

Establishing the National Seed Bank of Wales: collecting, conserving and restoring the Welsh flora

Kevin J. McGinn¹

Abstract

Seed banks are an efficient method of *ex situ* plant conservation, capable of conserving vast genetic diversity within a small space. In 2018, the National Botanic Garden of Wales (NBGW) started on a journey to establish a new seed bank focused on conserving the Welsh flora to expand its native plant conservation activities. Now equipped with facilities allowing professional long-term seed conservation within two lab spaces in NBGW's Science Centre, the National Seed Bank of Wales has become a valuable resource for plant conservation and research in Wales. This article describes how the new seed bank has evolved and how it operates, which may inform other small-to-medium size botanic gardens looking to develop seed collecting and banking activities. When based at a botanic garden, seed banks are an excellent resource uniting the horticultural, scientific and educational expertise of staff. Wider benefits beyond long-term seed conservation include improving short-term storage methods of seed grown by the horticulture department for conservation and display; and training horticulture and science students. An associated trial of harvesting and marketing seed from NBGW's wildflower-rich hay meadows for restoration purposes has also proved successful and commercially viable, helping to fund our conservation activities.

Introduction

With recent estimates suggesting that up to 40 per cent of the world's vascular plant species are threatened with extinction (Nic Lughadha *et al.*, 2020), the conservation of plant diversity *ex situ* is more important than ever. Seed conservation is a cost-effective, long-term method of safeguarding plant species and their genetic material (Li & Pritchard, 2009). Seed banks ensure that seed of suitable origin is available for future conservation and research uses, such as the reintroduction of plants that have become extinct in the wild, the facilitation of plant species recovery and the restoration of degraded ecosystems. When established at a

botanic garden, seed banks bring a wealth of specialist horticultural and scientific expertise to support the collection, production and application of native seeds, as well as abundant public engagement opportunities (Hardwick *et al.*, 2011).

Since opening in the year 2000, the National Botanic Garden of Wales (NBGW) has made a number of tangible contributions towards Welsh plant conservation through its scientific research and via its horticultural capabilities (de Vere, 2021; Ritchie, 2020). Past collaborative projects have included the propagation and reintroduction of *Salvia pratensis* (meadow clary) to its only Welsh site (Moughan *et al.*, 2021); propagating and

¹ Kevin McGinn is Science Officer for the Growing the Future project at the National Botanic Garden of Wales. Address: National Botanic Garden of Wales, Llanarthne, Carmarthenshire, SA32 8HG, UK. Email: kevin.mcginn@gardenofwales.org.uk; kevinmcginn@outlook.com

bolstering numbers of *Cotoneaster cambricus* (Great Orme berry), a species endemic to Llandudno where only six original individuals remain; growing *ex situ* collections of endemic *Sorbus* (whitebeam) species; and conducting research on the conservation genetics of *Succisa pratensis* (devil's bit scabious) (Jones, 2015) and *Campanula patula* (spreading bellflower) (Long, 2013). As a natural progression of NBGW's conservation activities, the creation of a professional seed bank had been a long-term vision, and in 2018 we started on a journey to establish a seed bank for the Welsh flora.

The Welsh landscape comprises a varied patchwork of terrestrial habitats rich in biodiversity, which support their fair share of declining and threatened plant species. Almost half of vascular plant species declined in their abundance and distribution in Wales over the period 1970–2013 (Hayhow *et al.*, 2016). Furthermore, the Vascular Plant Red Data List for Wales estimated that 38 plant species have become nationally extinct and an additional 256 (18 per cent) are threatened with extinction from Wales (Dines, 2008). Further declines are probable over the 14-year period since this Red Data List was published. Wales has 58 endemic plant species, with a notable diversity of *Sorbus* spp. and *Hieracium* spp. (hawkweeds) (Clubbe *et al.*, 2020). While some rare species such as *Gagea serotina* (Snowdon lily) and *Draba aizoides* (yellow whitlow grass) occur elsewhere in Europe, Wales is home to their only British populations. Internationally rare taxa such as *Gentianella amarella* subsp. *occidentalis* (dune gentian), *Centaureum scilloides* (perennial centauray) and *Asparagus officinalis* subsp. *prostratus* (wild asparagus) are also found in Wales. Additionally, many taxa reach their most southerly, northerly or westerly extent of distribution in Wales

(Dines, 2008) and such edge-of-range populations can be genetically distinct, representing important targets for the conservation of plant diversity (e.g. Vakkari *et al.*, 2020).

The Millennium Seed Bank Partnership (MSBP) run by the Royal Botanic Gardens, Kew has spearheaded global seed conservation, and their efforts include a key focus on the UK flora. At least one collection from 72 per cent of the UK's angiosperm native and archaeophyte flora and 78 per cent of all threatened taxa have been banked, including intraspecific taxa and taxonomically cryptic apomictic species (Chapman *et al.*, 2019; Clubbe *et al.*, 2020). There are now increasing efforts to bank multiple seed collections from across species' geographic ranges in order to capture and conserve intraspecific genetic diversity at population and regional levels (Chapman *et al.*, 2019). Genetic diversity holds the key to species' resilience and adaptation in the face of environmental changes (Breed *et al.*, 2013; Broadhurst *et al.*, 2008; Kelly, 2019), and the more genetic diversity conserved within *ex situ* seed collections, the wider the potential uses are for assisted recovery, reintroduction projects and research. Even local extinctions of plant species are a concern as this represents a loss of natural heritage, may result in genetic erosion (Rogers, 2004) and may cause knock-on effects to wider biodiversity (Schleuning *et al.*, 2016). Therefore, the more seed collections are banked and the wider their geographic origins, the better.

