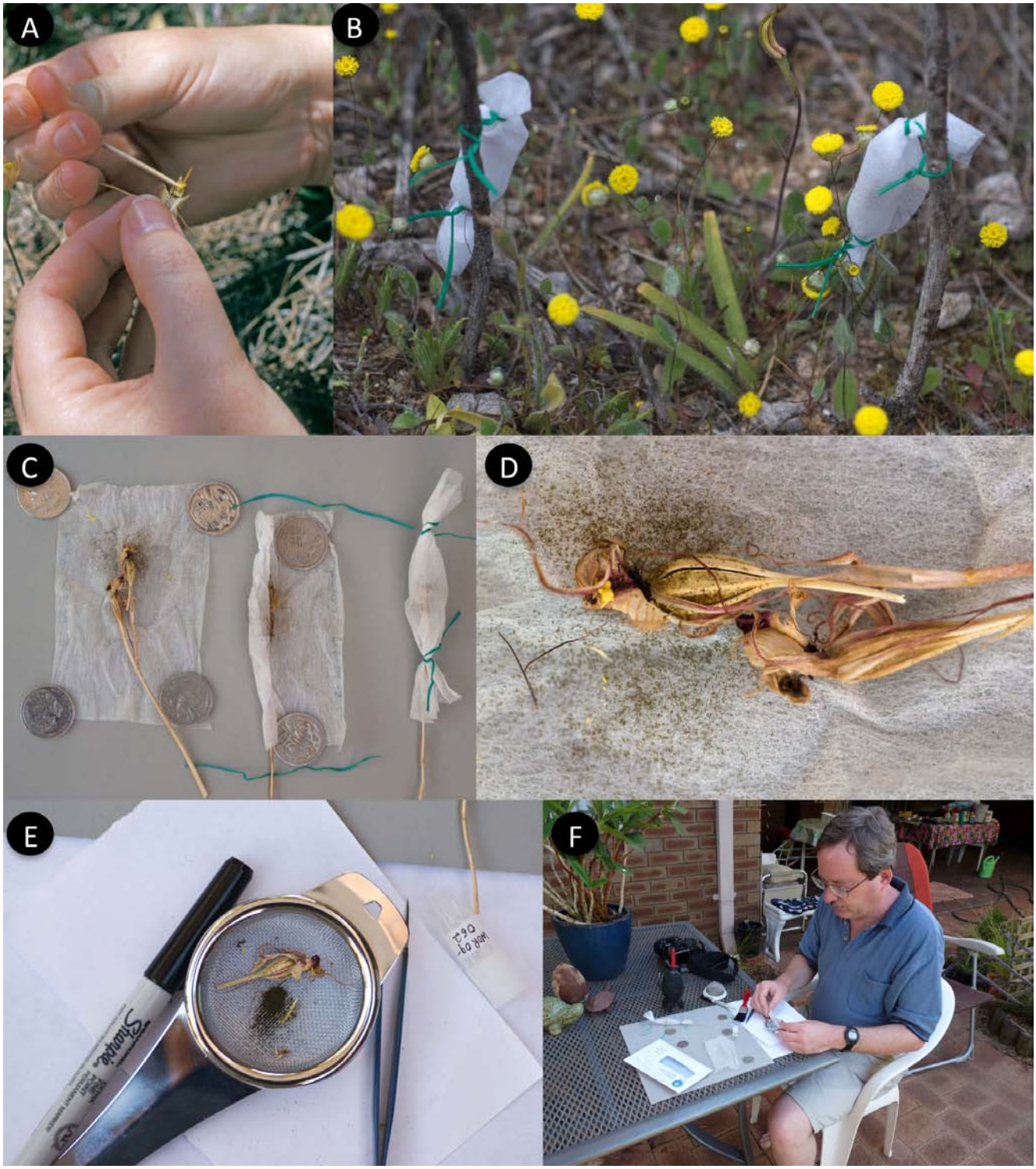

Figure 1. A. Artificial pollination of *Caladenia graniticola* was required in some cases to allow seed collection. **B-D.** Seed bags were sued to protect developing seedpods from grazing and prevent loss of seeds when pods open. **CD**. Opening seed bags retrieved from a field site. **EF**. Cleaning seed by sieving.

Table 1. Seed collections for rare *Caladenia* species from different populations over 4 years.

Name	*Species*	Population	2007	2008	2009	2010
Ballerina orchid	*C. melanema*	1	7	3	6	4
Ballerina orchid	*C. melanema*	2			1	
Ballerina orchid	*C. melanema*	3			1	
William's spider	*C. williamsiae*	2	3	2	2	3
Granite spider	*C. graniticola*	1	2	1		
Granite spider	*C. graniticola*	2	1			
Granite spider	*C. graniticola*	4		3		
Granite spider	*C. graniticola*	5	1	4	1	

