Royal Botanic Gardens Kew

Propagation Protocols for 10 Threatened Cornfield Annuals

Report for Colour in the Margins

Ted Chapman, Sarah Pocock & Rachael Davies

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Background

RBG Kew's UK Native Seed Hub (UKNSH) was commissioned and funded by Colour in the Margins to develop propagation protocols for ten cornfield annual species using existing Millennium Seed Bank (MSB) data supplemented in some cases by new germination and propagation trials. Colour in the Margins is a Back from the Brink project led by Plantlife.

All work was carried out by the UKNSH in the MSB and Wakehurst Plant Propagation and Conservation Unit. Data relating to seed collection dates, weight, longevity, dormancy-alleviation and germination requirements were sourced from the MSB's Seed Bank Database, accessed between April and October 2018. Data relating to seedling establishment and growth were sourced from horticultural records held by the UKNSH, accessed over the same period. References for all other sources of information are provided in the text.

About the UK Native Seed Hub

The UK Native Seed Hub seeks to mobilise the seed collections, facilities, technical knowledge and scientific expertise of RBG Kew to support conservation and habitat restoration in the UK.

We provide plants and seed, technical services and research to help conservation practitioners overcome constraints to sourcing, producing and using native plant materials in the UK.

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Propagation Protocol: *Adonis annua* L.

1 Ecological overview

1.1 Description

Adonis annua L. is an erect annual herb growing to 50cm (Wilson & King, 2003). Plants bear multiple, branched stems with finely dissected leaves. Flowers are 15-25mm in diameter with deep red petals and a dark basal spot (Wilson & King, 2003). Approximately 30 achenes (dry fruit) are produced per infructescence, each bearing one seed, which remains inside a hard covering structure (Figure 1). Achenes darken in colour and drop readily when mature. Usually no more than 30 infructescences are produced per plant (Plantlife, 2009). When grown as a cultivated population at the MSB in 2014, an average of 259 seed were produced per plant.

Figure 1. *Adonis annua* infructescence showing individual achenes each containing a single seed. ©RBG Kew

1.2 Phenology

Seeds germinate mainly in autumn, but also in spring (Wilson & King, 2003). Plants flower in June and July, dispersing seed shortly afterwards. The MSB's wild UK collection of *A. annua* was made in late July, with a spring-sown cultivated collection made during August.

1.3 Mating system

Adonis annua may be cross-pollinated by insects or self-pollinated within the bisexual flowers. Pollinator interactions are not described in detail, although the plant is known to be attractive to bees (Clapham, 1987). Plants grown without pollinators are also able to set a high proportion of viable seed, suggesting self-pollination is an important mechanism in *A. annua* reproduction (Thomann et al., 2015). Seed are heavy and generally remain on the soil surrounding the mother plant, restricting colonisation of new areas.

1.3 Distribution

Adonis annua is has never been abundant in the UK. The species' distribution has historically been linked to areas of chalk and limestone in southern England (Wilson & King, 2003). The UK is the northern limit of the species' distribution, with records across the Mediterranean region.

1.4 Habitat

Adonis annua grows on either on arable or other disturbed ground, often on loamy calcareous soils either on chalk or oolithic limestone (Wilson & King, 2003).

1.5 Co-occurrence with other CitM species

Adonis annua is known to co-occur with *Torillis arvensis* and *Galeopsis angustifolia* (Wilson & King, 2003).

1.6 Threats

Adonis annua cannot tolerate herbicides and is easily out-competed by fertilised crops and improved varieties (Wilson & King, 2003). Plants produce relatively few seeds, resulting in low seed numbers within soil seed banks (Wilson & King, 2003). Arable species with low seed production show increased probability of extinction compared to those with high seed production (Saatkamp et al., 2018), with winter annuals such as *A. annua* particularly vulnerable to herbicide sprays applied early in their life-cycle (Albrecht, 2003).

2 Seed ecology

2.1 Seed longevity

There is some uncertainty regarding the longevity of *A. annua* seed in the soil seed bank. Plantlife (2009) describe the seed as 'relatively short-lived', whilst Wilson and King (2003) use the term 'quite long-lived'. More recent research by Saatkamp et al. (2009) found that a sample of 40 seeds retained 96.5% viability after burial in the soil for two and a half years, the highest of all 38 arable species studied. Ongoing research at the MSB supports the view that *A. annua* has intermediate or long-term longevity.

2.2 Seed dormancy

Seed dormancy describes a range of mechanisms that prevent seeds germinating, even under favourable germination conditions. Dormancy may delay germination until the conditions are likely to support healthy plant growth and stagger germination over multiple growing seasons, helping the population recover from damaging short-term effects such as drought, disturbance or unfavourable management practices.

Adonis annua appears to display two forms of dormancy - morphological and physiological dormancy - commonly described together as morphophysiological dormancy (Baskin and Baskin, 2014).

As with many species in the Ranunculaceae family, seeds of *A. annua* have underdeveloped rudimentary embryos at dispersal, resulting in morphological dormancy (Baskin & Baskin, 2014; Figure 2). Embryos must fully develop inside the seed before germination can occur. The environmental conditions required to promote this development differ between species. As a cornfield annual species, *A. annua* seeds are dispersed onto exposed soils, remaining uncovered for the late summer months. These warm, dry conditions are likely to promote full embryo development.

Figure 2 *Adonis annua* seeds with and without the covering structure. Cut seed displays the underdeveloped, rudimentary embryo. ©RBG Kew

When the embryo has fully developed, physiological dormancy prevents immediate germination of the seed. *A. annua* seeds have a hard, thick covering structure which the radicle (root tip) is unable to penetrate. This mechanical restriction is overcome naturally by splitting or decay of the covering structures through repeated cycles of warm-dry and cool-wet conditions across the seasons.

2.3 Dormancy alleviation

Extensive research has been conducted at the MSB into the dormancy alleviation requirements for *A. annua.* The most successful protocol identified to date involves a period of dry after-ripening (DAR) in warm, dry conditions (60% relative humidity, 25°C) to overcome morphological dormancy, followed by a partial de-husk (removing a small piece of covering structure to expose the radical tip, but without piercing the seed coat). An alternating temperature of 20/10°C promotes germination following these dormancy-breaking techniques. In the most successful test, 94% of seed germinated within 105 days, with a mean time to germination of 36 days.

Step	Method	Dormancy
1	4 weeks DAR (60%RH, 25°C)	Morphological
2	Partial de-husk	Physiological
3	Germination temperature (20/10°C, 12/12 photoperiod)	-

Table 1. MSB lab protocol for germination of *A. annua* seeds, listing the corresponding dormancy mechanism being overcome in each step.

To achieve germination without the requirement for partial de-husking, an additional warm/dry and cool/wet cycle may be included. The most successful germination protocol without partial de-husking is described in Table 2 – an additional cycle of warm stratification at an alternating temperature of 30/10°C is followed by cooler alternating germination temperatures of 20/10°C (at which point some germination may occur), followed by a short period of re-drying at 60% RH and 40°C, before returning to the germination conditions once more.

Step	Method	Dormancy
1	4 weeks DAR (60%RH, 25°C)	Morphological
2	1 week warm stratification (30/10°C)	Physiological
3	4 weeks germination temperature (20/10°C, 12/12 photoperiod)	-
4	4 hours re-drying (60%RH, 40°C)	Physiological
5	Germination temperature (20/10°C, 12/12 photoperiod)	-

Table 2. MSB lab protocol for germination of *A. annua* seeds avoiding the need for partial dehusking, listing the corresponding dormancy mechanism being overcome in each step.

Several other dormancy-alleviation treatments have been applied to *A. annua* without success, summarised in Table 3.

Method	Result	Dormancy
DAR for longer than 4 weeks	Decreases germination	Morphological
Surgical methods carried out prior to DAR (and, thus, embryo growth)	May lead to the embryo becoming detached and dying or producing a tiny seedling.	Physiological
'Dry-heat shock' at 103°C	No germination	Physiological
Smoke solution	Germination not increased	Physiological

Table 3. Unsuccessful dormancy alleviation treatments for *A. annua*.

3 Cultivation

In 2014, a modified version of the MSB germination protocol outlined in Table 1 was applied to a wild seed collection, with plants grown on in the UKNSH production site to provide a large regenerated seed collection (Table 4).

Propagation work began in January 2014, with treatments reduced to two-week intervals to ensure seedlings were available for planting in spring. Ideally, seeds would begin the dormancy-breaking treatments in late summer, to allow the full after-ripening and dormancy-alleviating processes to be completed and enable germination in autumn. Germination percentage was 48% - it is likely allowing the full time for DAR and warm stratification would have achieved a higher germination rate.

Figure 3 Photographs of *A. annua* seedlings, showing the characteristic divided leaves. ©RBG Kew

Seedlings were grown in glasshouse conditions before being planted out into production beds at the end of May 2014 (Figure 3). Seeds were collected from this regenerated population between 12th August and 2nd September 2014. Following collection, seed material was temporarily stored in paper bags at 20°C and 60% RH to allow continued maturation for one week to ten days, before drying to 15% RH at 15°C for long-term storage. 282 plants were harvested, yielding 73,056 healthy seed with a viability of 94% - plants grown under less favourable field conditions are likely to be less productive.

Seed origin:	Arable field margin, Oxfordshire.
Date seed collection:	29/07/11
Length of storage:	2 years, 5 months
Pre-treatments:	Dry after-ripening at 25°C, 60% RH (2 weeks) Partial de-husk Warm stratification at 30°C (2 weeks)
Germination conditions:	20/10°C, 12/12 photoperiod
Time until germination:	Nine weeks (from initiation of pre-treatments)
Germination duration:	Three weeks, with sporadic germination thereafter.
Germination percentage:	48%
Germination media:	Sand
Growing conditions:	Glasshouse with minimum temperature of 16°C for two weeks, then glasshouse with minimum temperature of 2°C.
Growing media:	3:1 mix of Petersfield Peat Free Supreme compost and standard perlite, with 2g/L of Osmocote controlled release fertilizer. Seedlings pricked into 5cm pots then repotted into 9cm pots.
Planting:	Planted in open production beds on 28/05/14. Moisture retentive, slightly acidic clay loam with moderate drainage and no addition of fertiliser or organic matter.
Aftercare:	Irrigation until established, weeding.
Seed collection:	First collection after 31 weeks (12/08/14 - 02/09/14). Seeds fell readily into the hand when mature. Seed after-ripened at 20°C and 60% RH for one week to ten days before final drying, cleaning and storage in the MSB.
Seed yield and viability:	282 plants yielded 73,056 healthy (filled, uninfested) seed. Viability >94%.

Table 4. Cultivation data for an *A. annua* crop at Wakehurst

4 Implications for restoration

4.1 Establishing new populations

Adonis annua is suspected to have medium-long term persistence in the soil seed bank and may return with the reinstatement of management practices that provide adequate germination and establishment niches. If the species has not been recorded recently or has failed to return under a favourable management regime, reintroduction is likely to be required.

Sowing onto bare, cultivated ground in late summer mimics the timing of natural seed dispersal and enables seeds to experience the warm, dry conditions required for after-ripening and dormancy break. Seeds sown in late summer are more likely to germinate before winter than autumn-sown seed. Seeds which do not germinate before winter may have successfully overcome morphological dormancy but require further warm/dry and cool/wet cycling events to overcome physiological dormancy before germinating in spring. Experience at Wakehurst suggests autumn germinands form larger and more productive plants, although spring germinands can establish and perform well.

It may be possible to apply dormancy-breaking pre-treatments to stored seed to enhance germination and establishment in the field. Alternating seeds between warm/dry and cool/wet cycles under controlled conditions may allow slightly later sowing and increase the percentage of seed germinating in autumn, although this has not been demonstrated experimentally. Propagating seedlings in cultivation and introducing them as plug plants would be an alternative means of securing a first generation of flowering plants and direct dispersal of seed into the soil seed bank.

4.2 Sowing rate

Lang et al. (2016) suggest an optimal sowing rate for arable plant reintroductions of 50-100 seed/m^2.

Complex dormancy mechanisms mean only a small proportion of *A. annua* seed present in the soil are likely to germinate each year. To establish a large, healthy population it would be advisable to assume a low germination rate of <5% and increase the sowing rate accordingly – for example, sowing at least 1000 seed to achieve a population of 50 individuals.

1g of *A. annua* seed contains approximately 117 individual seeds.

4.3 Site management

Adonis annua is likely to benefit from management regimes that:

- ensure plants are able to disperse seed into the soil seed bank before the crop is cut;
- provide the exposed, open conditions required for seed after-ripening and dormancy-break;
- minimise disturbance of autumn germinating seedlings;
- limit competition from crop or weed species.

Cutting immediately after seed dispersal in July followed by a short fallow period and cultivation in August or early September will enable after-ripening on the soil surface and promote autumn germination, a regime consistent with the reported association between *A. annua* and autumn-sown winter cereals (Plantlife, 2009). As *A. annua* is suspected to form a persistent soil seed bank and can also germinate in spring, populations may also withstand spring cultivation if required to promote other arable species.

If growing *A. annua* within a crop, reduced fertiliser application and lower cereal density can increase light levels for arable species. Reduced cereal sowing has been shown to increase rare arable species richness in cereal crops (Wagner et al., 2017).

In non-arable habitats, disturbance should be carried out to in late summer or early autumn to expose seed and maintain open ground and high light levels.