In the UK, the MSBP's UK National Tree Seed Project, UK Flora and UK Threatened Flora Projects have been addressing this challenge by making multiple seed collections for a range of species (Chapman *et al.*, 2019). A gap analysis in 2018 identified that around 75 per cent of Welsh native and

archaeophyte angiosperm species were yet to be represented by Welsh-origin material in the Millennium Seed Bank's collections (O'Donnell, 2019). NGBW is working in partnership with the MSBP to increase the taxonomic and genetic diversity of Welsh-origin seed bank collections, prioritising threatened species and populations.

Establishing the seed bank

In 2018, the author spent a stimulating and enjoyable three weeks at the MSBP on a 'Seed Conservation Techniques' training course, learning all about seed science and conservation from the experts, with a group of international attendees. Armed with this knowledge, a feasibility study produced by Botanic Gardens Conservation International (BGCI) (O'Donnell, 2019) and advice from MSBP staff, the aim was to gradually establish seed conservation facilities in two labs in NGBW's Science Centre. These labs are now home to the National Seed Bank of Wales, a critical resource for conserving Wales' most threatened wild plants (Figs 1 & 2).

The project has proved very scalable, able to develop as and when funds have become available. A range of specialist equipment has been purchased to gradually expand our technical abilities and conservation standards (see Appendix). We now have a well-equipped facility, but there are often simple, cheaper methods available to substitute expensive lab equipment at least in the early stages – for example using household as opposed to lab-standard freezers for storage.

The seed banking process

MSBP standards for collecting, handling, processing and storing seeds (MSBP, 2019) are followed at NGBW. This process, briefly outlined below, allows us to conserve

genetically comprehensive, high-quality seed collections with high viability and longevity. For detailed guidance around the processes and standards, see MSBP's collection of Technical Information Sheets, available online (MSBP, 2021).

Seed banking starts at the desk – identifying priority target species and field sites, and investigating species' phenology. Landowner permissions need to be secured prior to field visits, and for collection within Sites of Special Scientific Interest and for protected species we also secure consent from Natural Resources Wales.

Fieldwork is then conducted to search for target populations (Fig. 3) in the hope that healthy ripe seed is available. Local experts such as conservation rangers and recorders from the Botanical Society of Britain and Ireland (BSBI) are invaluable in helping to locate target plants and to monitor seed maturity so that it can be collected at the optimum time. It is sometimes necessary to do recce trips, or revisit a population once seed has matured. Some trips, especially for threatened target species, may fail to locate populations, and other visits may find only infested or low-quality seed, but every trip is informative to understand and monitor target species.

Once a target species has been located, we assess the population size and the maturity, quality and quantity of the seed available (Way & Gold, 2014). Seed quality is assessed via a cut-test by sampling fruits from different plants and cutting 10–20 seeds in half to examine the contents. Healthy seeds generally have a firm, white interior. Predation or other factors such as stress or insufficient pollination can result in poor-quality or empty seeds. To ensure seeds are mature, they, or the fruits, are collected at the point of natural dispersal, as indicated by