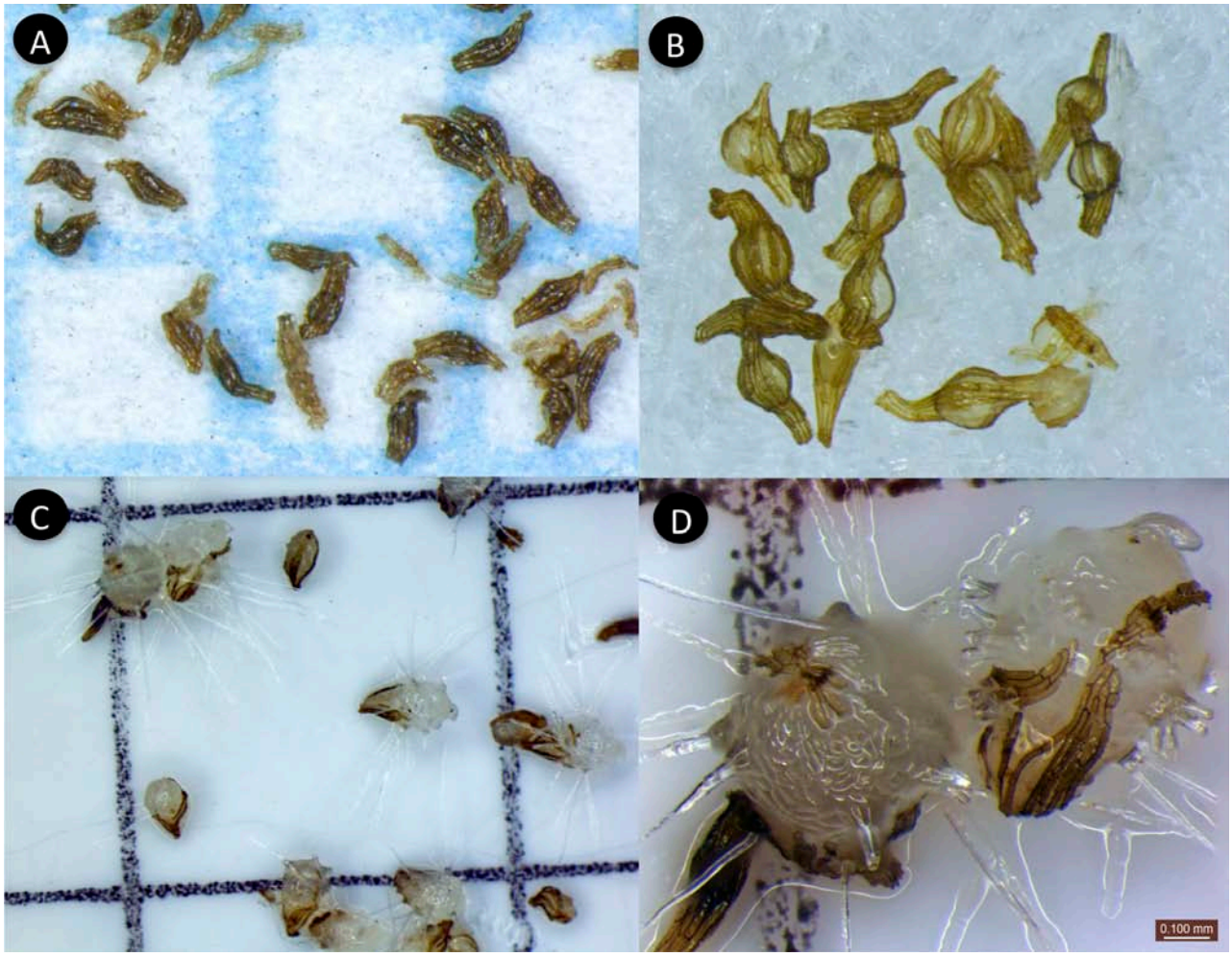

Figure 2. Orchid seed and seed baiting examples. **A**. Orchid seed on 1 mm graph paper. **B**. Imbibed seed ready for germination. **CD**. *Caladenia graniticola* protocorms growing over soil organic matter in an *ex situ* germination trial. These are highly magnified images (seedlings are up to 2 mm long).

Orchid seeds are only about 0.1 x 0.3 mm (called dust seeds) (Fig. 2A). These swell up in the presence of moisture (Fig. 2B), but only germinate into protocorms in the presence of a compatible fungus (Fig. 2CD), or in sterile culture (Fig. 4).

2. Seed Baiting Experiments

The *ex situ* seed baiting method tests if compatible mycorrhizal fungi are present by germinating orchid seeds over organic mater separated from soil from natural habitats (Brundrett et al. 2003).

Soil samples were collected from known or potential orchid habitats for baiting trials (Table 2). Common orchids were also included in trials to compare germination rates. The results of three major seed baiting experiments were similar, so only one is presented in Figure 3. Baiting trials with *C. melanema*, *C. williamsiae* and *C. graniticola* demonstrated that compatible mycorrhizal fungi capable of germinating seed were present in some soil samples from suitable looking habitats, but not others (Fig. 3). This method also confirmed that all the seed samples tested were highly viable, with close to 100% germination if fungi are present. Soils that supported germination were later used to provide compatible fungi for orchid propagation (Section 3).

As shown in Figure 3, rare orchids germinated in fewer soil samples than the common orchid *C. flava*, which germinated in more soils than any other orchid. The weedy orchid *Microtis media* also germinated in 3 soils (light coloured bars). Thus it seems that fungi compatible with rare orchids were often less common than those from common orchids, even in habitat areas where rare orchids occur.