5 References

Albrecht, H., 2003. Suitability of arable weeds as indicator organisms to evaluate species conservation effects of management in agricultural ecosystems. Agriculture, Ecosystems & Environment, 98 (1-3), pp. 201-211.

Baskin, J.M., Baskin, C.C., 2014. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination, 2nd edition. Academic Press.

Cheffings, C.M. & Farrell, L. (Eds), Dines, T.D., Jones, R.A., Leach, S.J., McKean, D.R., Pearman, D.A., Preston, C.D., Rumsey, F.J., Taylor, I. 2005. The Vascular Plant Red Data List for Great Britain. Species Status 7, 1-116. Joint Nature Conservation Committee, Peterborough.

Clapham, A., Tutin, T., Moore, D., 1987. Flora of the British Isles. Third Edition. Cambridge, CUP.

Lang, M., Prestele, J., Fischer, C., Kollmann, J., Albrecht, H., 2016. Reintroduction of rare arable plants by seed transfer. What are the optimal sowing rates? Ecology and Evolution, 6, 15, pp. 5506–5516.

Plantlife, 2009. Adonis annua species briefing sheet. Available at: www.plantlife.org.uk/uk/discover-wild-plants-nature/plant-fungi-species/pheasants-eye.

Saatkamp, A., Affre, L., Dutoit, T., Poschlod, P., 2009. The seed bank longevity index revisited: limited reliability evident from a burial experiment and database analyses. Annals of Botany, 104, 4, pp.715–724.

Saatkamp, A., Affre, L., Dutoit, T., Poschlod, P., 2018. Plant traits and population characteristics predict extinctions in a long-term survey of Mediterranean annual plants. Biodiversity and Conservation, 27, 10, pp. 1-14.

Thomann, M., Imbert, E., Cheptou, P., 2015. Is rapid evolution of reproductive traits in *Adonis annua* consistent with pollinator decline? Acta Oecologica, 69, pp. 161-166.

Wagner, M., Bullock, J.M., Hulmes, L., Hulmes, S., Pywell, R.F., 2017. Cereal density and N-fertiliser effects on the flora and biodiversity value of arable headlands. Biodiversity and conservation, 26, 1, pp. 85-102.

Wilson, P., King, M., 2003. Arable plants: a field guide. Wild Guides.

Propagation Protocol: *Bromus interruptus* (Hack.) Druce

1 Ecological overview

1.1 Description

Bromus interruptus is a robust annual grass, 20-100cm tall, gaining its name from the gaps that can sometimes be observed between the spikelets (Figure 1; Clayton et al., 2006;). The spikelets usually form in clusters of three and individually measure approximately 10-17mm in length, with the lemma bearing an awn up to 8mm long (Wilson & King, 2003). The entire panicle can reach 8cm (Rich & Lockton, 2002).

Figure 1 *Bromus interruptus* spike, growing in association with *Onobrychis vicifolia*. ©Plantsurfer at English Wikipedia

1.2 Phenology

Germination occurs in autumn and spring, with autumn-germinating seeds forming earlier-flowering and more productive plants (Rich & Lockton, 2002). Plants flower from May to July, sometimes extending into early autumn in warm, damp seasons, with seed dispersal in late summer. Seed collections from cultivated plants held in the MSB were made in August. Plants produce abundant seed which is often retained in the spikelets, causing seedlings to appear in small clumps.

1.3 Mating system

The mating system of *B. interruptus* is not described in detail. Rich and Lockton (2002) and Hubbard (1984) note that the anthers may protrude or be entirely enclosed within the floret, suggesting both outcrossing and self-pollination can occur. Cleistogamy (self-pollination within unopened flowers) is a common strategy in other annual bromes (for example, Meyer et al., 2013).

1.4 Distribution

Bromus interruptus is endemic to the UK and was first discovered in 1849 at Odsey in Cambridgeshire. Now extinct in the wild, the species' former range extended across south England and East Anglia (Wilson & King, 2003). The species was last recorded in the wild at Pampisford in Cambridgeshire in 1972. Introduced populations have been recorded in the Netherlands.

1.5 Habitat

Bromus interruptus is associated with agricultural crops and disturbed field edges, typically on calcareous soils but also occurring in neutral, sand and clay sites (Wilson & King, 2003; Rich and Lockton, 2004).

Historically it was particularly associated with Sainfoin and Clover crops, but also occurred in hay meadows, fallow land, roadsides and waste ground (Rich & Lockton, 2002).

1.6 Threats

As with many other arable species, improved seed cleaning, improved and competitive crop varieties and increasing use of artificial fertilisers and herbicides contributed to the decline of *B. interruptus* (Wilson & King, 2003). Plants are maintained in cultivation, with an introduced population at Plantlife's Ranscombe Farm reserve in Kent.

2 Seed ecology

2.1 Seed longevity

Rich and Lockton (2002) report there is 'some indication that seeds may be short-lived' and attempts to restore lost populations from the soil seed bank appear to have failed. Seed stored under ambient conditions is reported to have died in four years (Donald, 1980), although the seed can survive for long periods under optimal storage conditions. One collection has retained 100% viability after 50 years of storage at 15% equilibrium relative humidity (eRH) and -20°C in the MSB, for example, with two 40-year old collections retaining >93% viability.

Given the high conservation value of *B. interruptus* and the importance of the soil seed bank in the recovery and persistence of annual plant populations, it would be useful to conduct some longevity or seed aging experiments on this species.

2.2 Seed germination

The MSB holds ten collections of *B. interruptus* seeds, all of which have germinated successfully (>85% germination) at a range of temperatures from 10°C to an alternating temperature of 25/10. Tests were conducted in the presence of light, with total germination reached within one or two weeks.

3 Propagation

RBG Kew has not attempted to grow *B. interruptus* plants to maturity, although several other institutions have successfully established and maintained cultivated populations including Cambridge Botanic Garden, Ness Botanic Garden and a restored population at the Plantlife reserve at Ranscombe Farm.

4 Implications for restoration

4.1 Establishing new populations

With no recent recordings of *B. interruptus* in the wild, establishing new populations will require the introduction of seed or plant propagules from cultivated or restored populations.

Early autumn is the optimal time for sowing – autumn-sown *B. interruptus* plants are more vigorous and produce more seed – although seed may also be sown and germinated in spring. Seed should be sown onto moist, bare ground when the temperature is likely to remain at or above 10°C until germination is complete.

4.2 Sowing rate

Lang et al. (2016) suggest an optimal sowing rate for arable plant reintroductions of 50-100 seed/m^2.

Bromus interruptus does not display seed dormancy and a high proportion of viable seed exposed to suitable germination conditions may germinate. Initial sowing rates at the lower end of the range suggested by Lang et al. (2016) may therefore produce good results and make optimal use of scarce seed, although a higher rate is likely to result in a larger population and promote more rapid development of a large soil seed bank.

1g of *B. interruptus* seed contains approximately 382 individual seeds.

Seed production is high (Rich and Lockton, 2002) and populations may become self-sustaining relatively quickly where appropriate management is in place.

4.3 Long-term management

Bromus interruptus is likely to benefit from management regimes that:

- ensure plants are able to disperse seed into the soil seed bank before the crop is cut;
- avoid disturbing autumn-germinating seedlings and young plants;
- limit competition from crop or weed species.

Cultivation shortly after seed dispersal in late summer or autumn is likely to provide optimal conditions for *B. interruptus*, allowing germination and establishment before winter. To avoid disturbing autumn germinands, there should be no further disturbance until the following year. *Bromus interruptus* may also germinate in spring, suggesting periodic spring cultivation may be tolerated where necessary to control persistent weeds and benefit other arable species. To ensure populations are not lost, it would be prudent to cultivate no more than half of the site to ensure continuity of seed production.

If growing *B. interruptus* within a crop, reduced fertiliser application and lower cereal density can increase light levels for arable species. Reduced cereal sowing has been shown to increase rare arable species richness in cereal crops (Wagner et al., 2017).

In non-arable habitats, disturbance should be carried out in autumn to maintain open ground and high light levels.

5 References

Cheffings, C.M. & Farrell, L. (Eds), Dines, T.D., Jones, R.A., Leach, S.J., McKean, D.R., Pearman, D.A., Preston, C.D., Rumsey, F.J., Taylor, I., 2005. The Vascular Plant Red Data List for Great Britain. Species Status 7: 1-116. Joint Nature Conservation Committee, Peterborough.

Clayton, W.D., Vorontsova, M.S., Harman, K.T. and Williamson, H., 2006 onwards. GrassBase - The Online World Grass Flora. http://www.kew.org/data/grasses-db.html. [accessed 16 October 2018]

Donald, D., 1980. *Bromus interruptus* (Hack.) Druce. Dodo or phoenix? Nature in Cambridgeshire, 23, pp. 49– 51.

Lang, M., Prestele, J., Fischer, C., Kollmann, J., Albrecht, H., 2016. Reintroduction of rare arable plants by seed transfer. What are the optimal sowing rates? Ecology and Evolution, 6, 15, pp. 5506–5516.

Meyer, S., Ghimire, S., Decker, S., Merrill, K., Coleman, C., 2013. The ghost of outcrossing past in downy brome, an inbreeding annual grass. Journal of Heredity, 104, 4, pp. 476–490.

Rich & Lockton, 2002. *Bromus interruptus* (Hack.) Druce (Poaceae) - an extinct English endemic. Watsonia. 24, pp. 69–80.

Wagner, M., Bullock, J.M., Hulmes, L., Hulmes, S. and Pywell, R.F., 2017. Cereal density and N-fertiliser effects on the flora and biodiversity value of arable headlands. Biodiversity and conservation, 26, 1, pp. 85-102.

Wilson, P. and King, M., 2003. Arable plants: a field guide. Wild Guides.

Propagation Protocol: *Filago pyramidata* L.

1 Ecological overview

1.1 Description

Filago pyramidata is an annual herb growing to 30cm, with flowering stems branching from a basal rosette to grow upright or prostrate (Plantlife, 2006). The entire plant is covered in grey hairs (Wilson & King, 2003). Tiny flowers occur in clusters of around eight to fifteen dense heads, with yellow-tipped pointed bracts. Each flower produces a single achene (dry fruit) approximately 0.6mm long and bearing pappus hairs (Plantlife, 2006).

1.2 Phenology

Seeds may germinate at any time of year if exposed to sufficiently warm, moist conditions but peak germination occurs between October and December then again in March (Plantlife, 2006; Wilson & King, 2003; Rich, 1999). Flowering typically occurs from July to September, although some plants may flower later. Seed production follows shortly afterwards - the MSB's UK collections of *F. pyramidata* seed were made between August and October (Table 1). Autumn-germinating plants are larger and more productive, although the over-wintering rosettes may be lost in harsh winters (Rich, 1999).

1.3 Mating system

The mating system of *F. pyramidata* is not described in detail, although Rich (1999) reports that no pollinators have been observed visiting flowers and that the plants are probably self-fertile. The flowers are composed of bisexual and female florets, but lack petals or other structures to promote cross-pollination (Plantlife, 2006). Seed are dispersed by the wind, aided by the small pappus, and may travel several metres from the parent plant (Rich, 1999).

1.4 Distribution

Filago pyramidata was once recorded widely across south eastern Britain but is now restricted to a few sites (Wilson & King, 2003; Rich, 1999). The UK represents the northern edge of the species' global distribution, which is centred around west Asia, north Africa and southern and western Europe (Rich, 1999; Clapham et al., 1987).

1.5 Habitat

Filago pyramidata is associated with warm, dry sites with open vegetation and disturbed soil such as arable field margins, abandoned quarries, roadsides, paths and hedge banks. The species typically grows in calcareous, sometimes sandy soils, (Moyse, 2013; Wilson & King, 2003), although plants have been also recorded on neutral or acidic soil (Rich, 1999).

1.6 Co-occurrence with other CitM species

Associations with other CitM species are not described, although *Filago pyramidata* is associated with a number of other annual and perennial species associated with open, disturbed habitats including *Arenaria serpyllifolia, Anagallis arvensis*, *Hypericum perforatum* and *Medicago lupulina* (Rich, 1999).

1.6 Threats

The intensification of agriculture on arable land, including the increased use of chemical fertilisers and herbicides, has been a major driver of the species' decline (Wilson & King, 2003; Rich 1999). On non-arable land, urbanisation and infrastructure development, management changes and natural succession to coarse grassland, scrub and woodland have also reduced the area of suitably open, disturbed habitat. (Plantlife, 2006). The marginal nature of many sites means individuals of *F. pyramidata* are particularly vulnerable to chance events (Plantlife, 2006).

2 Seed ecology

2.1 Seed longevity

The longevity of *F. pyramidata* seed has not been established experimentally, although the literature suggests that the seed is long-lived and persistent in the soil seed bank. Large numbers of *F. pyramidata* individuals have been recorded at sites with no previous records (Moyse, 2013) or after an absence of 10-15 years (Rich, 1999), suggesting the species is able to initiate new populations from buried seed.

Given the high conservation value of *F. pyramidata* and the importance of the soil seed bank in the recovery and persistence of annual plant populations, it would be useful to conduct some longevity or seed aging experiments on this species.

2.2 Seed germination

The MSB holds germination data for 15 UK collections of *F. pyramidata* (Table 1). In general, seed germinate readily at temperatures between 10°C and 20°C, typical of autumn or spring in warmer parts of the UK, with total germination usually reached within three weeks. There is some variation in germination response between collections – such variation is not uncommon in native species and may be influenced by factors including environmental conditions at the collecting site, the time of collection, post-harvesting handling and storage (Baskin & Baskin, 2014).