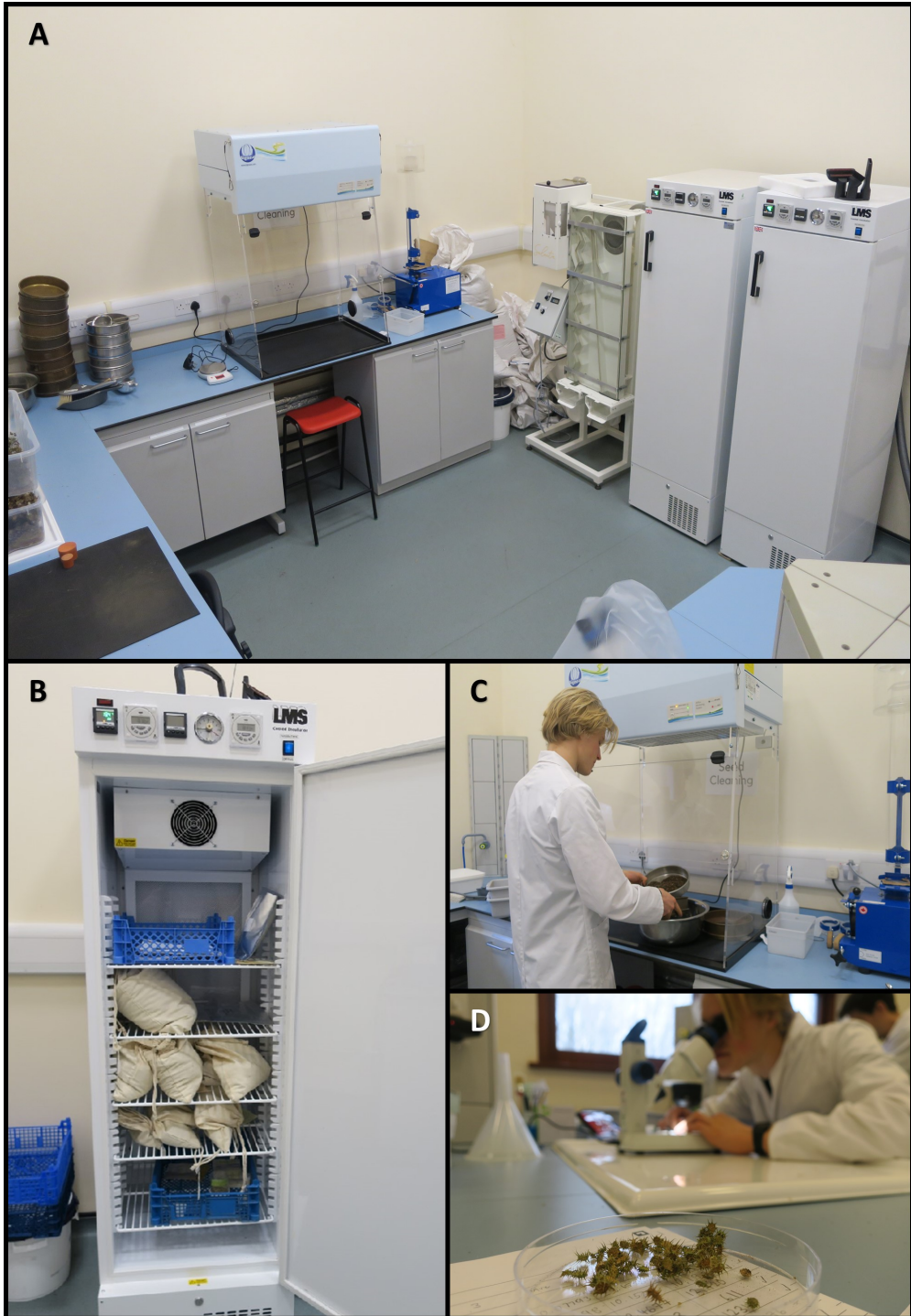


Fig. 1 The Seed Processing Lab (A), where incoming collections from the field are dried (B), cleaned (C), quality-checked and counted (D). Cooled incubators (B) are used to dry the seed, but can also be used as germination cabinets. Photos A & B: K. McGinn; photos C & D: E. Baker.

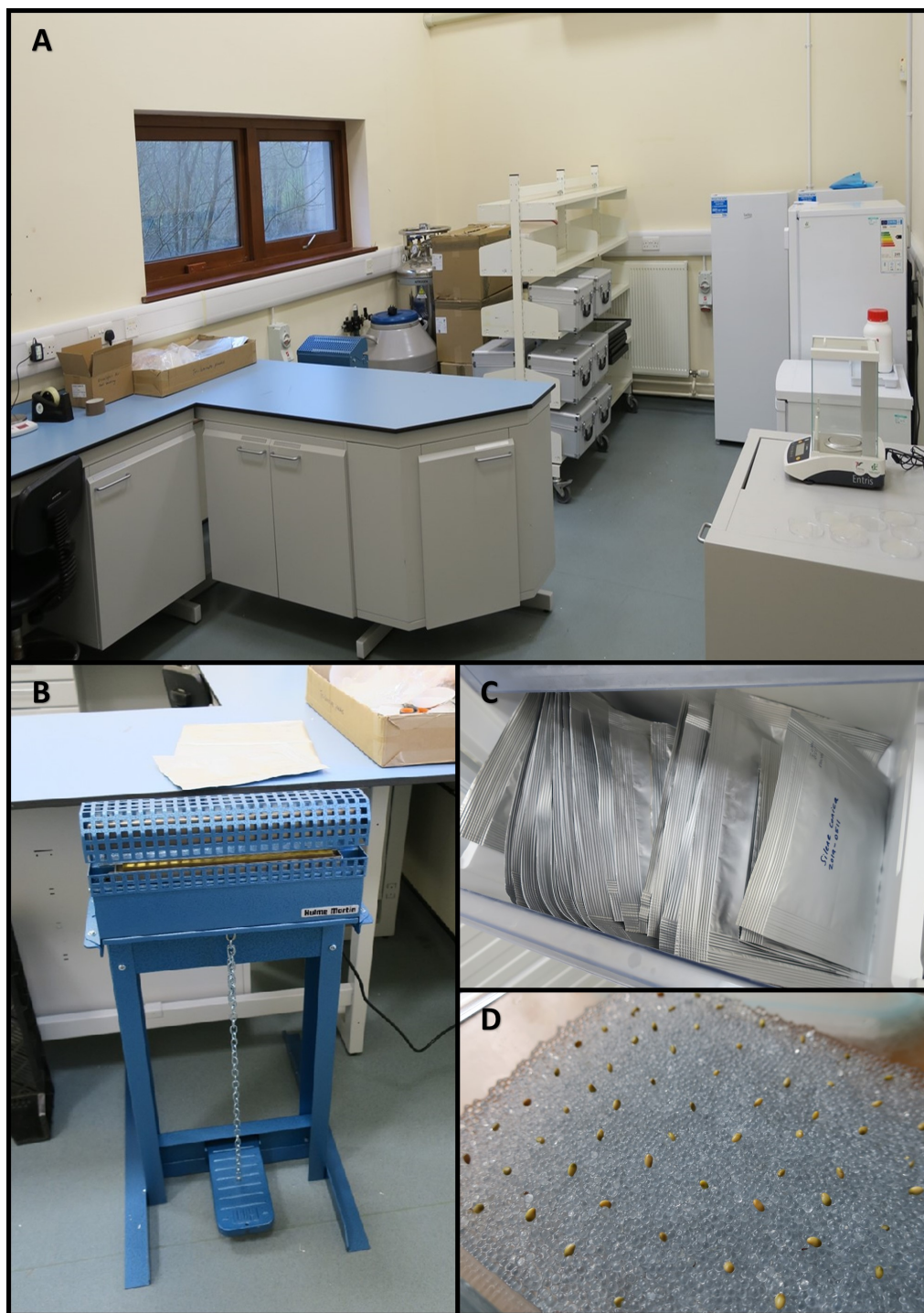


Fig. 2 The Seed Storage Lab (A), where a heat sealer (B) is used to package clean, dried seed in trilaminate foil pouches (C), after which it is placed in freezers at -20°C for long-term storage. This clean lab is also used to set up germination on agar plates or on glass beads (D). Photos: K. McGinn.



