Twinflower Restoration Handbook

A practical guide to planning and implementing targeted restoration action for twinflower in Scotland

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A. SUMMARY FOR PRACTITIONERS

This handbook aims to facilitate appropriate restoration action for twinflower (*Linnaea borealis*) in Scotland by recording, sharing and promoting best practice, and making this accessible to anyone with an interest in twinflower conservation. The following recommendations are based on the current state of knowledge, drawing on evidence from scientific research and field trials. The content is intended for guidance only, and may need to be adapted according to individual situations. Future updates will be made as understanding of best practice continues to evolve.

1. What is the objective of a twinflower restoration programme?

To create, by means of carefully planned translocation, and to maintain, by appropriate habitat management, a series of populations containing multiple, unrelated, and sexually compatible twinflower plants which are ecologically suited to the sites in which they grow. The aim is to restore fruit production, allowing the species to recolonize suitable habitat via seed dispersal and to adapt to environmental change. Whilst all twinflower populations should be cared for through appropriate habitat management (see Plantlife Scotland guidance) many are genetically depauperate and in need of restoration (SUPPORTING INFORMATION SECTIONS 1-3).

2. What is the twinflower resource available for translocation?

Twinflower plants which have the potential to provide material suitable for translocation are present both in wild populations and in *ex situ* clone collections (SUPPORTING INFORMATION SECTION 4). Detailed information on both is available from the Twinflower Restoration portal https://andrewrfr365.shinyapps.io/Latest/

3. How should twinflower translocation take place?

Two strategies can be used to achieve restoration. Twinflower plants can be translocated into release sites where the species is already present, i.e. within both its native and natural range, to *reinforce* existing patches. This provides assurance that ecological site conditions are suitable and may enhance the genetic variation within the population, but does not increase the number of twinflower populations. The alternative is to *reintroduce* twinflower plant material into release sites not currently containing twinflower (outside its native range but within its natural range) to create a new population (SUPPORTING INFORMATION SECTION 5.1). Success is critically dependent on choosing sites possessing appropriate ecological characteristics (SUPPORTING INFORMATION SECTION 5.2).

4. How many donor sites are required to supply plants for each release site?

Each *reinforced/reintroduced* population should comprise a minimum of 6 unique clones. To achieve this, as virtually all donor sites contain a single clone, material originating from plants from a minimum of 5 donor sites should be transplanted into each reinforcement site, and from a minimum of 6 donor sites into each reintroduction site. This will ensure sufficient genetic variation for future adaptation by the population. If more than one translocated population (i.e. release site) is to be created in the same local forest area, each one should ideally comprise plant material from a unique combination of donor sites (SUPPORTING INFORMATION SECTION 5.3).

5. How should donor sites be chosen for each release site?

Donor sites should be:

i) Ideally from the same geoclimatic and elevational zone as that of the release site (SUPPORTING INFORMATION SECTION 5.4.1).
ii) Located at least 1.5 km apart from each other and from any population that is *reinforced* to minimise relatedness among clones (SUPPORTING INFORMATION SECTION 5.4.2).
iii) Able to provide planting material without compromising donor patch survival (SUPPORTING INFORMATION SECTION 5.5).

6. What form of plant material should be transplanted?

Cuttings are the primary form of plant material available for translocation. A cutting comprises a section of twinflower shoot (up to 40 cm long) with at least one set of well-developed roots which have not yet penetrated the soil layer. Cuttings can be transplanted directly from wild patches or from *ex situ* clone collections. Where seedlings have been generated in *ex situ* 'seed orchards', these can provide an alternative form of transplant material (SUPPORTING INFORMATION SECTION 5.5).

7. How should transplants be laid out at release sites?

At the release site, cuttings or other transplants should be located in a standard manner with plants from each donor source in discrete single clone plots located between 5 to 10 metres from their neighbour. This design ensures that transplants can be easily re-found to measure their success and facilitates the use of uniform monitoring procedures for recording flowering and detecting seed production in the population (SUPPORTING INFORMATION SECTION 6.2). This design is recommended as the model for new restoration schemes, with appropriate modifications dependant on local circumstances.

8. How should release sites be documented and monitored?

Release sites should be documented at the time of establishment to record their location, the donor sites from which transplants have been introduced, the planting design, and relevant baseline ecological data. Release sites should be monitored in a standard manner to record establishment, spread, flowering and seed set of transplants in at least years one, five and ten after their creation (SUPPORTING INFORMATION SECTION 7) (APPENDIX 2).

9. How should release sites be managed?

The woodland at the release site should be managed to retain, over a long time scale (50+ years), a potentially dynamic mosaic of areas, from 0.25-5.00 ha in size, with moderate shade (canopy cover 60-80 %, basal area 30-50 m^2/ha). Implementation of suitable disturbance regimes should be considered at sites where there is limited bare ground for seedling establishment (SUPPORTING INFORMATION SECTION 8).