Some of the MSB's UK collections (including one from a chalk pit) respond positively to a high alternating temperature regime, possibly mimicking the hot, exposed habitat of origin.

Seed origin	Collection date	Germination >85%	Germination <85%
Cambridgeshire, England	02/10/73	10°C	
Cambridgeshire, England	19/09/78	15°C	
Cambridgeshire, England	19/09/78	15°C	
Essex, England	22/08/07	10°C, 15°C	
Kent, England	25/09/91	35/20°C	
Kent, England	16/09/08	5°C, 15°C, 35/20°C	
Oxfordshire, England	07/08/95	15°C	
Oxfordshire, England	05/09/97	15°C	
Surrey, England	24/08/09	35/20°C	15°C
Surrey, England	24/08/09	15°C	20°C
Surrey, England	24/08/09	20°C (with Gibberellic acid)	15°C, 20°C
West Sussex, England	30/08/91	25°C, 25/15°C	
West Sussex, England (chalk pit)	25/09/91	20°C, 35/20°C	5°C, 10°C, 15°C, 25°C, 25/10°C
West Sussex, England	25/09/91	15°C	
West Sussex, England	25/09/91	35/20°C	

Table 1 Germination data from the MSB's UK *F. pyramidata* collections. Tests were carried out with exposure to light with 8/16 and 12/12 photoperiods.

3. Propagation

RBG Kew has not attempted to grow *F. pyramidata* plants to maturity. The seed and seedlings are very small and difficult to handle and may be vulnerable to damping off and other fungal disease (Rich, 1999).

4 Implications for restoration

4.1 Establishing new populations

Filago pyramidata appears to form a persistent soil seed bank and has been restored following scrub clearance and soil disturbance at some former sites (Rich, 1999) although such attempts have not always succeeded (Plantlife, 2006). If the species has not been recorded recently or has failed to return under a favourable management regime, reintroduction is likely to be required.

Sowing seed in autumn mimics natural seed dispersal and takes advantage of the peak germination season between October and December (Rich, 1999). Seed may also be sown in spring, and it may be prudent to follow an autumn sowing with a second spring sowing if the over-wintering rosettes have been lost. Seed should be sown onto bare, recently cultivated ground.

4.2 Sowing rate

Lang et al. (2016) suggest an optimal sowing rate for arable plant reintroductions of 50-100 seed/m^2.

Filago pyramidata does not display complex seed dormancy and a high proportion of viable seed exposed to suitable germination conditions may germinate. Seedling establishment may be low, however, as the very small seeds and seedlings are likely to be vulnerable to disturbance, disease and herbivory, consistent with generally higher seedling mortality rates in small-seeded species (Moles and Westoby, 2014). This would justify sowing at the higher rate recommended by Lang et al. (2016) - preferably higher where sufficient seed is available.

1g of *F. pyramidata* seed contains approximately 20,000 individual seeds.

4.2 Long-term management

Filago pyramidata is likely to benefit from management regimes that:

- ensure plants are able to disperse seed into the soil seed bank before the crop is cut;
- bring buried seed to the soil surface;
- avoid disturbing seedlings and young plants;
- limit competition from crop or weed species.

Optimal management for *F. pyramidata* would allow the plants to disperse seed before annual cultivation in September, allowing seed to germinate and form over-wintering rosettes from October onwards (Rich, 1999). Later cultivation may result in a more limited spring germination – Plantlife (2006) recommend that all disturbances are complete by January, with no further disturbance until the following autumn. Annual cultivation may not be required in less competitive sites, with disturbance carried out as necessary to expose seed at the soil surface and maintain open ground and high light levels.

If growing *F. pyramidata* within a crop, reduced fertiliser application and lower cereal density can increase light levels for arable species. Reduced cereal sowing has been shown to increase rare arable species richness in cereal crops (Wagner et al., 2017).

5 References

Cheffings, C.M. & Farrell, L. (Eds), Dines, T.D., Jones, R.A., Leach, S.J., McKean, D.R., Pearman, D.A., Preston, C.D., Rumsey, F.J., Taylor, I. 2005. The Vascular Plant Red Data List for Great Britain. Species Status 7: 1-116. Joint Nature Conservation Committee, Peterborough.

Clapham, A.R., Tutin, T.G., Moore, D.M., 1987. Flora of the British Isles. 3rd edition. Cambridge University Press, Cambridge.

Lang, M., Prestele, J., Fischer, C., Kollmann, J., Albrecht, H., 2016. Reintroduction of rare arable plants by seed transfer. What are the optimal sowing rates? Ecology and Evolution, 6, 15, pp. 5506–5516.

Moles, A., Westoby, M., 2004. Seedling survival and seed size: a synthesis of the literature. Journal of Ecology, 92, pp. 372-383.

Moyse, R.I., 2013. Response of broad-leaved cudweed Filago pyramidata to cultivation under Environmental Stewardship at Ranscombe Farm Reserve, Kent, UK. Conservation Evidence, 10, pp.72-76.

Plantlife, 2006. Filago pyramidata species dossier. Available: https://www.plantlife.org.uk/uk/discover-wild-plants-nature/plant-fungi-species/broad-leaved-cudweed.

Rich, T., 1999. Conservation of Britain's biodiversity IV: *Filago pyramidata* (Asteraceae), broad-leaved cudweed. Edinburgh Journal of Botany, 56, 01, pp. 61-73.

Wagner, M., Bullock, J.M., Hulmes, L., Hulmes, S. and Pywell, R.F., 2017. Cereal density and N-fertiliser effects on the flora and biodiversity value of arable headlands. Biodiversity and conservation, 26, 1, pp.85-102.

Wilson, P. and King, M., 2003. Arable plants: a field guide. Wild Guides.

Propagation Protocol: *Galeopsis angustifolia* Ehrh. ex Hoffm.

1 Ecological overview

1.1 Description

Galeopsis angustifolia Ehrh. ex Hoffm. is an erect annual herb growing to 50cm. Flowers are bilaterally symmetrical, with a long corolla tube. Each flower produces four seeds (Figure 1; Wilson & King, 2003). Growing conditions significantly impact plant size and the number of flowers produced, with those growing on the most nutrient poor soils producing only a single flower (Wilson & King, 2003).

Figure 1 *G. angustifolia* inflorescence, showing a cluster of four immature seeds produced from a single flower. ©RBG Kew

1.2 Phenology

Seeds germinate in late spring (Wilson & King 2003; Stewart et al., 1994). Plants flower from July to October (Wilson & King, 2003), with a single plant capable of producing flowers throughout the summer. When mature, seeds darken in colour from green to black and dehisce readily. The MSB's wild UK collections of *G. angustifolia* were made between August and October, and the MSB's cultivated collections were made from July to early October.

1.3 Mating system

Galeopsis angustifolia is an outcrossing species, with pollen transfer by a wide range of insects (Gibson et al., 2006). Ensuring restoration sites contain a diverse range of more common flowering plants is likely to support healthy pollinator populations and promote the long-term persistence of *G. angustifolia*. Pollinator visitation appears to vary considerably between sites, however, with one population studied relying heavily on pollination by a single species (*Bombus pascuorum*, Common Carder Bee). In these cases, interventions targeted at promoting key pollinator species – augmenting populations of *G. angustifolia* and other food plants, for example – may be beneficial. (Gibson et al., 2006).

1.4 Distribution

Galeopsis angustifolia was once widespread and recorded across calcareous soils in England and Wales, however the species is now restricted to south and south-east England (Wilson & King 2003). The species' native range extends across western, central and southern Europe (Stewart et al., 1994).

1.5 Habitat

Plants grow in arable fields, coastal vegetated shingle or scree, and other disturbed ground on light, calcareous soils (JNCC, 2010; Wilson & King, 2003; Stewart et al., 1994).

1.6 Co-occurrence with other CitM species

Galeopsis angustifolia is known to co-occur with *Torilis arvensis* (Wilson & King 2003; Stewart et al., 1994;) and *Adonis annua* (Stewart et al., 1994).

1.7 Threats

Galeopsis angustifolia cannot tolerate herbicides and is poorly-competitive within fertilised crops and improved varieties (Wilson & King 2003; Stewart et al., 1994). As a spring-germinating species, the change from spring to autumn-sown crops may be a key factor in the species' decline – plants often fail to set seed before autumn-sown crops are harvested and the stubble ploughed, preventing the establishment of a persistent soil seed bank (Stewart et al., 1994).

2 Seed ecology

2.1 Seed longevity

No long-term longevity data are available from Kew databases, although the seed is believed to be long lived (Wilson & King, 2003).

Galeopsis ladanum, a close relative of *G. angustifolia*, has been shown to have intermediate seed longevity, with a sample of 40 seeds retaining 61.9% viability after burial for two and a half years (Saatkamp et al., 2009). Although not the same species, seed longevity trends may be similar across the *Galeopsis* genus.

Given the high conservation value of *G. angustifolia* and the importance of the soil seed bank in the recovery and persistence of annual plant populations, it would be useful to conduct some longevity or seed aging experiments on this species.

2.2 Seed dormancy

Seed dormancy describes a range of mechanisms that prevent seeds germinating, even under favourable germination conditions. Dormancy may delay germination until the conditions are likely to support healthy plant growth and stagger germination over multiple growing seasons, helping the population recover from damaging short-term effects such as drought, disturbance or unfavourable management practices.

Seeds of *G. angustifolia* appear to display complex and potentially variable physiological dormancy. Seeds have a hard seed coat, imposing a mechanical constriction on the developing embryo and preventing radical (root) emergence until the seed coat has been broken down or the embryo gains sufficient growth potential to force the seed coat open. In some cases, removal of the seed coat alone has enabled germination to take place – in others this had not been sufficient, suggesting additional physiological dormancy mechanisms, controlled within the internal seed tissue, may also influence *G. angustifolia* germination.

2.3 Dormancy alleviation

Extensive research has been conducted at the MSB into the dormancy alleviation requirements of *G. angustifolia* (Table 2), resulting in a successful germination protocol for laboratory and seed production use (Table 1). This involves removal of the seed coat and the endosperm tissue surrounding the embryo (Figure 2), before incubation at 15°C on agar or sand (Figure 3). Using sand appears to promote germination and seedling development over a range of incubation temperatures, possibly due to reduced fungal growth.

Germination is typically rapid, with maximum germination achieved within 1-3 weeks following embryo excision.

Step	Method
1	Full embryo excision, using forceps and scalpel to remove seed coat and endosperm
2	Incubation at 15°C on agar or sand, 12/12 photoperiod.

Table 1 MSB laboratory germination protocol for *G. angustifolia*

Previous germination trials have included the use of the plant growth sustance gibberellic acid (GA_3) in germination media, soaking seed in GA_3 prior to sowing and removal of the seed coat alone. Some of these trials resulted in acceptable levels of germination but the results were inconsistent and variable between collections, suggesting considerable intra-specific variation in dormancy and germination behaviour.

Figure 2 *G. angustifolia* seeds, showing (left-right) whole seed, seed with seed coat removed, seed with seed coat and endosperm removed. ©RBG Kew

The MSB is currently conducting a long-term 'move-along' experiment to better understand how dormancy in *G. angustifolia* is broken under natural conditions. This technique involves cycling multiple samples of untreated seed through a series of temperature regimes designed to replicate seasonal fluctuations at the seed dispersal site (Baskin & Baskin, 2003). This study, now in a second year, suggests germination occurs slowly and sporadically over many years.

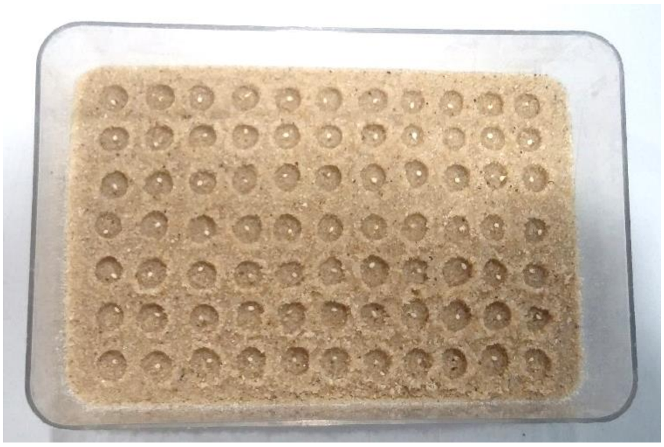

Figure 3 Excised *G. angustifolia* embryos on sand, prior to incubation at 15°C. ©RBG Kew

Pre-treatment	Temperature	Maximum Germination
GA_3 in germination media at 250mg/l	20°C	0%
	25/10°C	0%
GA_3 seed soaking at 5g/l	20/10°C	0%
GA_3 seed soaking at 10g/l		10%
	5°C	10%

Removal of seed coat, agar as germination media	10°C	30%
	15°C	87%
	20°C	30%
	15/5°C	40%
	20/10°C	67%
	25/15°C	40%
Removal of seed coat, sand as germination media	5°C	80%
	10°C	80%
	15°C	80%
	20°C	20%
	15/5°C	30%
	20/10°C	70%
	25/15°C	20%
Move-along experiment	Temperatures of 5°C, 20/10°C, 20°C and 10°C. Cycle lengths of 4, 8 and 12 weeks.	Ongoing. Low levels (4%) of germination after 1 year.

Table 2 Alternative MSB methods tested for overcoming dormancy in *G. angustifolia* seeds.