Fig. 3 Training horticulture staff and science students in seed collection (*Marrubium vulgare*, white horehound) on the Great Orme, Llandudno, together with Millennium Seed Bank staff. Photo: K. McGinn.

their colour and easy detachment from the parent plant.

To adequately capture genetic diversity, the aim for a seed bank collection is to reach 10,000 seeds per population, but this is not always possible, especially for threatened species. To ensure our seed collecting does not compromise future recruitment in the populations sampled, seeds are collected sensitively, with collection limited to a maximum of 20 per cent of the available seeds, reduced to 10 per cent for threatened species. Collections are handled carefully after harvest to prevent deterioration – kept cool and out of sunlight and, if damp, spread out thinly in ambient conditions as soon as possible. The moisture status of collections is monitored using a hygrometer, and the

handling is adjusted to reach < 50 per cent equilibrium relative humidity (eRH) for safe short-term storage during the collecting trip (Gold, 2014; Gold & Manger, 2014a).

Back at NBGW, the seed is taken to our Seed Processing Lab (Fig. 1) where it is carefully cleaned and dried. The aim of cleaning is to remove the bulk of fruiting structures and debris without causing damage to or loss of viable seed (Terry & Sutcliffe, 2014). Techniques vary depending on the species, but the process invariably involves low-tech manual methods like sieving, teasing seeds from dry capsules using a rubber bung or removing fruit pulp by gently mashing fleshy fruits and rinsing over a sieve. For dry collections, we also have mechanical aspirators that use air currents to separate healthy seeds from chaff and empty seeds, based on weight differences. Manual cleaning is carried out within a dust containment hood for health and safety reasons. If seeds are not quite fully mature, they are left at ambient conditions in the lab (65–80 per cent relative humidity) to after-ripen, although the aim in the field is always to collect fully mature seeds. Cleaning is taken to a reasonable end point, not necessarily removing all of the chaff, to avoid loss of viable seed.

Estimates of the total number of seeds per collection are calculated by weighing three samples of fifty seeds, taking an average, weighing the whole seed collection and multiplying up. To give an indication of the proportion of healthy seeds, results from cut-tests are used to adjust the seed totals in order to take into account the estimated proportion of empty or infested seeds (Terry & Sutcliffe, 2014).

The seed is then dried to 15 per cent eRH, the recommended moisture level for long-term conservation of orthodox seeds.

This is achieved using cooled incubators set on drying mode at 18 °C. The drying stage is crucial as it minimises the risk of ice crystals forming and damaging the seed tissue upon freezing, while also extending seed longevity, but retaining enough moisture to keep the tissues alive (Gold, 2014). A cheaper, more accessible drying option is to use an airtight container with colour-changing silica gel desiccant and a hygrometer to monitor the moisture status (Sutcliffe & Adams, 2014a, 2014b).

Once dried to 15 per cent eRH, seed collections are taken to the Seed Storage Lab (Fig. 2), where each collection is transferred into airtight trilaminate foil pouches. Trilaminate foil pouches can be purchased in large sheets, cut to size and sealed using a heat sealer (Gold & Manger, 2014b). Once sealed, the pouches are stored in freezers at –20 °C to slow respiration and the rate of ageing. Duplication is extremely important for safeguarding – half of each collection is stored in our seed bank and the other half is sent to the Millennium Seed Bank.

The research and conservation value of seed collections is heavily reliant on diligent data recording. Quality field data, including photos, are recorded on our botanic garden database. This includes details of the habitat, locality, associated species, sampling detail and germination data, along with collection permission forms. Herbarium voucher specimens are also taken in the field wherever possible and lodged at NBGW and Royal Botanic Gardens, Kew in case the taxonomic identity needs to be verified.

To monitor seed viability, germination tests are conducted by the MSBP on the duplicate collections. This provides NBGW with a good indication of the viability of their collections. Following MSBP protocols (Davies *et al.*, 2015a; Davies *et al.*, 2015b), these tests

are performed on each collection seven days to three months after storage at –20 °C to establish baseline viability. Tests are then conducted on each collection at least every 10 years to monitor viability. Pre-treatments such as cold stratification or scarification are sometimes needed to break dormancy (Davies *et al.*, 2015a). If monitoring identifies that seed viability has fallen below 85 per cent of initial viability (Davies *et al.*, 2015b), collections will be regenerated or recollected from the wild.

Facilities at the NBGW seed bank also enable germination and propagation. A laminar flow cabinet provides a sterile workspace to set up germination trials on agar or glass beads. The cooled incubators used at the drying stage are dual-purpose and can be switched to incubation mode and utilised as germination cabinets, with specific daylength and temperature regimes programmed to provide optimum conditions.

The process described here is for conventional seed banking, suitable for the conservation of desiccation-tolerant (orthodox) seeds of spermatophytes (seed-bearing plants), but not for desiccation-intolerant (recalcitrant) seeds. Although the UK flora has few native recalcitrant species, some important taxa such as *Quercus* fall into this category, and there are also many intermediate species that tolerate desiccation but have short lifespans under conventional storage. The Millennium Seed Bank has cryopreservation and micropropagation facilities and an active research programme to conserve desiccation-intolerant species (e.g. Ballesteros *et al.*, 2021).