10. What guidance is available, and what permissions are required for conducting a restoration programme?

The Scottish Code for Conservation Translocations provides valuable guidance for planning, conducting and documenting a restoration programme. Permission must first be obtained from landowners of all proposed release and donor sites, and if these fall within designated sites NatureScot Operations staff must also be consulted to establish whether consent is required. A

Scottish Code for Conservation Translocations – Translocation Project Form is then submitted to the NatureScot Licencing Team. They will assess whether the translocation programme can proceed, and if so, will grant a translocation licence where this is needed. An example of a translocation licence application is provided (SUPPORTING INFORMATION SECTION 9) (APPENDICES 1 & 2).

B. SUPPORTING INFORMATION

1. Species description and ecology

Twinflower (*Linnaea borealis* L.) is a perennial, evergreen, understory dwarf-shrub with a circumpolar distribution extending across the boreal regions of North America, Europe and Asia, with outlying populations present in more southerly montane areas throughout the range (Fig 1a). In Britain most populations are located in highland Scotland, with particular strongholds on Strathspey and Deeside (Fig.1b).

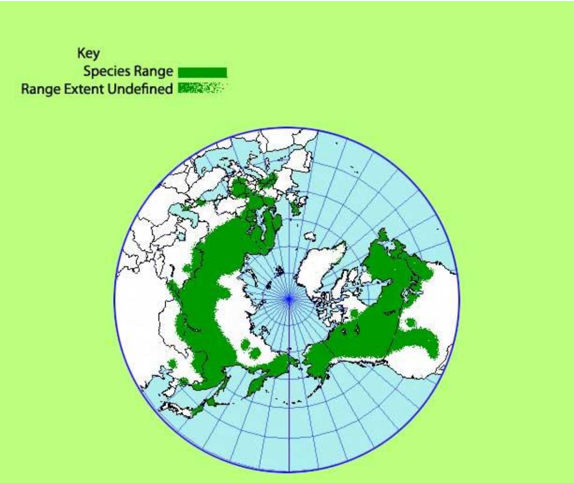

Figure 1a. Worldwide distribution of twinflower.

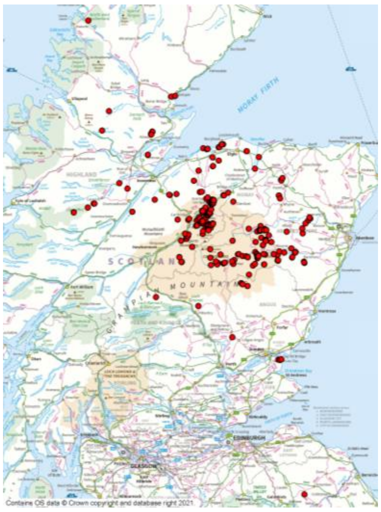

Figure 1b. Twinflower distribution in Britain and Ireland. Blue: Native populations; Red: Introduced populations (© BSBI).

Individual plants consist of a branching network of rooted stolons or shoots bearing opposite pairs of small (2cm), ovate leaves (Fig. 2). Stolons may grow up to 50 cm per year, and individual long-lived-clones may extend for distances of up to 100 m.

Figure 2. Vegetative and reproductive morphology of twinflower.

Twinflower is typically found as a component of the ground flora of conifer forests on soils of Poor to Very Poor soil nutrient regime, often growing with *Vaccinium* species in conditions of moderate shade. Where plants are found outside the forest, they are usually present on north facing slopes, often among boulders and shaded by other dwarf-shrubs.

Figure 3a. Twinflower inflorescence.

Figure 3b. Fruits of twinflower.

Some 2% of twinflower vegetative stolons give rise to flowering stems approximately 10 cm tall which bear a pair of bell-shaped flowers (Fig. 3a). Flowers are visited and pollinated mainly by muscid, syrphid and empid flies, which means that the vast majority of effective pollination events occur at short distances between individuals (<10 m) with very little occurring at distances greater than 30 m. Plants are self-incompatible, and high levels of seed set occur only when pollen is transferred between genetically distinct, compatible individuals. Following successful fertilization, the ovary of each flower ripens to produce a single seeded fruit partially enclosed within two bracts covered with viscid hairs (Fig. 3b). These structures provide the fruit with the potential for long distance dispersal attached to the fur of mammals and the feathers of birds. Seedling establishment is likely to require disturbed areas that are free of vegetation, such as those created by small mammals, large herbivores or timber extraction operations.

2. Conservation issues

In Scotland the habitat of twinflower has declined dramatically over many centuries and become highly fragmented due to destruction and overexploitation of the native pinewoods. As a consequence, the population of twinflower has become sub-divided into a series of isolated patches, each containing a single or a small number of clones. The distance between patches largely prevents inter-patch genetic exchange via pollen or seed.