3 Cultivation

In 2018, the MSB germination protocol outlined in Table 1 was applied to a wild seed collection and plants were grown on for the production of regenerated seed for the Colour in the Margins project (Table 3).

Germination following embryo excision is rapid, allowing propagation to take place in spring and provide mature plants for harvesting in the same year.

Seedlings were grown on in a growth chamber to maximise establishment before being transferred to a glasshouse (Figure 4). Plants were potted into one litre pots in May, approximately two months after germination, and began to flower approximately fourteen weeks after germination. Seed production began about two weeks after flowering (Figure 4) and were collected over a long period between 3rd July and 4th October 2018. Following collection, seed material was temporarily stored in paper bags at 20°C and 60% RH to facilitate continued maturation for one week to ten days, before drying to 15% RH at 15°C for long-term storage.

Figure 4 Photographs of *G. angustifolia* seedlings, showing tiny seedlings after transfer from agar (left) and established seedlings (right). ©RBG Kew

Seed origin:	Gloucestershire, England. Scree on limestone grassland.
Collection date:	01/09/14
Length of storage:	3 years, 7 months
Pre-treatments:	Rehydration, excision of embryo from the seed coat and endosperm.
Germination conditions:	15°C
Germination media:	Sand
Time until germination:	One week
Duration of germination:	Three weeks
Germination percentage:	60%
Growing media:	2:1 mix of Petersfield Peat Free Supreme compost and standard perlite, with 2g/L of Osmocote controlled release fertiliser. Seedlings pricked into 5cm pots then repotted into 9cm pots.

Growing conditions:	Growth chamber set at 17°C, followed by glasshouse with minimum temperature 16°C then unheated polytunnel.
Seedling establishment:	Seedlings repotted after two months into 1 litre pots containing Melcourt nursery compost with 4g/L Osmocote controlled release fertiliser.
Time to flowering:	14 weeks
Time to seed production:	16 weeks
Seed collection:	The first seed collection occurred on 3/7/18 and continued until the final collection on 4/10/18. Seeds change colour from green to brown and can be readily shaken from the calyx when mature. Seed was after-ripened at 20°C and 60% RH for one week to ten days before final drying, cleaning and storage in the MSB.
Seed yield:	The estimated seed yield in cultivation is approximately 350 seeds per plant (to be confirmed). Yield under natural conditions is likely to be substantially lower.

4 Implications for restoration

4.1 Establishing new populations

Galeopsis angustifolia is suspected to have intermediate to long-term persistence in the soil seed bank and may return with the reinstatement of management practices that provide adequate germination and establishment niches. If the species has not been recorded recently or has failed to return under a favourable management regime, reintroduction is likely to be required.

It is not possible to apply seed coat removal or embryo-excision techniques prior to sowing in the field. Seeds must therefore be sown without pre-treatment, resulting in a spread of germination across multiple years.

Sowing seeds on bare ground in autumn mimics natural seed dispersal and enables seeds to experience the seasonal temperature fluctuations that may be required to weaken the hard seed coat and promote internal chemical changes and break physiological dormancy.

4.2 Sowing rate

Lang et al. (2016) suggest an optimal sowing rate for arable plant reintroductions of 50-100 seed/m^2.

The long-term move along experiment described above and successful reintroduction work at Cleeve Common, Gloucestershire (Philips, 2017), suggest that only a small proportion of seed (<5%) is likely to germinate in the first spring following sowing, with sporadic germination over subsequent years. To establish a large, healthy population it would be advisable to assume a low germination rate of <5% and increase the sowing rate accordingly – for example, sowing at least 1000 seed to achieve a population of 50 individuals.

1g of *G. angustifolia* seed contains approximately 588 individual seeds.

4.3 Site management

Germination of *G. angustifolia* occurs entirely in the spring and is associated with spring-sown cereal crops (Plantlife, 2007; Wilson & King, 2003). Cultivation should therefore take place in spring, between February and April. Cutting should be carried out when at least some seed has been dispersed, with the plants allowed to regrow in the stubble to disperse additional seed in the late summer and autumn (Stewart et al., 1994). Fields should be left fallow over winter, leaving seed exposed on the soil surface to promote dormancy break.

If growing *G. angustifolia* within a crop, reduced fertiliser application and lower cereal density can increase light levels for arable species. Reduced cereal sowing has been shown to increase rare arable species richness in cereal crops (Wagner et al., 2017).

In non-arable habitats, disturbance should be carried out to in late summer or early autumn to maintain open ground and high light levels.

5 References

Baskin, J.M., Baskin, C.C., 2014. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination, 2nd edition. Academic Press.

Cheffings, C.M. & Farrell, L. (Eds), Dines, T.D., Jones, R.A., Leach, S.J., McKean, D.R., Pearman, D.A., Preston, C.D., Rumsey, F.J., Taylor, I. 2005. The Vascular Plant Red Data List for Great Britain. Species Status 7: 1-116. Joint Nature Conservation Committee, Peterborough.

Gibson, R. H., Nelson, I. L., Hopkins, G. W., Hamlett, B. J., Memmott, J., 2006. Pollinator webs, plant communities and the conservation of rare plants: arable weeds as a case study. Journal of Applied Ecology, 43, pp. 246-257.

JNCC, 2010. Species pages for 2007 UK BAP Priority Species: *Galeopsis angustifolia*. Available at: http://jncc.defra.gov.uk/_speciespages/319.pdf.

Lang, M., Prestele, J., Fischer, C., Kollmann, J., & Albrecht, H., 2016. Reintroduction of rare arable plants by seed transfer. What are the optimal sowing rates? Ecology and Evolution, 6, 15, pp. 5506–5516.

Philips, E., 2017. Email with Ellie Philips, 11/12/17.

Plantlife, 2007. *Galeopsis angustifolia* species briefing sheet. Available at: https://www.plantlife.org.uk/uk/discover-wild-plants-nature/plant-fungi-species/red-hemp-nettle.

Saatkamp, A., Affre, L., Dutoit, T., Poschlod, P., 2009. The seed bank longevity index revisited: limited reliability evident from a burial experiment and database analyses. Annals of Botany, 104, 4, pp. 715–724.

Stewart, A., Pearman, D.A. and Preston, C.D., 1994. Scarce plants in Britain. JNCC.

Wagner, M., Bullock, J.M., Hulmes, L. et al. 2017. Biodiversity Conservation, 26: 85

Wilson, P. and King, M., 2003. Arable plants: a field guide. Wild Guides.

Propagation Protocol: *Ranunculus arvensis* L.

1 Ecological overview

1.1 Description

Ranunculus arvensis is an erect annual herb growing to 50cm, with much-branched stems. Solitary flowers resemble those of other buttercup species, however they are smaller and paler in colour. Each flower produces up to eight achenes (dry fruit) each containing a single seed. Achenes are up to 8mm in length with characteristic spiny husks (Figure 1, Wilson & King, 2003).

Figure 1 Photograph of *R. arvensis* flower, showing multiple green achenes, each containing a single seed. ©RBG Kew

1.2 Phenology

Seed germinate in autumn and winter (Wilson & King, 2003), with a few seedlings also germinating in spring (Stewart et al., 1994). Flowers are borne from May until mid-June. Achenes mature from green to dark brown in colour and the covering structure develops into a woody husk. UK wild seed collections held in the MSB were collected in August, with harvesting from cultivated populations between early July and early September.

1.3 Mating system

Ranunuculus arvensis plants may be bisexual or gynomonoecious (bearing bisexual and female flowers on the same plant). Cross-pollination is by small flies (Clapham, 1987) and potentially other insects – pollinator interactions for this species are not described, although other *Ranunculus* species have been found take a generalist approach and attract a broad range of insects (Steinbach & Gottsberger, 1996). Although plants may display protandry (male parts of the flowers develop first) and other mechanisms to favour cross-pollination these are not present in all cases (Clapham, 1987) and some self-fertilisation is likely to take place.

1.4 Distribution

Ranunculus arvensis was widespread across lowland England and Wales, extending to Scotland, however healthy populations are now rare, with scattered sites across the south-west midlands and southern England (Wilson & King, 2003; Stewart et al., 1994;). The species occurs throughout central and southern Europe, north Africa and west Asia, though is also declining in north-west Europe (Stewart et al., 1994).

1.5 Habitat

Ranunculus arvensis is associated with arable crops and field margins, although it also occurs on disturbed ground such as roadsides, most frequently on heavy clay and sometimes calcareous soils (Stewart et al., 1994; Wilson & King, 2003).

1.6 Co-occurrence with other CitM species

Ranunculus arvensis is known to co-occur with *Torilis arvensis* (Stewart et al., 1994; Wilson & King, 2003) and *Valerianella rimosa* (Stewart et al., 1994).

1.7 Threats

Improved seed cleaning techniques, broad-spectrum herbicides and more competitive crops have led to significant declines in *R. arvensis*. Winter annuals such as *R. arvensis* are particularly vulnerable to herbicide sprays applied early in the plant's life cycle (Albrecht, 2003).

2 Seed ecology

2.1 Seed longevity

Accelerated aging experiments under controlled conditions (50°C, 60%RH) in the MSB found *R. arvensis* was the longest-lived of 16 *Ranunculus* species tested (Bird, 2006). Although these tests do not replicate the conditions experienced by seed in the field, they do support the general view that buried *R. arvensis* seed may remain viable in the soil many years (Wilson & King, 2003; Stewart et al., 1994).

2.2 Seed dormancy

Seed dormancy describes a range of mechanisms that prevent seeds germinating, even under favourable germination conditions. Dormancy may delay germination until conditions are likely to support healthy plant growth and stagger germination over multiple growing seasons, helping the population recover from damaging short-term effects such as drought, disturbance or unfavourable management practices.

Ranunculus arvensis appears to display two forms of dormancy - morphological and physiological dormancy - commonly described together as morphophysiological dormancy (Baskin and Baskin, 2014).

As with many species in the Ranunculaceae family, seeds of *R. arvensis* have underdeveloped rudimentary embryos at dispersal, resulting in morphological dormancy (Baskin & Baskin, 2014; Figure 2). Embryos must fully develop inside the seed before germination can occur. The environmental conditions required to promote this development differ between species, but may include a period of warm, dry after ripening following seed dispersal.

When the embryo has fully developed, physiological dormancy prevents immediate germination of the seed. *Ranunculus arvensis* seeds have a hard, thick covering structure which the radicle (root tip) is unable to penetrate. This mechanical restriction is overcome naturally by splitting or decay of the covering structures through repeated cycles of warm-dry and cool-wet conditions across the seasons.

2.3 Dormancy alleviation

Research at the MSB has identified a germination protocol for *R. arvensis* suitable for use in laboratories or nursery environments (Table 1). To achieve high levels of germination quickly, surgical pre-treatment is necessary. Removing a section of the hard-covering structure allows the seed to imbibe water and begin the internal metabolic processes which enable the embryo to elongate unrestricted by the husk. This partial de-husk should ideally be carried out using forceps and a scalpel under a microscope. Crucially, only the covering structure should be removed, and the seed coat itself should not be pierced. The material to be removed should not be adjacent to the embryo, as underdeveloped embryos can detach from the seed and may die or form tiny seedlings.

In addition to the surgical pre-treatment, high germination rates are promoted by the addition of the plant growth substance gibberellic acid (GA_3) to the germination medium. Although the use of GA_3 can lead to deformed or unhealthy seedling growth, these impacts were reduced by removing seedlings from the germination medium as soon as germination was observed and providing high light levels to prevent etiolation and support healthy growth.

Step	**Method**	**Type of dormancy**
1	Partial de-husk	Physiological
2	Incubation at 10^{o}C on agar containing 250mg/l GA_3	Morphological & physiological

Table 1 MSB laboratory germination protocol for *R. arvensis.*

Soaking seeds in higher concentrations of GA_3, and incubating the seed at alternative temperatures, did not increase total germination of *R. arvensis*.

Ranunculus arvensis may achieve high germination percentages without the use of pre-treatments over longer periods of time. Incubation at 5^{o}C for periods of up to 6 months has promoted germination in some collections but proved unsuccessful in others, suggesting considerable intra-specific variation in dormancy and germination behaviour.

3 Propagation

In 2018, the MSB germination protocol outlined in Table 1 was applied to a wild seed collection and plants were grown on for the production of regenerated seed for the Colour in the Margins project (Table 2).

Artificially alleviating dormancy enabled a fast germination response and the production of mature plants from a spring sowing in March. Germination largely occurred within three weeks, with some additional sporadic germination for fifteen weeks.

Seedlings were pricked out and grown on in a heated glasshouse and then unheated polytunnel. Plants were repotted into one litre pots throughout May (Figure 2) and began to flower approximately 12 weeks after germination. Seed production began a week later, with collection between 9th July and 3rd September 2018. Following collection, seed material was stored in paper bags at 20°C and 60% RH to facilitate continued maturation for one week to ten days, before drying to 15% RH at 15°C for long-term storage.

Figure 2 Photographs of *R. arvensis* seedlings, showing seedlings after transfer from agar (left) and later once seedlings were established and repotted (right). ©RBG Kew

Seed origin:	Former arable land, Cambridgeshire.
Collection date:	01/08/1978
Length of storage:	39 years, 6 months
Pre-treatments:	Partial de-husk
Germination conditions:	10°C, 12/12 photoperiod
Germination media:	Agar with 250mg/L of GA_3
Time until germination:	11 days
Duration of germination:	Three weeks, with sporadic germination to 15 weeks
Germination percentage:	84%
Growing media:	2:1 mix of Petersfield Peat Free Supreme compost and standard perlite, with 2g/L of Osmocote controlled release fertiliser. Seedlings pricked into 5cm pots then repotted into 1L pots.
Growing conditions:	Glasshouse with minimum temperature 16°C, glasshouse with minimum temperature 4°C, unheated polytunnel.