Species conserved to date

Working closely with Horticulture and Science staff, NBGW's Growing the Future and Biophilic Wales projects have enabled

targeted seed-collecting trips. Our collection efforts have focused on species lacking Welsh-origin collections at the Millennium Seed Bank, on threatened species and populations, and on key grassland species for restoration.

So far, 68 seed collections have been banked, representing 60 different taxa. This includes a collection from *Campanula patula* (spreading bellflower), a critically endangered species in Wales, having undergone drastic declines across the UK and a loss of genetic diversity (Long, 2013) (Fig. 4). A collection of *Galeopsis angustifolia* (red hemp nettle) has been made from Gower, where only 200 plants grow on a pebble beach – one of only two extant populations in Wales (Fig. 4). Seed from *Genista pilosa* (hairy greenweed) was collected from coastal heathland in Pembrokeshire, one of only six isolated

populations of this declining species across the UK (Fig. 4). By being closely in touch with issues and networks on our doorstep, we are able to conserve population-level diversity that may otherwise disappear – for example, a collection of 530 seeds was made from a threatened population comprising only 12 plants of *Silene gallica* (small-flowered catchfly) in Burry Port Harbour, an area earmarked for development.

As well as safeguarding species in the long term, our seed collections have already contributed to immediate conservation action. We are thrilled that 150 seeds of *Galatella linosyris* (goldilocks aster) collected on the Great Orme were dispatched to help with a reintroduction project in Cumbria where the species is believed to have become extinct (Heritage Fund, 2019), Great Orme being where the closest extant population is located.

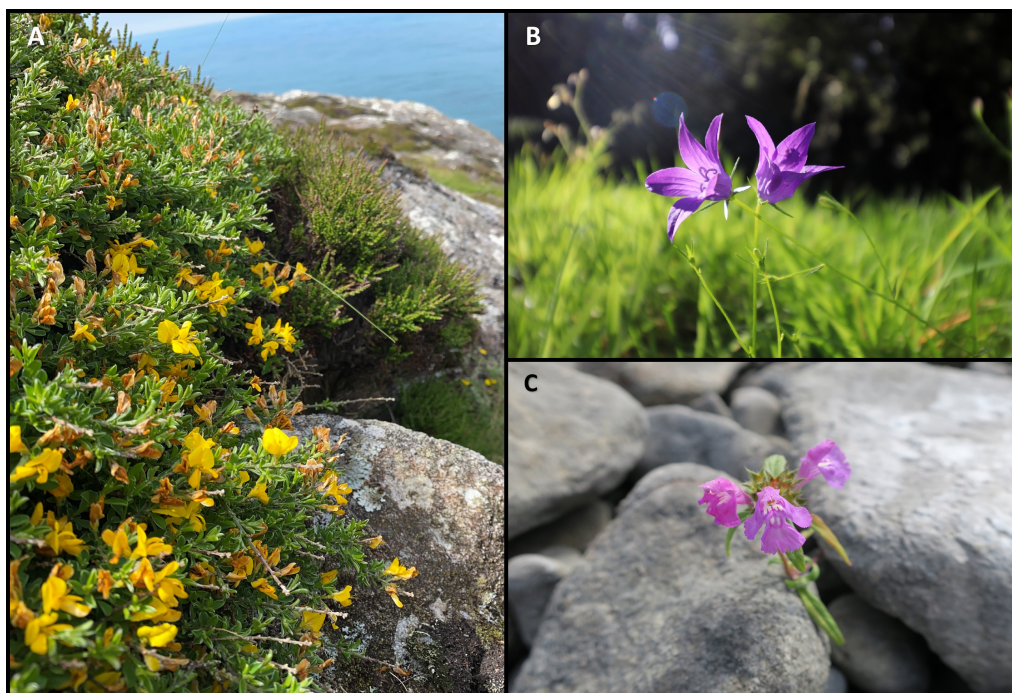


Fig. 4 Examples of localised, threatened and declining species seed banked to date: (A) *Genista pilosa* (hairy greenweed); (B) *Campanula patula* (spreading bellflower); (C) *Galeopsis angustifolia* (red hemp-nettle). Photos A & B: E. Waters; photo C: K. McGinn.



Fig. 5 Short-term storage of seed for the Horticulture Department: breathable packets in glass clip-top jars containing a small amount of colour-changing silica gel, stored in a fridge at 5 °C.

Wider benefits of the seed bank at NBGW

The seed bank has enabled the improved short-term storage (< 18 months) of seed used by the Horticulture Department to grow plants for the living collections. For this, seed is dried in the same way as for long-term conservation collections. Seed in breathable packets is then stored in glass clip-top jars with rubber seals, containing a small amount of colour-changing silica gel as an indicator of moisture status (see Gold & Manger, 2014b) and placed in a refrigerator at 5 °C (see de Vitis *et al.*, 2020; Hong & Ellis, 1996) (Fig. 5).

NBGW has been working on a series of ecological growing contracts with a restoration and conservation focus, for example growing *Succisa pratensis* (devil's bit scabious) to plant at a mitigation site to support populations of the endangered marsh fritillary butterfly following a

road-widening scheme. Seeds collected from these developments are in medium-term storage at the seed bank to maintain their viability until required for propagation.