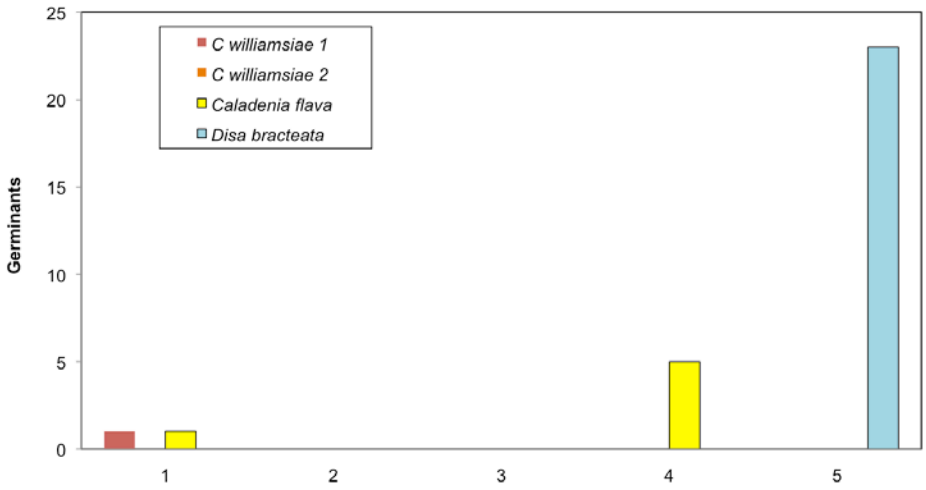

Figure 3A. Seed germination for 2 *Caladenia williamsiae* seed sources in comparison with 2 common orchids in 5 soil samples from habitats where *C. williamsiae* occurs.

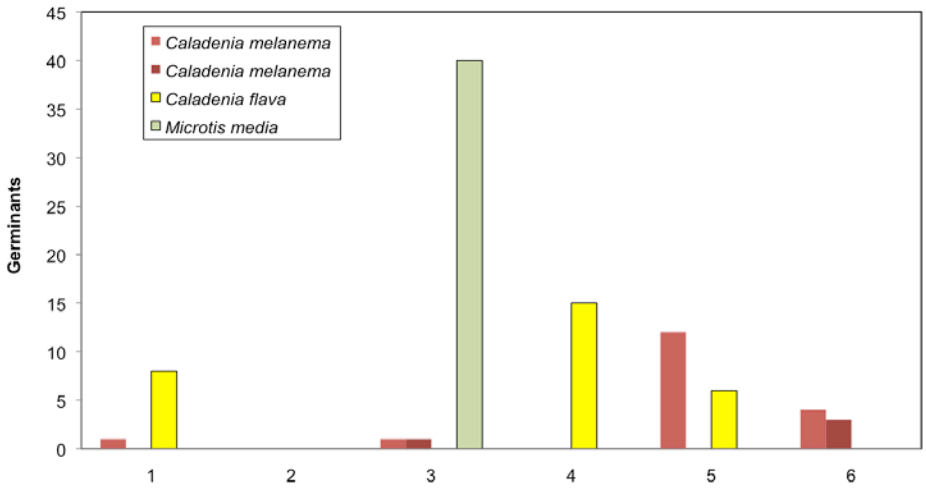

Figure 3B. Seed germination for 2 *Caladenia melanema* seed sources in comparison with the 2 common orchids in 6 soil samples habitats where *C. melanema* occurs.

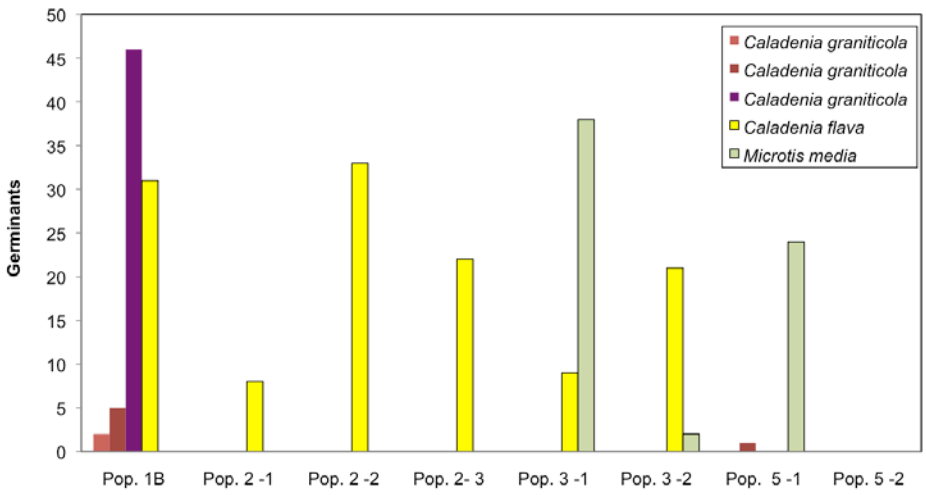

Figure 3C. Seed germination for 3 *Caladenia graniticola* seed sources in comparison with the 2 common orchids in 8 soil samples habitats where *C. graniticola* occurs from 3 nature reserves.

Table 2. Summary of soils used in one seed baiting experiment (Fig. 3).