Time to flowering:	12 weeks
Time to seed production:	13 weeks
Seed collection:	The first seed collection occurred on 9/7/18 and continued until the final collection on 3/9/18. Seeds dry and change colour from green to brown when mature and can be easily pulled or shaken from the plant.
Seed yield:	The estimate seed yield in cultivation is 250 seed per plant (to be confirmed). Seed yield under natural conditions is likely to be substantially lower.

Table 2 Propagation protocol for *R. arvensis.*

4 Implications for restoration

4.1 Establishing new populations

Ranunculus arvensis is believed to have intermediate or long-term persistence in the soil seed bank and may return with the reinstatement of management practices that provide adequate germination and establishment niches. If the species has not been recorded recently or has failed to return under a favourable management regime, reintroduction is likely to be required.

Artificial dormancy alleviation techniques for *R. arvensis* are not practicable for large-scale use. Reintroduction should therefore take advantage of natural opportunities for dormancy release and germination, with germination likely to be spread over multiple years.

Sowing seeds in autumn mimics natural seed dispersal and enables seeds to experience the seasonal temperature fluctuations that may be required to weaken the hard covering structure, promote internal hormonal changes and break physiological dormancy.

4.2 Sowing rate

Lang et al. (2016) suggest an optimal sowing rate for arable plant reintroductions of 50-100 seed/m^2.

The dormancy and germination behaviour of *R. arvensis* appears to be quite variable, making it hard to predict germination and establishment success. Whilst high levels of germination are possible, to establish a large, healthy population it may be prudent to sow at the higher rate recommended by Lang et al. (2016), or higher where the supply of seed permits.

1g of *R. arvensis* seed contains approximately 89 individual seeds.

4.3 Site Management

Ranunculus arvensis is likely to benefit from management regimes that:

- ensure the maximum number of seed are able to enter the soil seed bank before the crop is cut;
- provide an opportunity for seed after-ripening and dormancy-break on the soil surface;
- minimise disturbance of autumn germinating seedlings.

Cutting immediately after seed dispersal in August followed by a short fallow period and cultivation in September or early October is likely to enable after-ripening on the soil surface and promote autumn germination, a regime consistent with the association between *R. arvensis* and autumn-sown winter cereals (Wilson & King, 2003; Stewart et al., 1994). As *R. arvensis* is believed to form a persistent soil seed bank and can also germinate in spring, populations may also withstand periodic spring cultivation if required for other arable species (Plantlife, 2009).

If growing *R. arvensis* within a crop, reduced fertiliser application and lower cereal density can increase light levels for arable species. Reduced cereal sowing has been shown to increase rare arable species richness in cereal crops (Wagner et al., 2017).

In non-arable habitats, disturbance should be carried out to in autumn to maintain open ground and high light levels.

5 References

Albrecht, H., 2003. Suitability of arable weeds as indicator organisms to evaluate species conservation effects of management in agricultural ecosystems. Agriculture, Ecosystems & Environment, 98, 1-3, pp. 201-211

Baskin, J.M., Baskin, C.C., 2014. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination, 2nd edition. Academic Press.

Bird, A. E., 2006. Thermal methods and comparative seed longevity in the genus *Ranunculus* L. PhD Thesis, School of Biological and Chemical Sciences, Birkbeck College, University of London.

Cheffings, C.M. & Farrell, L. (Eds), Dines, T.D., Jones, R.A., Leach, S.J., McKean, D.R., Pearman, D.A., Preston, C.D., Rumsey, F.J., Taylor, I., 2005. The Vascular Plant Red Data List for Great Britain. Species Status 7, 1-116. Joint Nature Conservation Committee, Peterborough.

Clapham, A., Tutin, T., Moore, D., 1987. Flora of the British Isles. Third Edition. Cambridge, CUP.

Plantlife, 2009. Ranunculus arvensis species briefing sheet. Available: https://www.plantlife.org.uk/uk/discover-wild-plants-nature/plant-fungi-species/corn-buttercup.

Steinbach, K., Gottsberger, G., 1994. Phenology and pollination biology of five *Ranunculus* species in Giessen, Central Germany. Phyton; annales rei botanicae, 34, pp. 203-218.

Stewart, A., Pearman, D.A. and Preston, C.D., 1994. Scarce plants in Britain. JNCC.

Wagner, M., Bullock, J.M., Hulmes, L., Hulmes, S. and Pywell, R.F., 2017. Cereal density and N-fertiliser effects on the flora and biodiversity value of arable headlands. Biodiversity and conservation, 26, 1, pp. 85-102.

Wilson, P. and King, M., 2003. Arable plants: a field guide. Wild Guides.

Propagation Protocol: *Silene gallica* L.

1 Ecological overview

1.1 Description

Silene gallica is an erect annual growing to 30cm. Young plants form a basal rosette followed by a framework of occasionally-branched stems (Wilson & King, 2003). Upper parts are covered in sticky, glandular hairs. Multiple flowers form along stem branches, each up to 15mm across with five yellowish-white to pink petals. Seed capsules reach approximately 10mm long and open at the tip to release the seeds (Figure 1, Wilson & King, 2003). Each capsule produces an average of 48 seeds, each weighing approximately 0.4mg (Plantlife, 2007; Salisbury, 1961). Seeds are dark brown, kidney-shaped and approximately 0.8mm in diameter (Wilson & King, 2003).

Figure 1 *Silene gallica* capsule, showing individual seeds dispersing and sticking to glandular hairs. ©RBG Kew

1.2 Phenology

Seeds germinate in autumn and spring (Wilson & King, 2003; Stewart et al., 1994). Flowers are produced over a long period from June-October (Plantlife, 2007), with seed dispersal following shortly afterwards. The MSB's wild and cultivated UK collections of *S. gallica* seed were made between July and October.

1.3 Mating system

Silene gallica flowers are predominantly bisexual, although gynomonoecious (bearing bisexual and female flowers on the same plant) individuals have been reported (Casimiro-Soriguer et al., 2015). Cross-pollination by a range of *Lepidoptera* and bees is reported (CABI, 2018) although pollinator interactions for this species are not described in detail. Some self-fertilisation is also likely to take place (Plantlife, 2008). Although most seed is dispersed close to the parent plant (Plantlife, 2008), viscid stems and capsules may aid seed dispersal by animals or human disturbance (Guthrie-Smith, 1953) and the small, light seed may be carried by wind.

1.3 Distribution

Although never abundant, *S. gallica* was widespread across lowland southern UK and Wales, with limited sites extending to Scotland. The species has declined across its range and is now highly restricted to south-west coastal areas of England and Wales (Wilson & King, 2003; Stewart et al., 1994). *Silene gallica* is also found in dunes in the Channel Islands (Stewart et al., 1994). The southerly distribution is likely influenced by the inability of *S. gallica* to tolerate temperatures below -10°C (Stewart et al., 1994).

1.4 Habitat

Silene gallica grows in light, sandy soils in arable fields of spring and winter crops, wastelands, open coastal grassland and sand dunes (Preston et al., 2002; Stewart et al., 1994).

1.5 Co-occurrence with other CitM species

Silene gallica is known to co-occur with *Filago pyramidata* and *Valerianella rimosa* (Plantlife, 2008).

1.6 Threats

Silene gallica does not compete well with fertilised crops and improved varieties (Wilson & King, 2003). Seeds are easily cleaned from crop harvests, and abandonment of arable land and conversion of field margins to grassland has led to declines in suitable arable habitat (Plantlife, 2007). Increased tourism and clifftop erosion also threaten coastal populations (JNCC, 2010; Plantlife, 2007).

2 Seed ecology

2.1 Seed longevity

In accelerated aging studies conducted at the MSB, S. *gallica* seeds incubated at 60%RH and 45^{o}C showed no significant loss in viability until they had been stored for more than 100 days, suggesting the seed is comparatively long-lived. The MSB also holds a *S. gallica* collection that has retained 98% viability for 36 years under storage conditions of 15% eRH (equilibrium relative humidity) and -20°C. Whilst controlled trials and long-term dry storage do not replicate conditions experienced by seed in the soil, these data support the general view (for example, Plantlife 2008) that *S. gallica* seed are long-lived and persistent in the soil seed bank.

2.2 Germination

The MSB holds germination data for three UK collections of *S. gallica*, displaying variable germination requirements (Table 1). In two collections, germination has been straightforward with no evidence of dormancy and high germination rates at alternating temperatures of 20/10^{o}C. Seeds from the Cornwall collection, by contrast, show low germination unless pre-treated with a radicle chip - removing a small portion of seed coat adjacent to the radicle (root) (Figure 2). Germination is then very rapid, even at the lower temperature of 10^{o}C. Research is underway to understand potential dormancy mechanisms in this collection and develop a propagation protocol without the need for a radical chip. Intra-specific variation in seed dormancy and germination is not uncommon in native species and may be influenced by factors including environmental conditions at the collecting site, the time of collection, post-harvesting handling and storage (Baskin & Baskin, 2014).

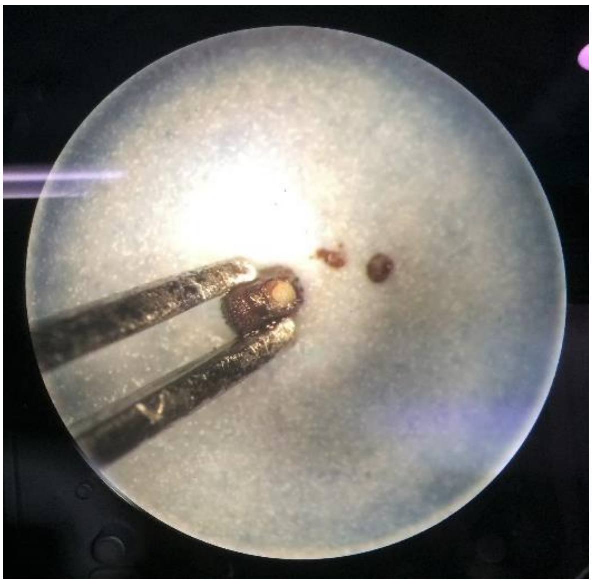

Figure 2 *Silene gallica* radicle chip using forceps and a scalpel under a stereoscope. ©RBG Kew

Silene gallica seed require light to germinate. Even low levels of shading – for example by young crop seedlings - can prevent germination (Battla et al., 2000).

Seed Origin	**Collection Date**	**Successful Protocol**
Pembrokeshire, Wales	01/08/68	20/10^{o}C (100% germination)
Essex, England	01/07/95	20^{o}C (100% germination)
Cornwall, England	20/10/99	Radicle chip, 10^{o}C (100% germination)

Table 1 Germination data from MSB's UK *S. gallica* collections. Tests were carried out with exposure to light with 8/16 and 12/12 photoperiods.

3 Propagation

In 2018, seeds from the MSB collection of *S. gallica* originating in Cornwall were germinated and grown on for the production of regenerated seed for the Colour in the Margins project (Table 2).

Propagation began in late March with a radicle chip and sowing on compost in a heated glasshouse. Germination was rapid, beginning after one week and continuing for three weeks. Seedlings were sown directly into 5cm pots to minimise root disturbance then transferred to one litre pots in May, approximately two months after germination (Figure 3). Seedlings of *S. gallica* are extremely small and susceptible to disturbance and herbivory - in cultivation, sciarid fly pose a particular threat - which can lead to total defoliation of the young plants.

Figure 3 *Silene gallica* seedlings growing in 5cm plug trays (left) and at the flowering stage (right). ©RBG Kew

Plants began to flower approximately twelve weeks after germination (Figure 3), and seed production began about three weeks later. Seeds were collected from this regenerated population between 18th July and continued at the time of writing in mid-October 2018. Entire flowering stems were cut when at least 50% of the seeds were at the point of dispersal. Following collection, seed material was temporarily stored in paper bags at 20°C and 60% RH to facilitate continued maturation for one week to ten days, before drying to 15% RH at 15°C for long-term storage.

Seed origin:	Arable field, West Pentire, Cornwall
Collection date:	20/10/99
Length of storage:	18 years, 5 months
Pre-treatment:	Radicle chip (removing a portion of the seed coat adjacent to the radicle)
Germination conditions:	Heated glasshouse (min 16°C).
Germination media:	2:1 Petersfield Peat Free Supreme potting compost and standard perlite in 5cm plug trays. Seeds covered with a thin layer of fine vermiculite.
Time until germination:	One week
Duration of germination:	Three weeks
Germination percentage:	46%
Growing media:	Seedlings repotted 15/5/18, 7 weeks after sowing, into 1 litre pots containing Melcourt nursery compost with 4g/L Osmocote controlled release fertiliser.
Growing conditions:	Established seedlings moved to a cool glasshouse (min 4°C).
Time to flowering:	12 weeks
Time to seed production:	15 weeks
Seed collection:	The first seed collection occurred on 18/7/18 and was continuing at the time of writing in mid-October 2018. Plants simultaneously bear unopened buds, flowers, immature and mature seed. Seeds change colour from green to brown when mature and can be easily shaken from the capsule.
Seed yield:	The seed yield in cultivation is likely to be in excess of 1000 seed per plant (to be confirmed). Yield under natural conditions is likely to be lower.