To increase the size of some smaller seed collections and broaden their potential future conservation use, small amounts of seed have been cultivated in NBGW's living collections to increase seed volumes. The plants have been grown in NBGW's Conserving Welsh Plants area for display and used for engagement and education.

Meadow seed harvesting

Seed banks in botanic gardens are ideally placed to help overcome issues surrounding the availability and application of native seed for ecological restoration (Hardwick *et al.*, 2011). The commercial availability of Wales-origin seeds for ecological restoration is limited (O'Donnell, 2019) in both quantity

and species diversity. Due to this limitation, restoration projects in Wales are likely to rely on seed sources from further afield. The use of geographically distant seed sources risks the introduction of new invasive pests and pathogens. Such seed may also be less suited to the local growing conditions through lacking local adaptation (Bucharova *et al.*, 2017) and may potentially reduce the fitness of natural populations through outbreeding depression, although there is scientific debate around these factors (Broadhurst *et al.*, 2008, 2016; Jones, 2013).

The Waun Las National Nature Reserve (NNR) is run as a commercially viable organic farm by NBGW, with real emphasis on managing the land to nurture biodiversity. Forty acres (16 ha) of wildflower-rich meadows (Fig. 6) provides a unique opportunity to sustainably harvest and market Welsh-provenance meadow seed. Wildflower-rich lowland hay meadows, once widespread in the UK, are now a very rare and important habitat – 97 per cent having been lost in the UK between the 1930s and 1980s (Fuller, 1987). Within Wales, the trend has been just as dramatic: the extent of Welsh semi-natural grasslands has decreased by 91 per cent since the 1930s (Blackstock *et al.*, 2010). Interest in restoring lowland hay meadows is increasing in the UK, and there is an expanding market for seed of native species across Europe (de Vitis *et al.*, 2017).

In 2020 and 2021, brush harvesting was piloted on Waun Las NNR (Figs 7 and 8). As a sustainable method of collecting mixed seed from species-rich grassland and heathland, brush-harvesting collects ripe seeds without cutting the sward, allowing flower heads and unripe fruits to remain for natural regeneration. We use a Logic MSH320 UTV ATV Seed Harvester, towed by a quad bike, which sweeps seeds and seed heads

off using a rotary brush with stiff bristles and collects these into a hopper (Fig. 7). The harvested seed is swiftly moved to a storage shed before it heats up, where it is spread thinly on tarpaulins and rotated every day or two to encourage drying (Fig. 8). Large stalks are removed by raking, and the remainder is sieved manually. The final product contains fairly high levels of chaff due to the wide range of seed sizes present, but this helps the seed go further upon sowing. The moisture status and environmental conditions are monitored during the process using a datalogger hygrometer (Gold & Manger, 2014a). Once dried to below 70 per cent eRH, the level below which rapid deterioration via fungal attack is prevented (de Vitis *et al.*, 2020), the seed is then bagged and stored indoors under cool, dry ambient conditions (< 18 °C; < 70 per cent RH). It is dispatched for use as soon as possible, ideally by the end of December, and is never kept from one season to the next. Reasonably rapid sowing is especially important for the key hemiparasitic meadow species *Rhinanthus minor* (yellow rattle) as its seed needs a long period of cold stratification to overcome physiological dormancy (Marin *et al.*, 2017).

The experience in seed conservation gained by staff through managing the seed bank has been transferable to the brush harvesting process, for example in understanding and monitoring seed moisture status and the effect of post-harvest handling practices on seed viability. The seed bank labs have also been used as a storage facility until seed is dispatched, and as a space to weigh bags of seed for dispatch. In 2020 and 2021, harvest volumes totalled 30 kg and 200 kg respectively, all of which sold out rapidly to a range of customers including private landowners, conservation organisations, local authorities and road agencies. The initial