Plate	Soil No.	Date	Location	*Genus**	*Species*
A. Granite spider orchid habitats					
1	Soil08-9	16/09/08	Near Pingaring	*Caladenia*	*graniticola*
2	Soil08-17	16/09/08	Near Pingaring	*Caladenia*	*radialis*
3	Soil08-18	16/09/08	Near Pingaring	*Allocasuarina*	*sp.*
4	Soil08-19	16/09/08	Near Pingaring	*Caladenia*	*graniticola*
5	Soil08-20	16/09/08	Near Pingaring	*Caladenia*	*roei*
6	Soil08-1	17/09/08	Dragon Rocks NR	*Caladenia*	*graniticola*
7	Soil08-5	17/09/08	Dragon Rocks NR	*Caladenia*	*falcata*
8	Soil08-6	17/09/08	Dragon Rocks NR	*Allocasuarina*	*sp.*
9	Soil08-8	17/09/08	Dragon Rocks NR	*Ericksonella*	*saccharata*
10	Soil08-2	18/09/08	Dragon Rocks NR	*Caladenia*	*graniticola*
11	Soil08-3	18/09/08	Dragon Rocks NR	*Caladenia*	*graniticola*
12	Soil08-10	18/09/08	Dragon Rocks NR	*Ericksonella*	*saccharata*
13	Soil08-4	19/09/08	Dragon Rocks NR	*Caladenia*	*graniticola*
14	Soil08-7	19/09/08	Dragon Rocks NR	*Caladenia*	*graniticola*
15	Soil08-11	19/09/08	Dragon Rocks NR	*Caladenia*	*falcata*
16	Soil08-12	19/09/08	Dragon Rocks NR	*Caladenia*	*graniticola*
17	Soil08-13	20/09/08	Near Karlgarin	*Pterostylis*	*nana*
18	Soil08-14	20/09/08	Near Karlgarin	*Caladenia*	*graniticola*
19	Soil08-15	20/09/08	Wave Rock	*Allocasuarina*	*sp.*
20	Soil08-16	20/09/08	Wave Rock	*Allocasuarina*	*sp.*
B. Ballerina orchid habitats					
21	Soil08-21	15/09/08	Near Pingrup	*Melaleuca*	*lateriflora*
22	Soil08-24	25/08/08	Near Pingrup	*Melaleuca*	*lateriflora*
23	Soil08-25	25/08/08	Near Pingrup	*Caladenia*	*melanema*
24	1A	22/11/07	Near Pingrup	*Caladenia*	*melanema*
25	4A	22/11/07	Near Pingrup	*Caladenia*	*melanema*
C. William's spider orchid habitats					
26	Soil08-22	20/09/08	Near Brookton	*Caladenia*	*falcata*
27	Soil08-23	20/09/08	Near Brookton	*Caladenia*	*flava*
28	6B	21/11/07	Near Brookton	*Caladenia*	*williamsiae*
29	7	21/11/07	Near Brookton	Woodland	

*Genus and species refers to dominant orchid or overstorey vegetation present at soil collection site.

3. Orchid Propagation

Several methods are routinely used to propagate terrestrial orchids for horticulture or conservation. Of these, asymbiotic and symbiotic sterile germination on agar plates with sterile culture media are most commonly used methods, but require expensive sterile culture facilities. An alternative method, where symbiotic fungi germinate orchid seeds in a non-sterile environment was first suggested by Brundrett et al. (2003), but had not been trailed on a large scale until now.

The primary objective of propagation trails in 2009 was to produce seedlings of orchids for translocation trials. Comparative studies of orchid propagation methods used the same seed lots for each species of orchid across a range of different propagation types (Table 3). All experiments included one or more co-occurring common *Caladenia* species along with the rare species required for translocation to allow the relative growth capacity of rare and common species to be compared. Emily Ager, a 4th year student at UWA, conducted these experiments with the author (MB) and compiled the comparative growth data shown in Table 4 (Ager 2009). Standard orchid propagation methods are not described in detail here (see Batty et al. 2001, 2006).

In 2009, the WOR project (MB) developed an innovative new germination pouch system to protect and track growth of very small orchid seedlings (Fig. 3). This method enclosed germinating orchid seeds are in permeable mesh and transparent plastic packets placed in contact with organic matter containing highly active mycorrhizal fungi (symbiotic fungi required by orchids for germination and growth). After germination, protocorms were transplanted into larger permeable pouches placed in potting mix inoculated with the same mycorrhizal fungi (Fig. 4).

Once seedlings were 0.5 - 1 cm long, seedlings were transplanted into new pouches for further growth in a growth cabinet until they reach sufficient size for establishment in soil (Fig. 5). Larger orchid seedlings were then used in translocations or grown in the glasshouse in pots containing soil from the same field sites (WOR Report 8). Older seedlings were placed in large pouches (10 x 10cm) to allow room for dropper and tuber growth in the glasshouse or field. The leaf and dropper length of seedlings were measured weekly.

Figure 4. Seedlings of *Caladenia williamsiae* growing on synthetic media W3 media from Western Laboratories (www.westernorchids.com.au). This is a standard culture media used for Australian orchids grown in the absence of mycorrhizal fungi.