Table 2 Propagation protocol for *S. gallica*.

4 Implications for restoration

4.1 Establishing new populations

Silene gallica is likely to have long-term persistence in the soil seed bank and may return with the reinstatement of management practices that expose buried seed to the light and reduce competition with other plants. If the species has not been recorded recently or has failed to return under a favourable management regime, reintroduction is likely to be required.

Sowing in early autumn mimics natural seed dispersal and provides an opportunity for autumn and spring germination. As a poor competitor, *S. gallica* should be reintroduced to bare, recently cultivated ground, ensuring seed are exposed to high light levels and are neither buried in the soil nor shaded by other plants. Seeds which do not germinate in the autumn/winter after sowing may germinate in the following spring.

4.2 Sowing rate

Lang et al. (2016) suggest an optimal sowing rate for arable plant reintroductions of 50-100 seed/m^2.

In general, *S. gallica* does not display complex seed dormancy and a high proportion of viable seed exposed to suitable germination conditions (moist soil, high light availability and moderate temperatures) may germinate. Seedling establishment is likely to be low, however, as experience at Wakehurst suggests the small seedlings are vulnerable to disturbance and herbivory, consistent with generally higher seedling mortality rates in small-seeded species (Moles and Westoby, 2014). This would justify sowing at the higher rate recommended by Lang et al. (2016) - preferably higher where sufficient seed is available.

1g of *S. gallica* seed contains approximately 3077 individual seeds.

4.2 Long-term management

Silene gallica is likely to benefit from management regimes that:

- ensure plants are able to disperse seed into the soil seed bank before the crop is cut;
- expose buried seed to light;
- avoid disturbing seedlings and young plants;
- limit competition from crop or weed species.

Silene gallica can germinate following cultivation in mid-autumn or early-spring, provided plants are able to complete their life cycle before the ground is disturbed again (Plantlife, 2008). As a poor competitor, reduced fertiliser application and lower cereal density is likely to be particularly beneficial for *S. gallica,* consistent with the general association between reduced cereal density rare arable species richness (Wagner et al., 2017).

Silene gallica may also persist in headlands, field margins and other habitats where large vegetation gaps and ongoing disturbance create opportunities for seeds to geminate and establish. Where natural processes (rabbit grazing, unstable soils etc.) are insufficient to create these niches, additional annual disturbance may be necessary (Plantlife, 2008).

5 References

Albrecht, H., 2003. Suitability of arable weeds as indicator organisms to evaluate species conservation effects of management in agricultural ecosystems. Agriculture, Ecosystems & Environment, 98, 1-3, pp.201-211.

Baskin, J.M., Baskin, C.C., 2014. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination, 2nd edition. Academic Press.

Batlla, D., Kruk, B.C. and Benech-Arnold, R.L., 2000. Very early detection of canopy presence by seeds through perception of subtle modifications in red: far red signals. Functional Ecology, 14, 2, pp. 195-202.

CABI, 2018. Invasive Species Compendium: *Silene gallica* datasheet. Available: https://www.cabi.org/isc/datasheet/117143.

Casimiro-Soriguer, I., Buide, M., Narbona, E., 2015. Diversity of sexual systems within different lineages of the genus *Silene*. AoB Plants, 7.

Cheffings, C.M. & Farrell, L. (Eds), Dines, T.D., Jones, R.A., Leach, S.J., McKean, D.R., Pearman, D.A., Preston, C.D., Rumsey, F.J., Taylor, I., 2005. The Vascular Plant Red Data List for Great Britain. Species Status 7, 1-116. Joint Nature Conservation Committee, Peterborough.

Guthrie-Smith H, 1953. Tutira - The Story of a New Zealand Sheep Station, 3rd edition. William Blackwood and Sons, Edinburgh, pp. 282-285.

JNCC, 2010. Species pages for 2007 UK BAP Priority Species: *Silene gallica*. Available: http://jncc.defra.gov.uk/_speciespages/575.pdf

Lang, M., Prestele, J., Fischer, C., Kollmann, J., Albrecht, H., 2016. Reintroduction of rare arable plants by seed transfer. What are the optimal sowing rates? Ecology and Evolution, 6, 15, pp. 5506–5516.

Moles, A., Westoby, M., 2004. Seedling survival and seed size: a synthesis of the literature. Journal of Ecology, 92, pp. 372-383.

Plantlife, 2007. *Silene gallica* species briefing sheet. Available: https://www.plantlife.org.uk/uk/discover-wild-plants-nature/plant-fungi-species/small-flowered-catchfly.

Plantlife, 2008. *Silene gallica* species dossier. Available: www.plantlife.org.uk/uk/discover-wild-plants-nature/plant-fungi-species/small-flowered-catchfly.

Preston, C.D., Pearman, D.A., Dines, T.D., 2002. New Atlas of the Flora of Britain and Ireland. Oxford University Press, London.

Salisbury E., 1961. Weeds and Aliens. Collins, London.

Stewart, A., Pearman, D.A., Preston, C.D., 1994. Scarce plants in Britain. JNCC.

Wagner, M., Bullock, J.M., Hulmes, L., Hulmes, S., Pywell, R.F., 2017. Cereal density and N-fertiliser effects on the flora and biodiversity value of arable headlands. Biodiversity and conservation, 26, 1, pp.85-102.

Wilson, P., King, M., 2003. Arable plants: a field guide. Wild Guides.

Propagation Protocol: *Torilis arvensis* (Huds.) Link

1 Ecological overview

1.1 Description

Torilis arvensis is an annual, rarely biennial, herb growing to 40cm. Inflorescences form umbels approximately 10-25mm in diameter. Individual flowers are small, approximately 2mm across, and bear white or pale pink petals. Fruit form in late summer and are borne in pairs, are 4-6mm long and covered with bristly hairs with incurved tips (Figure 1; Wilson & King, 2003).

Figure 1 Photograph of *T. arvensis* fruits, showing bristly hairs covering the outer husk. ©RBG Kew

1.2 Phenology

Torillis arvensis seed germinate in late autumn and winter, between October and December (Wilson & King, 2003), with seedlings forming an overwintering rosette. Flowers are produced from July to September (Wilson & King, 2003), with seed production shortly afterwards. The MSB's UK wild collection of *T. arvensis* was made in late September. Overwintering rosettes may not survive very harsh winters (Stewart et al., 1994).

1.3 Mating system

Torillis arvensis is an andromonecious species, bearing bisexual and staminate (male) flowers on the same plant. The species is also weakly protandrous, with male parts of the flower maturing first. Whilst these are typically strategies to promote cross-pollination, male and female flowering phases have been observed to overlap – this, together with the small flower size and lack of obvious pollinator interactions, suggests self-fertilisation is widespread (Koul, et al., 1993; Gupta, et al., 1985). Incurved hairs on the seed husk promote dispersal by animals.

1.4 Distribution

The species was formerly widespread across lowland chalk areas of southern and eastern England but has declined across its range and is currently most frequent in Somerset and East Anglia (Wilson & King, 2003). *T. arvensis* is most commonly found on heavy calcareous clay soils (Stewart et al., 1994). The UK is at the northern edge of the species' range, which extends to western, southern and central Europe and into south-western Asia (Stewart et al., 1994).

1.5 Habitat

Torilis arvensis is associated with the field margins of winter-sown cereal crops and disturbed ground such as wasteland and railway sidings adjacent to arable land (Plantlife, 2006; Stewart et al., 1994).

1.6 Co-occurrence with other CitM species

Torilis arvensis is known to co-occur with *Ranunculus arvensis, Adonis annua, Valerianella rimosa* and *Galeopsis angustifolia* (Plantlife, 2006; Wilson & King, 2003; Stewart et al., 1994).

1.7 Threats

Torilis arvensis does not compete well with fertilised crops and improved varieties. Early harvesting and ploughing may prevent this late-flowering species forming and dispersing seed (Wilson & King, 2003) and, like other winter annuals, seedlings are vulnerable to herbicides applied early in their life cycle (Albrecht, 2003; Stewart et al., 1994).

Improved arable seed cleaning and the succession of arable field margins to grassland – either as a deliberate policy or abandonment - may also threaten *T. arvensis* populations (Plantlife, 2006).

2 Seed ecology

2.1 Seed longevity

There is some disagreement in the literature regarding the longevity of *T. arvensis* in the soil seed bank, although the available research suggests seed can persist for at least three to four years and potentially longer (Plantlife, 2006).

No long-term longevity data are available from Kew databases and, given the importance of the soil seed bank in the recovery and persistence of annual plant populations, it would be useful to conduct some longevity or seed aging experiments on this species.

2.2 Seed germination

Torilis arvensis seeds do not display any dormancy mechanisms and will germinate readily when the germination conditions are met. The MSB's UK wild collection of *T. arvensis* has been tested under three conditions (Table 1). Incubation at a constant temperature of 10°C or alternating temperatures of 25/10°C both resulted in high germination (≥90%). Germination began within two weeks and continued sporadically for up to three months. Seed exposed to a period of cold stratification period of eight weeks followed by incubation at an alternating temperature regime of 25/10° was more prone to rotting and did not germinate readily.

Incubation temperature	Germination
8wks 5°C stratification, followed by 25/10°C	0%
10°C	90%
25/10°C	98%

Table 1 Germination data from the MSB's UK *T. arvensis* collections. Tests were carried out with exposure to light in 8/16 or 12/12 photoperiods.

3 Propagation

In 2018, seed from the MSB's wild UK collection of *T. arvensis* was propagated to produce a regenerated seed for the Colour in the Margins project (Table 2).

Seeds were sown in January 2018 on agar plates and incubated at an alternating temperature of 25/10°C. Germination began after twelve days, with final germination reached after three months.

Seedlings were grown on in 5cm pots in a growth chamber providing supplementary lighting to sustain growth until natural light levels increased in late March. Seedlings were transferred to a glasshouse and then an unheated polytunnel, and were potted into one litre pots in April, three months after germination began (Figure 2). Plants began to flower in July, however the entire population was suddenly lost – rotting at the roots and stem base suggest a short period of over-watering combined with exceptionally high summer temperatures may have been the cause. The plants also proved very susceptible to aphid infestation in cultivation.

Figure 2 *Torilis arvensis* seedlings, showing cotyledons and characteristic true leaves (left), and established plants shortly before flowering (right). ©RBG Kew

Seed origin:	Edge of arable field, Acton, Suffolk
Collection date:	22/09/98
Length of storage:	Twenty years, four months
Pre-treatment:	None
Germination conditions:	25/10°C, 12/12 photoperiod
Germination media:	Agar
Time until germination:	Twelve days
Duration of germination:	Three months

Germination percentage:	92%
Growing media:	2:1 Petersfield Peat Free Supreme potting compost and standard perlite in 5cm plug trays.
Growing conditions:	Growth chamber set at 17°C, followed by a glasshouse with minimum temperature 16°C then an unheated polytunnel.
Establishment:	Seedlings repotted after three months into 1 litre pots 1 litre pots containing Melcourt nursery compost with 4g/L Osmocote controlled release fertiliser.

Table 2 Propagation protocol for *T. arvensis*. To be updated with the results of a second attempt in 2019.

4 Implications for restoration

4.1 Establishing new populations

Torilis arvensis is believed to have at least medium-term persistence in the soil seed bank and may return with the reinstatement of management practices that provide suitable germination niches and reduced competition with other plants. If the species has not been recorded recently or has failed to return under a favourable management regime, reintroduction is likely to be required.

Sowing in early-mid autumn mimics natural seed dispersal and permits rapid germination in autumn and early winter. As a poor competitor, T. *arvensis* should be reintroduced to bare, recently cultivated ground. Although the species is generally understood to germinate exclusively in autumn some studies have also reported limited spring germination (Plantlife, 2006).

4.1 Sowing rate

Lang et al. (2016) suggest an optimal sowing rate for arable plant reintroductions of 50-100 seed/m^2.

Torilis arvensis does not display seed dormancy and a high proportion of viable seed exposed to suitable germination conditions may germinate. Initial sowing rates at the lower end of the range suggested by Lang et al. (2016) may produce good results, although a higher rate is likely to result in a larger population and promote more rapid development of a large soil seed bank.

1g of *T. arvensis* seed contains approximately 500 individual seeds.

4.2 Long-term management

Torilis arvensis is likely to benefit from management regimes that:

- ensure plants are able to disperse seed into the soil seed bank before the crop is cut;
- avoid disturbing seedlings and young plants;
- limit competition from crop or weed species.

Torilis arvensis has a relatively long life-cycle, producing seed later than most other cornfield annuals. Where possible, cutting should be delayed until the seed has been dispersed, with stubble left unploughed to allow plants to continue flowering and dispersing seed. Cultivation should take place in autumn, with no further disturbance until the following autumn. Plantlife (2008) suggest periodic spring cultivation may be tolerated where necessary to control persistent weeds and benefit other arable species - to ensure populations are not lost, it would be prudent to cultivate no more than half of the site to ensure continuity of flowering and seed production.

If growing *T. arvensis* within a crop, reduced fertiliser application and lower cereal density can increase light levels. Reduced cereal sowing has been shown to increase rare arable species richness in cereal crops (Wagner et al., 2017).

In non-arable habitats, disturbance should be carried out in autumn to maintain open ground and high light levels.

5 References

Albrecht, H., 2003. Suitability of arable weeds as indicator organisms to evaluate species conservation effects of management in agricultural ecosystems. Agriculture, Ecosystems & Environment, 98, 1-3, pp. 201-211.