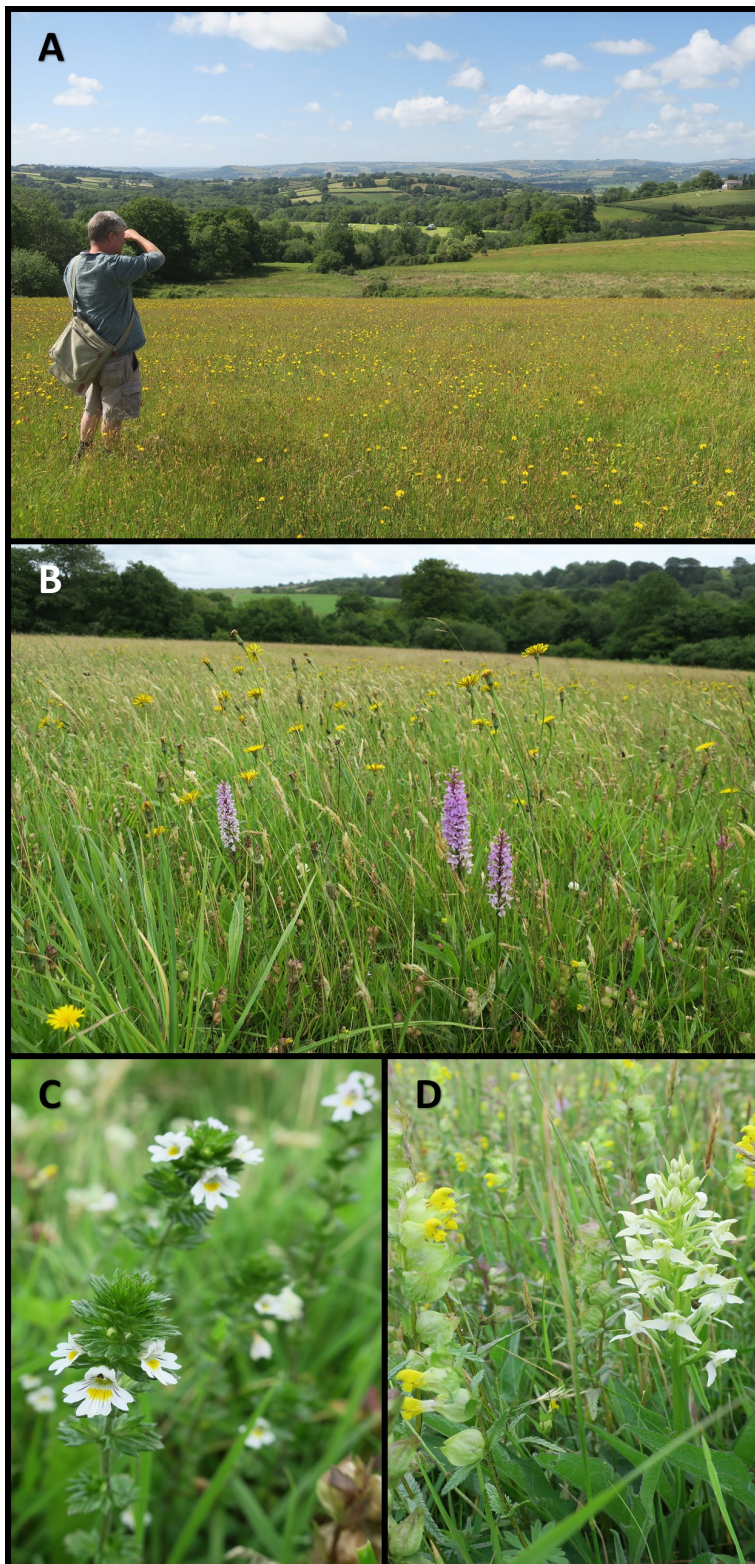


Fig. 6 Wildflower-rich hay meadows on the Waun Las National Nature Reserve at the National Botanic Garden of Wales (A, B), including (B) *Dactylorhiza fuchsii* (common spotted orchid), (C) *Euphrasia* sp. (eyebright), (D) *Rhinanthus minor* (yellow rattle) and *Platanthera chlorantha* (greater butterfly orchid). Photos: K. McGinn.