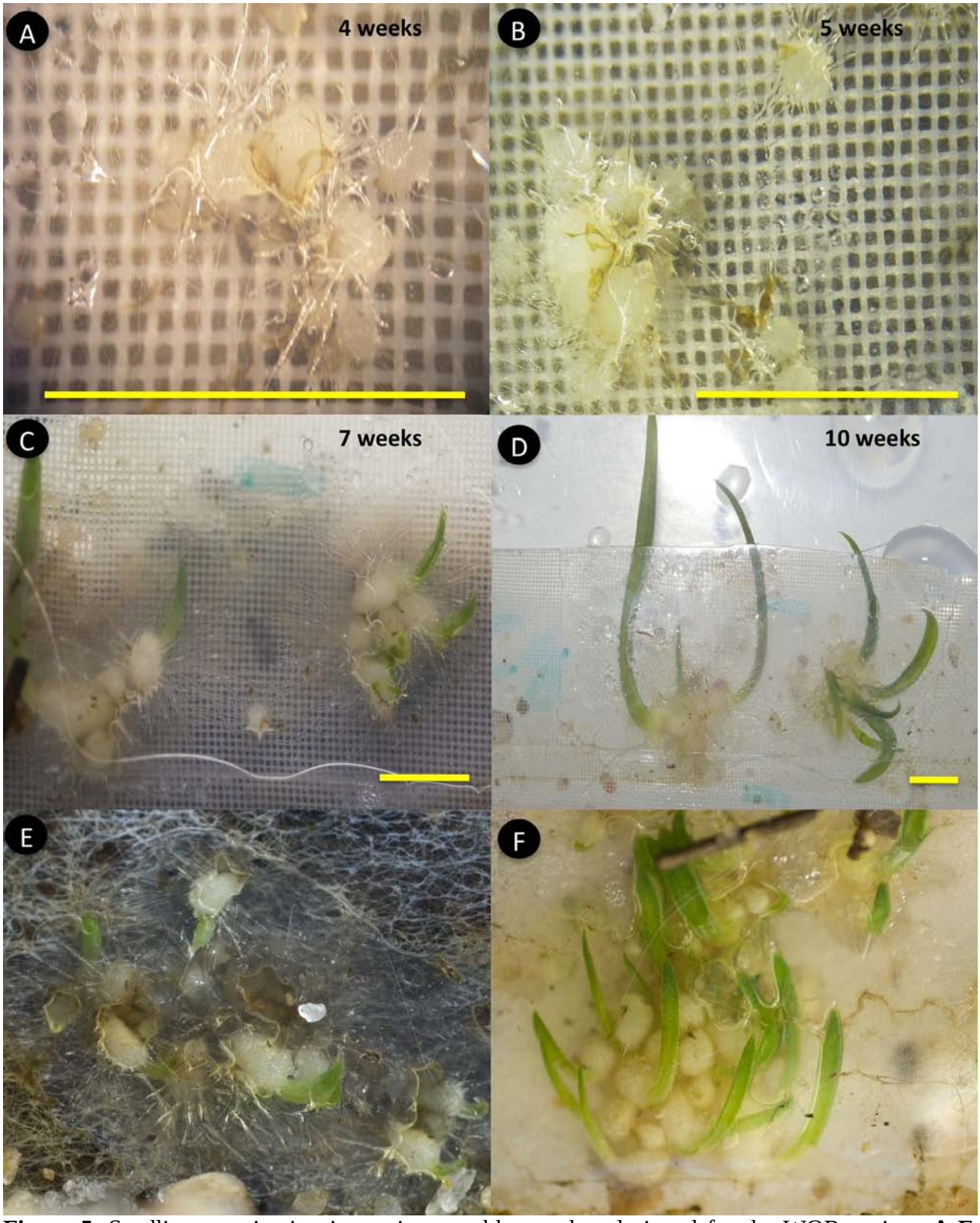

Figure 5. Seedling germination in semi-permeable pouches designed for the WOR project. **A-F**. The same seedlings of the granite spider orchid *Caladenia graniticola* observed over time in a germination pouch (scale bar = 5 mm). **E.** Seedlings in pouches about 2 months old that are up to 1 cm long. **F**. Three month old spider orchid seedlings in germination pouches were up to 3 cm tall.

We compared growth rates from the different propagation methods by measuring protocorm width or seedling length in photographs taken weekly (Fig 6, Table 4). Results showed a high degree of variation between species for each propagation method, but the asymbiotic media (sterile agar with mineral salts, sugar and organic supplements) was much slower and more erratic than other methods overall. Non-sterile symbiotic germination in seed packets was the most time and cost efficient method in these trials and resulted in hundreds of large seedlings for translocation trials (Table 5). In comparative studies within each propagation method, rare species were not harder to propagate than common orchids overall, but some orchids were exceptions (see WOR Report 8).

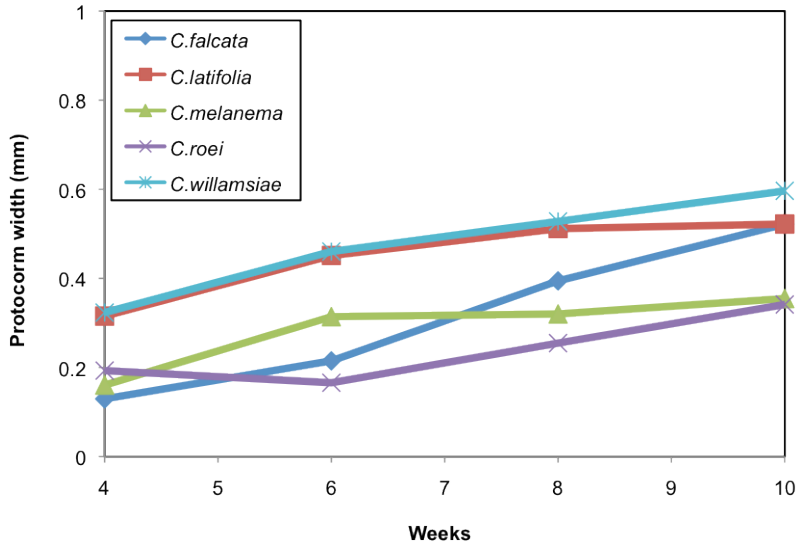

Figure 6A. Comparison of protocorm growth rates for 5 *Caladenia* species in asymbiotic germination media (W3).