Cheffings, C.M., Farrell, L., (Eds), Dines, T.D., Jones, R.A., Leach, S.J., McKean, D.R., Pearman, D.A., Preston, C.D., Rumsey, F.J., Taylor, I., 2005. The Vascular Plant Red Data List for Great Britain. Species Status 7, 1-116. Joint Nature Conservation Committee, Peterborough.

Gupta, S.K., Hamal, I.A., Koul, A.K. Proc., 1985. Reproductive biology of *Torilis arvensis* (Hudson) Link. Indian Acad. Sci. (Plant Sci.), 95, 227.

Koul, P., Sharma, N., Koul, A., 1993. Pollination biology of *Apiaceae*. Current Science, 65, 3, pp. 219-222.

Lang, M., Prestele, J., Fischer, C., Kollmann, J., Albrecht, H., 2016. Reintroduction of rare arable plants by seed transfer. What are the optimal sowing rates? Ecology and Evolution, 6, 15, pp. 5506–5516.

Plantlife, 2006. *Torilis arvensis* species dossier. Available: https://www.plantlife.org.uk/uk/discover-wild-plants-nature/plant-fungi-species/spreading-hedge-parsley.

Stewart, A., Pearman, D.A. and Preston, C.D., 1994. Scarce plants in Britain. JNCC.

Wagner, M., Bullock, J.M., Hulmes, L., Hulmes, S. and Pywell, R.F., 2017. Cereal density and N-fertiliser effects on the flora and biodiversity value of arable headlands. Biodiversity and conservation, 26, 1, pp.85-102.

Wilson, P. and King, M., 2003. Arable plants: a field guide. Wild Guides.

Propagation Protocol: *Valerianella rimosa* Bastard

1 Ecological overview

1.1 Description

Valerianella rimosa is a slender, branched annual herb growing to 30cm (Plantlife, 2008). Inflorescences form in terminal clusters, usually with additional single flowers in the branch axils. Flowers grow to 2mm in diameter with five white petals which are occasionally tinged pink. Fruits of *V. rimosa* are approximately 1.5mm across and resemble a grape pip, with a single tooth at the apex (Wilson & King, 2003).

Figure 1 *Valerianella rimosa* fruit, each containing a single seed. ©RBG Kew

1.2 Phenology

Seed germinate predominantly in autumn but may also germinate in spring (Plantlife, 2008; Wilson & King, 2003). Plants form a rosette from which a single, erect stem forms in summer and branches to bear flowers between June and August. Autumn germinands may flower earlier than spring germinands (Plantlife, 2008) and may be more productive. Seed is dispersed shortly after flowering – four of the MSB's UK collections of *V. rimosa* seed were made in July and August, with one collection in early September.

1.3 Mating system

The mating system of *V. rimosa* is not described in detail. Flowers are bisexual (Stace, 2010) and it is likely both cross and self-pollination occur, as observed in related *Valerianella* species (for example, Muminovic et al., 2018). The fruits do not have any obvious adaptations to aid dispersal (Plantlife, 2008).

1.4 Distribution

Although the species was never abundant, *V. rimosa* was formerly scattered across lowland southern England and Wales. The species has now declined to a handful of sites, concentrated in southern and south-west England (Plantlife 2008; Wilson & King, 2003).

1.5 Habitat

Valerianella rimosa is strongly associated with arable fields and field margins and may be found within both winter and spring-sown crops. Plants have also been recorded at coastal sites and other disturbed ground, such as quarries and chalk tracks (Plantlife, 2008). Historically associated with calcareous soils, remaining populations are found on a variety of acidic, sandy, clay and silt soils (Plantlife, 2008; Wilson & King, 2003).

1.5 Co-occurrence with other CitM species

Valerianella rimosa is known to occur with *Torilis arvensis, Silene gallica* and *Ranunuclus arvensis* (Plantlife, 2008; Wilson & King, 2003)

1.6 Threats

Valerianella rimosa is susceptible to herbicides and is easily out-competed by fertilised crops and improved crop varieties (Wilson & King, 2003). Seeds are easily cleaned from crop harvests and natural succession of arable field margins to grassland may also threaten *V. rimosa* populations (Plantlife, 2008). At coastal sites, populations may be vulnerable to cliff erosion (Plantlife, 2008)

2 Seed ecology

2.1 Seed longevity

The longevity of *V. rimosa* seed has not been established experimentally, although other *Valerianella* species are believed to be long-lived (Plantlife, 2008).

Given the high conservation value of *V. rimosa* and the importance of the soil seed bank in the recovery and persistence of annual plant populations, it would be useful to conduct some longevity or seed aging experiments on this species.

2.2 Seed germination

The MSB holds germination testing data for five UK collections of *V. rimosa* (Table 1). In general, these data suggest that *V. rimosa* will germinate without pre-treatment at temperatures of 10°C and alternating temperatures of 25°C and 10°C, although the germination rate and percentage is variable between collections. In one collection, germination was promoted by a period of cool incubation at 10°C for four weeks followed by an alternating temperature regime of 25/10°C. Separate germination peaks were observed at each temperature – a first within 2 weeks of incubation at 10°C, then a second 2 weeks into incubation at the alternating temperature regime.

Variation within and between collections of the same native species is not uncommon and may be influenced by factors including environmental conditions at the collecting site, the time of collection, post-harvesting handling and storage (Baskin & Baskin, 2014).

The addition of gibberellic acid (GA_3), a plant growth substance, to the growing media has promoted higher and faster levels of germination. More research is required to determine whether dormancy mechanisms are present in some collections and whether the failure of seed to germinate in winter is due to the imposition of dormancy (Plantlife, 2008) or temperatures below the germination threshold.

Seed origin	Collection date	Germination >85%	Germination <85%
Somerset, England	14/08/98	25/10°C GA3 10°C GA3	20°C GA3
Oxfordshire, England	13/07/01	10°C then 25/10°C	
Oxfordshire, England	13/07/01	25/10°C GA3	10°C 4wks > 25/10°C GA3 5°C 8wks > 25/10°C GA3

Hampshire, England	11/08/06	10°C 25/10°C	
Hampshire, England	14/07/16	10°C	10°C 4wks > 25/10°C

Table 1 MSB germination data for UK collections of *V. rimosa.*

3. Propagation

RBG Kew has not attempted to grow *V. rimosa* plants to maturity.

4 Implications for restoration

4.1 Establishing new populations

Valerianella rimosa is believed to be at least moderately long-lived in the soil seed bank (Plantlife, 2008) and may return with the reinstatement of management practices that provide adequate germination and establishment niches. If the species has not been recorded recently or has failed to return under a favourable management regime, reintroduction is likely to be required.

Sowing seed in autumn mimics natural seed dispersal and provides an opportunity for autumn germination and the establishment of larger and more productive plants (Plantlife, 2008). Seed which does not germinate in autumn may remain dormant or quiescent in the soil and germinate in spring, when further sowing may also take place. Seed should be sown onto bare, recently cultivated ground.

4.2 Sowing rate

Lang et al. (2016) suggest an optimal sowing rate for arable plant reintroductions of 50-100 seed/m^2.

The germination behaviour of *V. rimosa* appears to be variable, making it hard to predict germination and establishment success. Whilst high levels of germination are possible, to establish a large, healthy population it may be prudent to sow at the higher rate recommended by Lang et al. (2016), or higher where the supply of seed permits.

1g of *V. rimosa* seed contains approximately 814 individual seeds.

4.2 Long-term management

Valerianella rimosa is likely to benefit from management regimes that:

- ensure plants are able to disperse seed into the soil seed bank before the crop is cut;
- bring buried seed to the soil surface;
- avoid disturbing seedlings and young plants;
- limit competition from crop or weed species.

Optimal management for *V. rimosa* would allow the plants to disperse seed before annual cultivation in September, allowing seed to germinate before winter with no further disturbance until the following autumn. As seed is also able to germinate in spring, populations may withstand periodic spring cultivation if necessary to control problematic weeds or promote other rare annual species.

If growing *V. rimosa* within a crop, reduced fertiliser application and lower cereal density can increase light levels for arable species. Reduced cereal sowing has been shown to increase rare arable species richness in cereal crops (Wagner et al., 2017).

5 References

Cheffings, C.M. & Farrell, L. (Eds), Dines, T.D., Jones, R.A., Leach, S.J., McKean, D.R., Pearman, D.A., Preston, C.D., Rumsey, F.J., Taylor, I. 2005. The Vascular Plant Red Data List for Great Britain. Species Status 7, 1-116. Joint Nature Conservation Committee, Peterborough.

Lang, M., Prestele, J., Fischer, C., Kollmann, J., Albrecht, H., 2016. Reintroduction of rare arable plants by seed transfer. What are the optimal sowing rates? Ecology and Evolution, 6, 15, pp. 5506–5516.

Muminovic, J., Melchinger, E., Lübberstedt, T., 2018. Genetic diversity within lamb's lettuce (*Valerianella locusta* L.) and across related species determined by AFLP markers. Plant Breeding, 123, pp. 460-466.

Plantlife, 2008. Valerianella rimosa species dossier. Available: http://www.plantlife.org.uk/uk/discover-wild-plants-nature/plant-fungi-species/broad-fruited-cornsalad.

Wagner, M., Bullock, J.M., Hulmes, L., Hulmes, S. and Pywell, R.F., 2017. Cereal density and N-fertiliser effects on the flora and biodiversity value of arable headlands. Biodiversity and conservation, 26, 1, pp. 85-102.

Wilson, P. and King, M., 2003. Arable plants: a field guide. Wild Guides.

Propagation Protocol: *Veronica triphyllos* L.

1 Ecological overview

1.1 Description

Veronica triphyllos is a low-growing annual herb reaching 15cm. Flowers are 3-4mm in diameter, with deep blue petals that are shorter than the surrounding calyx. Fruit form in capsules, each 6-7mm long, deeply lobed and bearing two fruit (Wilson & King, 2003).

1.2 Phenology

Germination occurs in early autumn (Plantlife, 2009; Wilson & King, 2003) with seedlings emerging from the soil in January (Albrecht et al., 2000; Farrell, n.d.). Bare soil is essential, as *V. triphyllos* will not tolerate winter competition (Farrell, n.d.). Flowers appear as early as February, with most produced between March and May. Local environmental conditions influence seed maturation and dispersal, which typically occur from April to June (Farrell, n.d.). The MSB holds collection data for six UK collections of *V. triphyllos*, one of which was made in April and five in May. Plants die and rapidly disappear after seed dispersal.

1.3 Mating system

In a study using pollen/ovule ratios and other reproductive traits to infer mating systems in *Veronica* species (Scalone & Albach, 2013), *V. triphyllos* is described as a self-compatible, facultative autogamous species – self-pollination is the primary form of reproduction, but outcrossing may also occur. Seed is not adapted for widespread dispersal, so colonisation of new sites is restricted (Albrecht et al., 2000).

1.4 Distribution

Veronica triphyllos has historically been restricted to a few sites in East Anglia and Yorkshire, but now remains at two sites in the UK (Wilson & King, 2003). The species is more widespread in continental Europe in dry grassland and disturbed habitats, with a distribution extending south from Sweden and Latvia. Although rare in southern Europe, populations have been recorded from North Africa and western Asia (Farrell, n.d.).

1.5 Habitat

Veronica triphyllos is associated with arable field margins and other disturbed habitats including tracks, waste land and gravel pits, typically on calcareous or slightly acidic sandy soils (JNCC, 2010; Wilson & King, 2003; Farrell, n.d.).

1.6 Co-occurrence with other CitM species

None known.

1.7 Threats

A poorly-competitive species, *V. triphyllos* is very sensitive to competition from fertilised crops and improved varieties (Wilson & King, 2003; Farrell, n.d.). Spring cultivation can disturb plants before they are able to disperse seed (Albecht et al., 2000) and several former sites have been lost to development (Wilson

& King, 2003; Farrell, n.d.). Winter annuals, such as *V. triphyllos*, are also particularly vulnerable to herbicide sprays applied early in their life-cycle (Albrecht, 2003).

2 Seed ecology

2.1 Seed longevity

The longevity of *V. triphyllos* seed in the soil seed bank has not been established experimentally, although Albrecht et. al. (2000) found moderate levels of *V. triphyllos* seed in the soil in years when no seed producing plants were recorded, concluding that the species is able to form a persistent soil seed bank.

Given the high conservation value of *V. rimosa* and the importance of the soil seed bank in the recovery and persistence of annual plant populations, it would be useful to conduct some longevity or seed aging experiments on this species.

Under optimal ex situ storage conditions *V. triphyllos* seed appear to be long-lived – two UK collections held in the MSB have retained >95% viability after 25 years under storage conditions of 15% eRH (equilibrium relative humidity) and -20°C.

2.2 Seed dormancy

Seed dormancy describes a range of mechanisms that prevent seeds germinating, even under favourable germination conditions. Dormancy may delay germination until the conditions are likely to support healthy plant growth and stagger germination over multiple growing seasons, helping the population recover from damaging short-term effects such as drought, disturbance or unfavourable management practices.

The MSB holds germination data for four UK collections of *V. triphyllos* (Table 1). Variation in embryo size was recorded during testing, suggesting some embryos may not be fully developed at the point of dispersal, resulting in morphological dormancy. The environmental conditions required to promote embryo development and break morphological dormancy vary from species to species (Baskin & Baskin, 2014). Although these have not been established experimentally for *V. triphyllos,* exposing imbibed seed to warm summer temperatures promoted ripening and dormancy release in the related winter annual *V. hederifolia* (Roberts & Neilson, 1981).