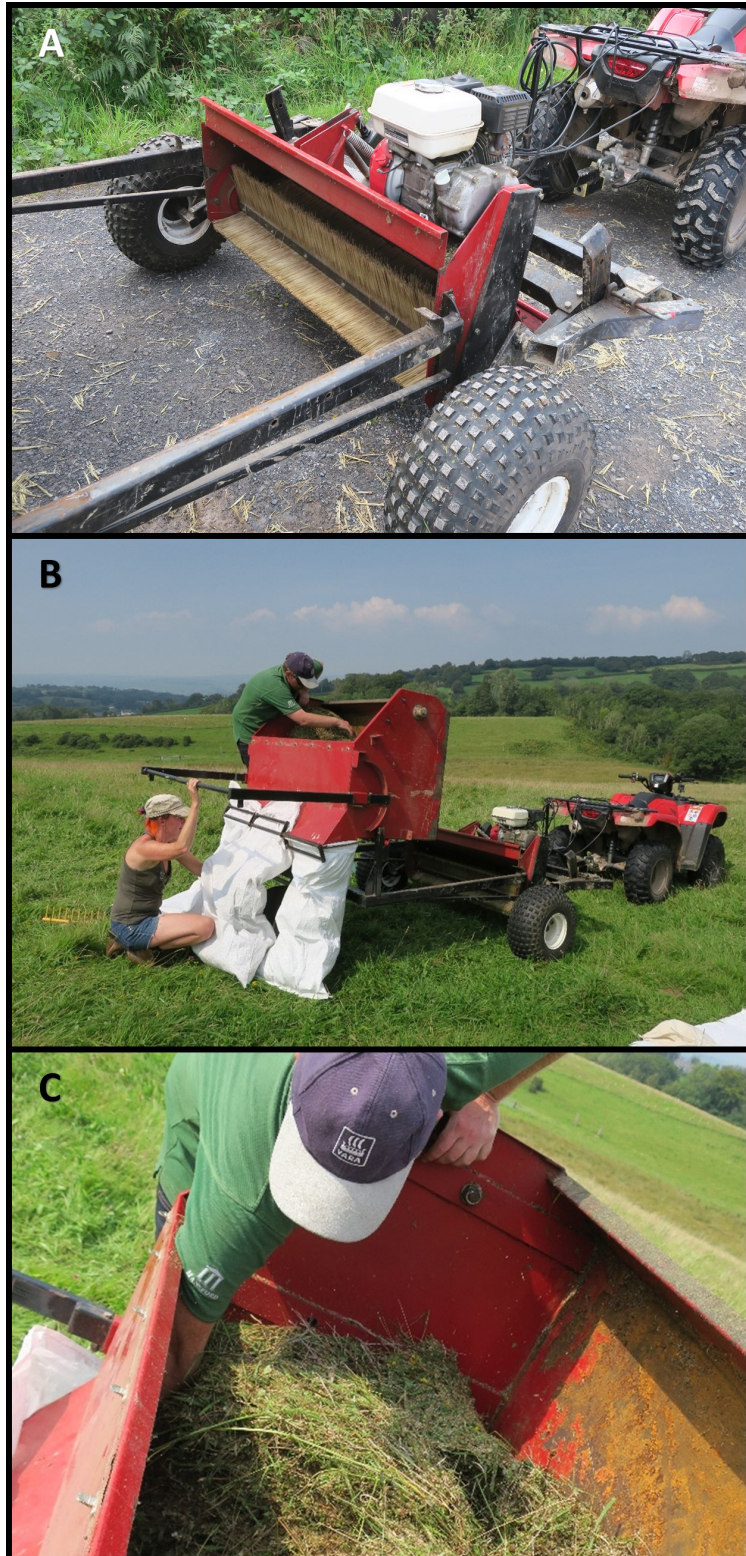


Fig. 7 Mechanical collection of meadow seed from Waun Las National Nature Reserve using a brush harvester towed by a quad bike (A, B). Once full, the hopper is emptied onto a sheet or into breathable sacks (B, C). Photos: K. McGinn.



Fig. 8 Processing brush-harvested meadow seed: spread out thinly undercover and out of direct sunlight to dry (A), monitoring equilibrium relative humidity using a hygrometer (B), sieving stalks and the bulk of the chaff (C) and storing the processed seed (D). Photos: K. McGinn.

capital outlay for a brush harvester, costing approximately £6,000, was paid back through income within the first year. Beyond that, costings of the staff hours and consumables have shown the harvesting to be commercially viable, providing a genuine source of income to fund NBGW conservation work, while also contributing to the conservation of existing meadows and the creation of new meadows. Note that wild-harvested meadow seed is subject to government regulation as a 'preservation mixture' and a licence is required from the Animal and Plant Health Agency to market it in the UK. To ensure that the seed harvesting does not compromise the conservation value of the meadows, particularly on the abundance of annual species, we will monitor for impacts. If required, a two-year rotation cycle around the meadows will be implemented.

Acknowledgements

NBGW is extremely grateful to the Millennium Seed Bank Partnership for providing training, technical support and guidance, in particular to Stephanie Miles, Ted Chapman, Jenny Peach and Clare Trivedi. We are also very grateful to Katherine O'Donnell and Suzanne Sharrock of BGCI for producing a feasibility study, via the LEADER programme, funded through the Welsh Government Rural Communities–Rural Development Programme 2014–2020, which is funded by the European Agricultural Fund for Rural Development and the Welsh Government. Within NBGW, I thank all staff involved with the project, particularly Dr Natasha de Vere, Will Ritchie, Elliot Waters and Carly Green. Thanks also go to all attendees of the Seed Conservation Techniques course 2018 for sharing experience and passion. Very many thanks to

Ted Chapman, an anonymous reviewer and Editor Kate Hughes for critically reviewing the article, and providing valuable comments and suggestions. The seed bank project would not have been possible without the Growing the Future project, funded through the Welsh Government Rural Communities–Rural Development Programme 2014–2020, which is itself funded by the European Agricultural Fund for Rural Development and the Welsh Government; and the Biophilic Wales project, funded by the Welsh Government’s Enabling Natural Resources and Well-being in Wales Grants (ENRaW).

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Appendix

The table below lists the capital equipment needed, with estimated costs (correct at the time of writing), to set up a seed bank within a botanic garden. A range of smaller items of equipment are required – secateurs, pruning pole, hand lenses, nail clippers (for seed cut-tests), weather-writer clipboards, a dissection kit, a range of sieve sizes, a vacuum cleaner – as well as a range of consumables: cotton bags for seed collection, trilaminate

foil bags for storage, colour-changing silica gel (sachets and loose), glass clip-top jars for storage, germination supplies and herbarium supplies. For detailed guidance, including lists of models/products and suppliers, see the Millennium Seed Bank Partnership's Technical Information Sheets that cover all aspects of seed conservation practices and facilities (MSBP, 2021).

| Item | Use and notes | Basic | Stage 2 | Stage 3 |
|--|---|---------------|---------------|----------------|
| Datalogger(s) | To monitor the humidity and temperature of seed collections in the field, during processing and before storage. Cost is per unit – more than one may be required. | £300 | | |
| Cooled incubator(s) | To dry seed to 15 per cent equilibrium relative humidity before long-term storage. Models fitted with lights can double up as germination cabinets. Cost is per unit – more than one may be required. | £4,500 | | |
| Dissecting microscope | To examine seed quality. | £350 | | |
| Balance | To weigh seed collections and generate seed count estimates. | £1,300 | | |
| Heat sealer with stand | To seal trilaminate foil pouches for long-term storage. | £1,200 | | |
| Freezers | For long-term storage at –20 °C. Household freezers may be used for basic setup. Costs stated are for single units (Stage 2 quote based on a 450 L lab model). More than one unit may be required. | £500 | £1,200 | |
| Fridges | For short-term storage of seed for the Horticulture Department at 5 °C. Household freezers may be used for basic setup, and lab standard fridges for Stage 2. Cost is per unit – more than one may be required. | £200 | £800 | |
| Dust containment workstation | For health and safety, to trap dust while seed is manually cleaned. | | £3,000 | |
| Agriculex aspirator | Mechanical seed cleaning – small volumes. | | £3,500 | |
| Laminar flow cabinet | To provide a sterile workstation for germination testing on agar. | | | £2,500 |
| Selecta Zig-Zag Gravity Seed Separator | Mechanical seed cleaning – larger volumes. | | | £16,600 |
| | | £8,350 | £8,500 | £19,100 |