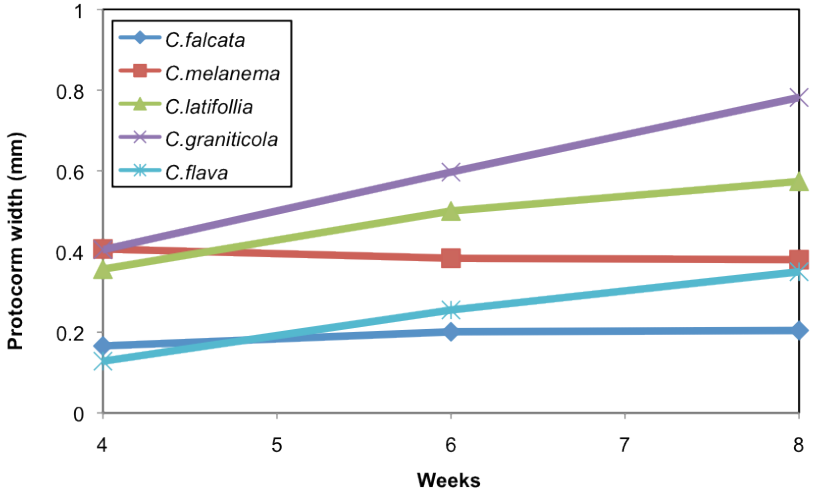

Figure 6B. Comparison of protocorm growth rates for 5 *Caladenia* species in soil baiting plates, a semi-natural symbiotic environment.

Success and efficiency of seed germination methods vary dramatically (Table 4). There was also a high degree of variation between species within each propagation method. It was more difficult to maintain the growth of seedlings produced by the asymbiotic method (sterile culture using synthetic media in Petri plates or jars) once transferred to potting mix than for other methods. In seedling pouches, growth rates were linked to germination rates in seed baiting trials using the same soils, since both are dependent on the activity of symbiotic fungi. These fungi were transferred from germination to growing containers along with orchid seedlings. The rate of leaf growth declined substantially for some orchids after transplanting and this was linked to dropper formation (stems that extend downwards and form tubers) or death of some seedlings.

Table 3. Seeds used in comparative growth trials using different propagation methods.

Vial Number	Date	*Genus*	*Species*	Location	Plants	Seed pods
WOR-07-032B	21/11/07	*Caladenia*	*melanema*	Near Pingrup	5	5
WOR08-051	2/12/08	*Caladenia*	*graniticola*	Dragon Rocks NR	4	4
WOR08-043	1/12/08	*Caladenia*	*williamsiae*	Near Brookton	1	2
WOR08-006	16/09/08	*Caladenia*	*roei*	Near Pingaring	1	2
WOR08-045	1/12/08	*Caladenia*	*falcata*	Near Brookton	5	5
WOR08-046	1/12/08	*Caladenia*	*flava*	Near Brookton	3	3
WOR08-035	1/12/08	*Caladenia*	*latifolia*	Near Pingrup	1	2
WOR-07-026	21/11/07	*Caladenia*	*graniticola*	Near Karlgarin	1	1

Table 4A. Average growth rate of protocorms of *Caladenia* species resulting from different propagation methods (mm / week).

Species	Sterile culture	Soil baiting	Seed packets
Caladenia williamsiae	0.04	0	0.07
Caladenia melanema	0.03	0.02	0.09
Caladenia graniticola	0	0.09	0.08
Caladenia falcata	0.07	0.01	0.14
Caladenia roei	0.03	0	0.15
Caladenia latifolia	0.03	0.05	0.09
Caladenia flava	0	0.06	0
Average	**0.04**	**0.05**	**0.10**

Table 4B. Average size of protocorms of *Caladenia* species resulting from different propagation methods (mm).

Caladenia species	Sterile culture	Soil baiting	Seed packets
Caladenia williamsiae	0.04	0	0.07
Caladenia melanema	0.03	0.02	0.09
Caladenia graniticola	0	0.09	0.08
Caladenia falcata	0.07	0.01	0.14
Caladenia roei	0.03	0	0.15
Caladenia latifolia	0.03	0.05	0.09
Caladenia flava	0	0.06	0
Average	**0.04**	**0.05**	**0.1**

Table 4C. Total numbers of protocorms of *Caladenia* species resulting from different propagation methods.

Caladenia species	Sterile culture	Soil baiting	Seed packets
Caladenia williamsiae	129	0	0
Caladenia melanema	94	36	42
Caladenia graniticola	0	32	49
Caladenia falcata	9	28	25
Caladenia roei	29	0	90
Caladenia latifolia	96	94	10
Caladenia flava	0	29	10
Total	**357**	**219**	**226**