2.2 Seed germination

Germination in European populations of *V. triphyllos* has been shown to occur at a range of constant temperatures between 3°C and 20°C and alternating temperatures of 5/15°C and 10/25°C. Optimal temperatures are between 7°C and 15°C, with germination strongly suppressed above 20°C (Albrecht et al., 2000). This is consistent with germination in autumn, although it is not known whether seeds used in this study were freshly harvested or had been exposed to a period of after-ripening or storage.

In the laboratory, high germination rates were secured by the addition of the plant growth substance gibberellic acid (GA_3) to the germination media and incubation at alternating temperatures of 25/10°C or 20/10°C. Germination typically began within 7 days, with maximum germination within 3 weeks.

Seed origin	Collection date	Germination >85%	Germination <85%
Norfolk, England	24/05/76	20/10°C GA3 5°C GA3	15°C 20°C 25/10°C GA3 25°C 4wks > 25/10°C 5°C 4wks > 25/10°C GA3 25/10°C 4wks > 15°C
Norfolk, England	24/05/76	25/10°C 25/10°C GA3	15°C 20°C 25°C 2°C 4wks > 25/10°C
Norfolk, England	24/05/76	25/10°C GA3	15°C 20°C 25°C 25/10°C 2°C 4wks > 20°C
Norfolk, England		25/10°C GA3	15°C 20°C 25°C 25/10°C 2°C 4wks > 25/10°C

Table 1 MSB germination data for UK collections of *V. triphyllos.*

3 Propagation

RBG Kew has not attempted to grow *V. triphyllos* plants to maturity.

4 Implications for restoration

4.1 Establishing new populations

Veronica triphyllos is believed to be at least moderately long-lived in the soil seed bank and may return with the reinstatement of management practices that provide adequate germination and establishment niches. Albrecht et. al. (2000), however, note that the soil seed bank may be quickly exhausted if management practices allow buried seed to germinate but prevent the plants completing their life cycle. If the species has not been recorded recently or has failed to return under a favourable management regime, reintroduction is likely to be required.

Sowing in mid to late summer would expose seed to the seasonal temperature cycles that may be required to promote embryo-development and dormancy release before the cool germination temperatures prevail in autumn. Seed should be sown on bare, recently cultivated ground with minimal competition from crops or other plants.

4.2 Sowing rate

Lang et al. (2016) suggest an optimal sowing rate for arable plant reintroductions of 50-100 seed/m^2.

The possible presence of dormancy in *V. triphyllos*, the lack of certainly around how these mechanisms operate and the sensitivity of the species to competition make it hard to predict germination and establishment success. Whilst high levels of germination are possible, to establish a large, healthy population it may be prudent to sow at the higher rate recommended by Lang et al. (2016), or higher where the supply of seed permits.

Albrecht et. al. (2000) report that where at least four individuals are observed per square metre, seed production can reach 500/m^2 in productive years, although is more often below 100/m^2.

1g of *V. triphyllos* seed contains approximately 2,336 individual seeds.

4.2 Long-term management

Veronica triphyllos is likely to benefit from management regimes that:

- ensure plants are able to complete their life cycle and disperse seed before crops are cut or disturbed;
- expose freshly-dispersed or buried seed to warm summer temperatures at the soil surface;
- avoid disturbing seedlings and young plants;
- severely limit competition from crop or weed species while the plant is in growth.

Veronica triphyllos is strongly associated with winter cereal crops (Albrecht, 2002), benefitting from autumn cultivation between September and November with no further disturbance until seed dispersal is complete in May-June the following year (Plantlife, 2009; Wilson & King, 2003;). Allowing a short fallow period for seed to ripen at the soil surface may be helpful, as may cultivation in early autumn to bring buried seed to the soil surface while temperatures are still relatively high. Populations may be severely reduced by weed control in early spring and lost altogether where fields are ploughed in spring (Albrecht, 2002).

If growing *V. triphyllos* within a crop, reduced fertiliser application and lower cereal density is likely to be beneficial for this non-competitive species. In general, reduced cereal sowing rates have been shown to increase rare arable species richness in cereal crops (Wagner et al., 2017).

5 References

Albrecht, H., Mayer, F., Mattheis, A., 2000. *Veronica triphyllos* L. in the Tertiärhügelland landscape in southern Bavaria – an example for habitat isolation of a stenoeceous plant species in agroecosystems. Zeitschrift für Ökologie und Naturschutz, 8, 4, pp.219-226.

Albrecht, H., 2003. Suitability of arable weeds as indicator organisms to evaluate species conservation effects of management in agricultural ecosystems. Agriculture, Ecosystems & Environment, 98, 1-3, pp. 201-211.

Cheffings, C.M. & Farrell, L. (Eds), Dines, T.D., Jones, R.A., Leach, S.J., McKean, D.R., Pearman, D.A., Preston, C.D., Rumsey, F.J., Taylor, I. 2005. The Vascular Plant Red Data List for Great Britain. Species Status 7, 1-116. Joint Nature Conservation Committee, Peterborough.

JNCC, 2010. Species pages for 2007 UK BAP Priority Species: *Veronica triphyllos*. Available: http://jncc.defra.gov.uk/_speciespages/2688.pdf.

Farrell, L, no date. *Veronica triphyllos* species account, Online Atlas of the British and Irish Flora. Available: https://www.brc.ac.uk/plantatlas/plant/veronica-triphyllos.

Plantlife, 2009. *Veronica triphyllos* species briefing sheet. Available: https://www.plantlife.org.uk/uk/discover-wild-plants-nature/plant-fungi-species/fingered-speedwell.

Roberts, H., Neilson, J., 1982. Role of Temperature in the Seasonal Dormancy of Seeds of *Veronica hederifolia* L. The New Phytologist, 90, 4, pp. 745-749.

Scalone, R. , Kolf, M. and Albach, D. C. (2013), Mating system variation in *Veronica* (Plantaginaceae): inferences from pollen/ovule ratios and other reproductive traits. Nordic Journal of Botany, 31, pp. 372-384.

Wagner, M., Bullock, J.M., Hulmes, L., Hulmes, S. and Pywell, R.F., 2017. Cereal density and N-fertiliser effects on the flora and biodiversity value of arable headlands. Biodiversity and conservation, 26, 1, pp. 85-102.

Wilson, P. and King, M., 2003. Arable plants: a field guide. Wild Guides.

Propagation Protocol: *Veronica verna* L.

1 Ecological overview

1.1 Description

Veronica verna is an erect annual herb growing to 15cm with sometimes branched flowering stems. Flowers are 2-3mm in diameter, with sky-blue petals and short stalks. Fruits form in lobed, yellowish-brown capsules, with each capsule containing two fruit, (Wilson & King, 2003).

1.2 Phenology

Veronica verna seed predominantly germinate in spring, although autumn germination may occur in wet years (Wilson and King, 2003; Farrell, n.d.). Flowers are produced between late March and June, with the inflorescence extending over the season to form a fruiting raceme. Seed is ripened and dispersed throughout the season. The MSB holds detailed collecting data for six UK collections of *V. verna*, five of which were made in June and one in early July.

1.3 Mating system

The reproductive system and pollinator interactions of *V. verna* are not described. Mating systems within the *Veronica* genus are extremely diverse but studies on similar annual *Veronica* species (Scalone et. al., 2015) suggests *V. verna* is likely to be at partially or predominantly self-pollinated.

1.3 Distribution

There are very few records of *V. verna* in Great Britain, and the species is now restricted to a cluster of small populations in Suffolk (Farrell, n.d.). Populations in Norfolk and introduced populations in Devon appear to have been lost. The species is widely scattered across continental Europe, western Asia and Morocco (Farrell, n.d.).

1.4 Habitat

Veronica verna is associated with short grassland and open, sparsely-vegetated habitats on infertile, sandy, calcareous soils (Wilson & King, 2003; Farrell, n.d.). The species is less typical of arable sites that *V. triphyllos* and other CitM target species, relying on naturally stony ground and grazing to maintain sufficiently open conditions (Farrell, n.d.).

1.5 Co-occurrence with other CitM species

None known.

1.6 Threats

Threats to *Veronica verna* are not described in detail, although the species has never been common in the UK and has further declined as suitable habitat has been lost to agricultural intensification (Wilson & King, 2003).

2 Seed ecology

2.1 Seed longevity

The longevity of *V. verna* seed in the soil seed bank has not been established experimentally, although the seed is believed to be long lived (Farrell, n.d.).

Given the high conservation value of *V. rimosa* and the importance of the soil seed bank in the recovery and persistence of annual plant populations, it would be useful to conduct some longevity or seed aging experiments on this species.

Under optimal ex situ storage conditions *V. verna* seed are long-lived – two UK collections held in the MSB have retained 100% viability after 36 years under storage conditions of 15% eRH (equilibrium relative humidity) and -20°C.

2.2 Seed germination

The MSB holds germination data for four UK collections of *V. verna* (Table 1). These tests consistently show that *V. verna* will readily germinate across a range of constant and alternating temperatures typical of spring and autumn in the UK. Germination begins quickly, with total germination after two weeks of incubation. No dormany-breaking pre-treatments were applied, and neither warm nor cold stratification increased germination percentage or germination rate.

Seed origin	Collection date	Germination >85%	Germination <85%
Suffolk, England	23/07/77	2°C 20°C 25°C 20/10°C 25°C 4wks > 10°C	
Suffolk, England	03/06/76	20/10°C	
Suffolk, England	01/06/76	20/10°C 25/10°C	
Suffolk, England	1974	15°C 20°C 25°C 25/10°C 2°C 4wks > 20°C	

Table 1 MSB germination data for UK collections of *V. verna.*

3 Propagation

RBG Kew has not attempted to grow *V. triphyllos* plants to maturity.

4 Implications for restoration

4.1 Establishing new populations

Veronica verna is believed to be long-lived in the soil seed bank and may return with the reinstatement of management practices that provide adequate germination and establishment niches. If the species has not been recorded recently or has failed to return under a favourable management regime, reintroduction is likely to be required.

Seed may be sown in autumn or spring – although most germination occurs in spring, sowing in autumn mimics natural seed dispersal and provides an opportunity for autumn germination in wetter years. Seed which does not germinate in autumn may remain quiescent in the soil and germinate in spring. Seed should be sown onto disturbed ground with substantial areas of bare soil.

4.1 Sowing rate

Lang et al. (2016) suggest an optimal sowing rate for arable plant reintroductions of 50-100 seed/m^2.

Veronica verna does not display complex seed dormancy and a high proportion of viable seed exposed to suitable germination conditions (moist soil, high light availability and moderate temperatures) may germinate. Seedling establishment may be low, however, as the small seeds and seedlings are likely to be vulnerable to disturbance, disease and herbivory, consistent with generally higher seedling mortality rates in small-seeded species (Moles and Westoby, 2014). This would justify sowing at the higher rate recommended by Lang et al. (2016) - preferably higher where sufficient seed is available.

1g of *V. verna* seed contains approximately 8,333 individual seeds.

4.2 Long-term management

Veronica verna is likely to benefit from management regimes that:

- ensure plants are able to complete their life cycle and disperse seed into the soil seed bank;
- maintain a short, open vegetation structure with areas of bare, disturbed ground.

It is essential that sites managed for *V. verna* are maintained as open habitats (JNCC, 2010), with relatively intensive grazing by sheep or rabbits (Farrell, n.d.). Concentrating grazing when *V. verna* is not in active growth - between July and March – may help reduce damage to plants. Routine cultivation is not desirable (Farrell, n.d), although small-scale manual disturbances in autumn or spring may help create germination niches in densely-vegetated sites.

5 References

Cheffings, C.M. & Farrell, L. (Eds), Dines, T.D., Jones, R.A., Leach, S.J., McKean, D.R., Pearman, D.A., Preston, C.D., Rumsey, F.J., Taylor, I. 2005. The Vascular Plant Red Data List for Great Britain. Species Status 7, 1-116. Joint Nature Conservation Committee, Peterborough.

Farrell, L, no date. *Veronica verna* species account, Online Atlas of the British and Irish Flora. Available: https://www.brc.ac.uk/plantatlas/plant/veronica-verna.

JNCC, 2010. Species pages for 2007 UK BAP Priority Species: *Veronica verna*. Available: http://jncc.defra.gov.uk/_speciespages/2689.pdf.

Lang, M., Prestele, J., Fischer, C., Kollmann, J., Albrecht, H., 2016. Reintroduction of rare arable plants by seed transfer. What are the optimal sowing rates? Ecology and Evolution, 6, 15, pp. 5506–5516.

Moles, A., Westoby, M., 2004. Seedling survival and seed size: a synthesis of the literature. Journal of Ecology, 92, pp. 372-383.

Scalone, R., Kolf, M., Albach, D., 2013. Mating system variation in *Veronica* (Plantaginaceae): inferences from pollen/ovule ratios and other reproductive traits. Nordic Journal of Botany, 31, 372-384.

Wagner, M., Bullock, J.M., Hulmes, L., Hulmes, S. and Pywell, R.F., 2017. Cereal density and N-fertiliser effects on the flora and biodiversity value of arable headlands. Biodiversity and conservation, 26, 1, pp. 85-102.

Wilson, P. and King, M., 2003. Arable plants: a field guide. Wild Guides.