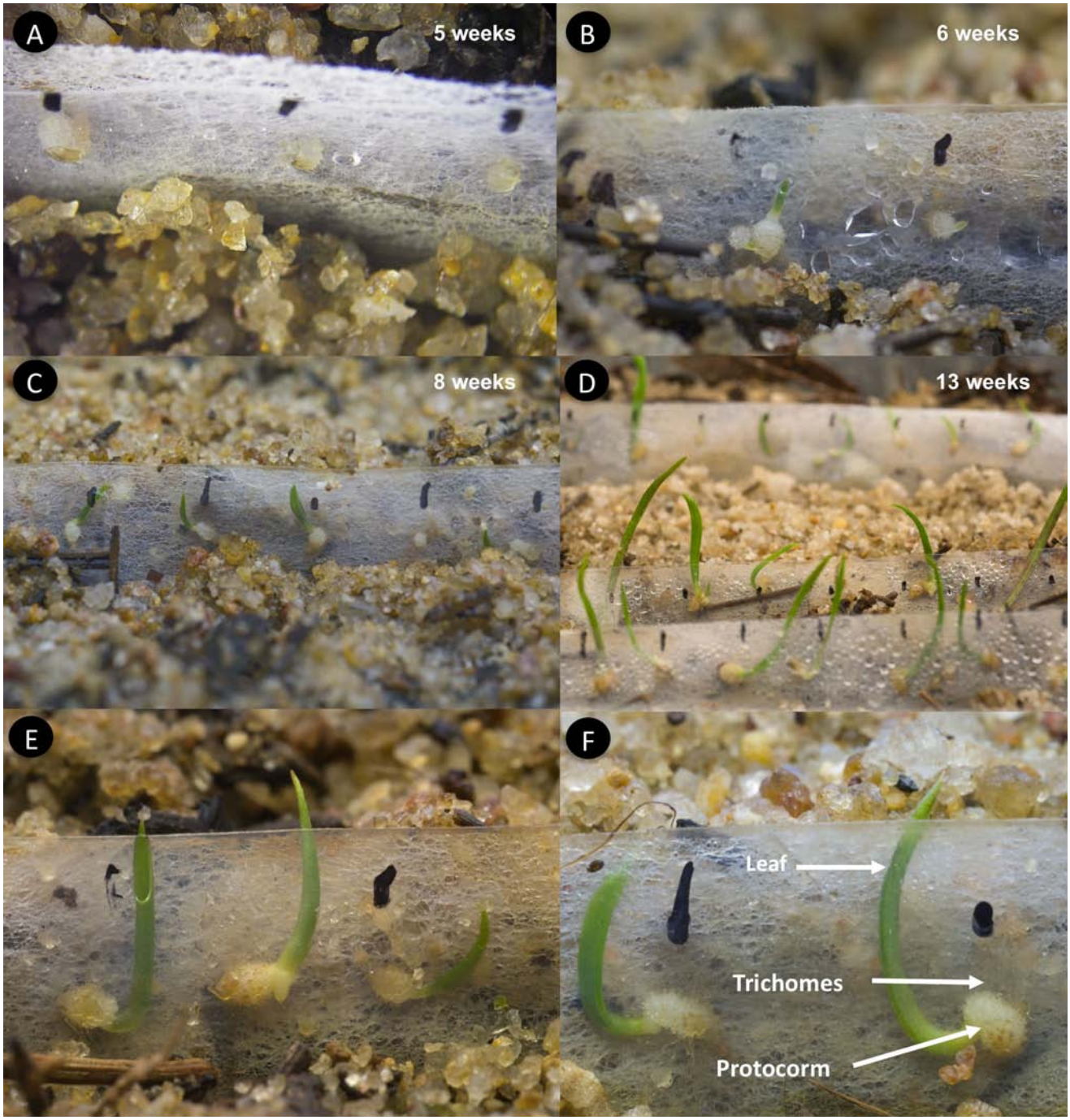

Figure 7. Seedling of the granite spider orchid *(Caladenia graniticola)* in growing pouches within potting mix containing compatible fungi. These images show stages in the development of *Caladenia* seedlings using the FORGE system over a 2-month period. Seedlings were grown in transparent semi-permeable FORGE pouches that allow fungal hyphae access while reducing water loss.

The development of pouches to protect seedlings by the WOR project is an important development in rare orchid propagation for conservation. These methods are collectively referred to as “FORGE” [Fungal induced - Orchid seed - Realistic (non-sterile) environment - Germination - in Enclosures (pouches)]. Seedlings were grown in a growth cabinet after transfer to pasteurised potting mix inoculated with soil organic matter from baiting trials (Fig. 8). Tuber formation was observed using translocation pouches and window pots (WOR Report 8) and a second year of growth from dormant tubers formed in 2009 occurred in 2010 (Fig. 8D).

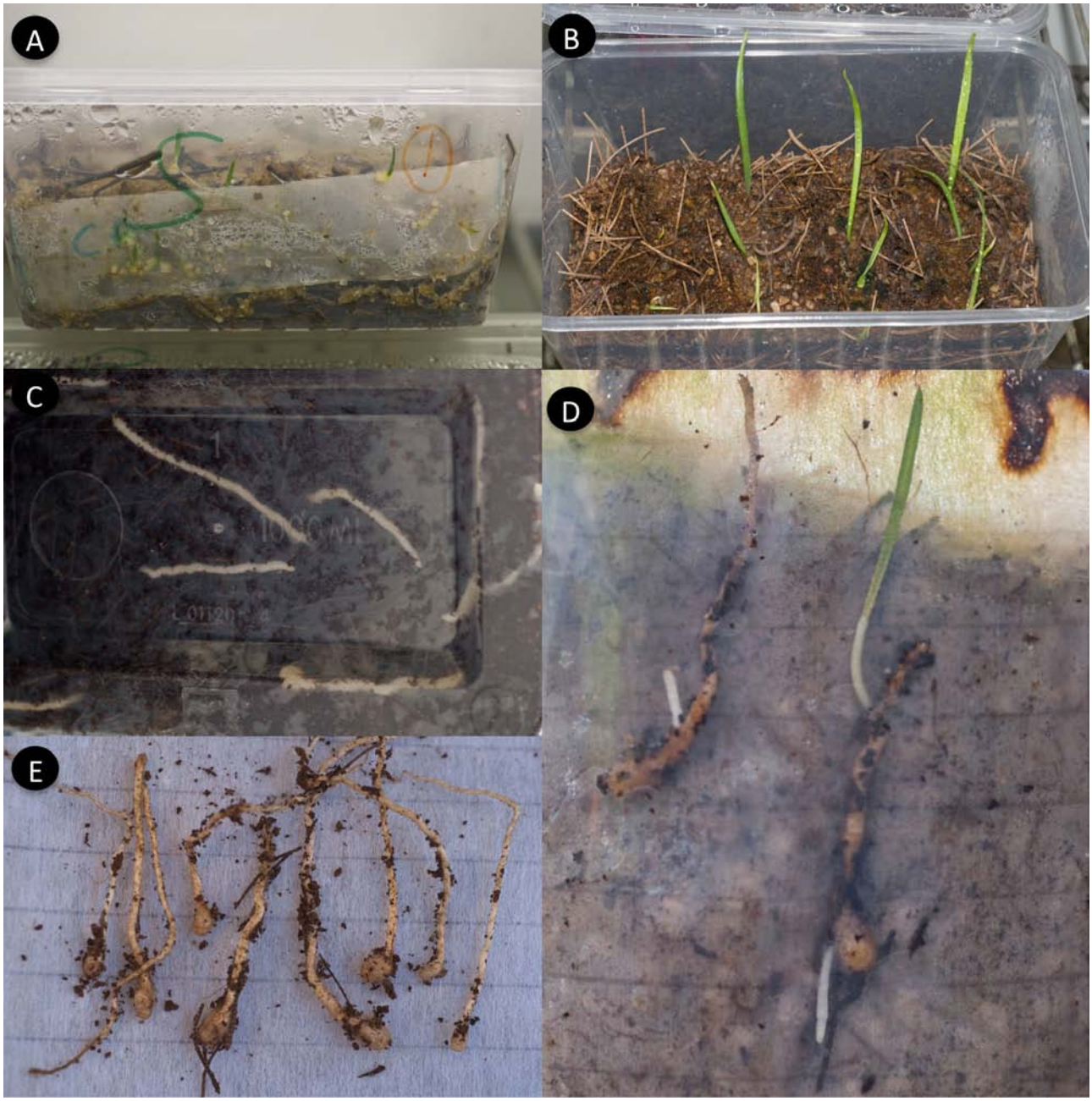

Figure 8. Later propagation stages for spider orchid seedlings. **AB**. Orchid seedlings growing in soil with active fungi in an incubator. **C-E**. Older *Caladenia* seedlings with tubers forming on a dropper under the protocorms. **D**. Two year old *Caladenia roei* plants grown from the same dormant tubers.

Table 5. Cost benefits analysis summary of different propagation methods (Ager 2009).

	Asymbiotic	**Soil Baiting**	**Seed Packets**
Relative Cost (financial)	high	low	low
Preparation (ordering, collecting)	2	3	3
Set up time (days)	2	1	1
Maintenance time (hrs/week)	1	1	1
Complexity of methods	high	medium	low
Loss due to contamination or similar	50%	15%	10%
Survival in field or glasshouse	low	low	high
Total hours	5	5	5
Overall Ranking	*	**	***

Window pots were used to measure seedling growth and tuber formation in the glasshouse (Fig. 8D). Permeable translocation pouches were found to protect growing seedlings in the field and glasshouse (WOR Report 8). Further work is required to optimize methods for seedling production and translocation.

The Friends of Kings Park Orchid Carers Group had a key role in propagating rare orchids for the WOR project in 2009 and 2010 (Fig. 9). They (Lyn Rowland, Lyn Roberts, Val Preston, Phylis Robertson and Mary-Ann Andrews) attended regular propagation sessions at the University of Western Australia for seed cleaning, assembling pouches, pricking out seedlings into growing pouches, as well as preparing potting mix and window pots for the growth of larger seedlings. It is anticipated that some of the techniques learned for the establishment of propagated seedlings will be of benefit during future work at Kings Park. The Orchid Carers also helped to translocate rare orchid seedlings into nature reserves in the wheatbelt in 2010 (WOR Report 8).

Figure 9. The Friends of Kings Park Orchid Carers Group assisted the author (MB) in propagation of rare orchids for translocation in 2010.

4. Conclusions and Recommendations

Results of orchid propagation trials are summarised in Tables 4 and 5. Overall, the new non-sterile symbiotic (FORGE) method developed by Mark Brundrett the WOR project was found to be the most successful and efficient method for propagation rare or common *Caladenia* species (Table 5). A major advantage of the FORGE system was that seedlings grew more rapidly in semi-natural conditions than in fully sterile culture. This represents a substantial breakthrough in our capacity to propagate rare Australian orchids, since it is easier to propagate them and they developed more rapidly than in sterile culture (from seed to translocated seedlings within 6 months). However, we found there were major differences in the consistency with all of the methods trialled, due to variations between and within orchid species. Consequently, additional research is required to further optimise growing conditions using FORGE methods (potting mixes, fungal inoculum sources and pouch designs) and to determine replication and time required to grow sufficient seedlings for translocation. A more detailed list of conclusions from propagation and translocation trials is presented in WOR Report 8.

5. Acknowledgements

Orchid seedlings were produced by Mark Brundrett, Emily Ager (UWA student) and Nur Koshkuson (volunteer) in 2009. Volunteers of the Friends of Kings Park Orchid Carers Group had a major role in orchid propagation, especially in 2010. The University of Western Australia provided facilities for orchid propagation. Gary Cass, Rob Creasy, Tim Morald, Margaret Collins and Tammy Edmonds also provided assistance.

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