In some natural populations seed set has been possible and new plants have established within the patch. However, due to the close relatedness of the parent clones and small number of compatible clones available for crossing, the immediate effect of this regeneration has been to increase inbreeding.

In other patches, where few clones remain, recruitment of new genotypes has ceased, and there has been loss of individual clones over time. The net result is that not only has there been continuous loss of twinflower sites, but of the sites that remain in the Cairngorms core area, some 79% comprise only a single clone. The single clone patches are unable to set seed due to their self-incompatibility and the large distances between patches which prevent cross-pollination by insects.

In the absence of high-quality seed production there can be no recruitment of new individuals into the existing population; twinflower in Scotland has therefore largely become a static collection of clones maintained almost solely by vegetative reproduction. The prognosis is that the existing inventory of twinflower clones will be gradually eroded by loss, through disease, or as a consequence of further habitat loss or degradation. Consequently, without intervention, the species will eventually become extinct in Scotland.

3. Overview of restoration action required

The overall aim of the restoration programme is to create within Scotland an ecologically and evolutionarily dynamic population of twinflower that is once again capable of producing outcrossed seed of high fitness, and able, through this seed production, to recruit new outcrossed clones locally, disperse seed to neighbouring patches, and establish new populations at suitable sites via natural colonisation.

To achieve these aims a number of management actions are required. The first is the creation and maintenance of a large number of twinflower patches in suitable habitat, each of which contains multiple unrelated clones and is capable of producing outcrossed seed. The second is the management of these sites so as to retain twinflower in the understorey and provide an appropriate environment for flowering and seed set. The third is the establishment of field layer disturbance regimes that will facilitate recruitment and establishment of twinflower via seed and thereby restore an ecologically and evolutionarily dynamic population.

4. The twinflower resource available for restoration– *in situ* and *ex situ*

4.1. *In situ* resource

As a result of a series of intensive survey efforts approximately 400 patches of twinflower have been identified across northern Scotland covering the full range of biogeographical zones in the region. Information on these sites can be accessed via the TWINFLOWER PORTAL https://andrewrfr365.shinyapps.io/Latest/. Genetic studies have demonstrated conclusively that as a whole this *in situ* Scottish population is highly genetically variable, and that individuals from different patches are genetically unique (distinct clones). Clones occurring within 1.5 km are relatively closely related (first cousin level) but beyond this distance kinship values rapidly drop to zero.

These results indicate that the genetic variation needed to build a resilient population is present in Scotland, and there is no need to import genetic material from outside Scotland for the restoration programme. Geographic patterns of relatedness suggest that when selecting clones for reinforcement or reintroduction of twinflower, it is desirable to choose clones that come from patches more than 1.5 km apart to avoid possible problems with inbreeding.

4.2. *Ex situ* resource

A number of *ex situ* clone banks have been created by collecting and rooting cuttings taken from the wild population. The presence of particular clones in existing clone banks is indicated in the TWINFLOWER PORTAL https://andrewrfr365.shinyapps.io/Latest/ .

5. Planning a conservation translocation restoration programme

5.1. Reinforcement or reintroduction?

5.1.1. Reinforcement

Reinforcement involves adding additional clones to an existing twinflower patch to increase the total number of clones that are present. It is particularly applicable in situations where a patch contains a large, successful, but isolated single clone. Here, without intervention, there is no prospect of fruit production. The advantage of reinforcement in these situations is that there is a high probability that the ecological conditions will be suitable for twinflower, and translocation will be successful. The disadvantage is that it does not result in a greater number of twinflower populations and therefore does not increase connectivity across the landscape (in the short term).

For reinforcement to be considered, the following criteria should be met:

i) Existing twinflower patch is persisting well vegetatively (minimum ground cover of shoots 25-50%) and the level of flowering is high (≥50 infl/sq m).

ii) Existing patch is isolated from neighbouring patches by distances >50 m, and seed set levels are low or zero (<<5% of flowers setting seed).

iii) Habitat conditions are favourable (see SECTION 5.2) both within the immediate area of the twinflower patch and in areas across the wider surrounding woodland – sufficient to accommodate the establishment and spread of transplanted clones and future recruitment of seedlings once seed set and seed dispersal are restored.

iv) Long-term favourable management of the site is secured (see SECTION 8).

5.1.2. Reintroduction

Reintroduction of twinflower in the landscape requires careful ecological selection of the release site to optimise the chances of successful establishment and maintenance of transplants (see SECTION 5.2). Reintroduction can enable us to select more favourable locations for the establishment and spread of twinflower compared to some relict natural sites which are often in sub-optimal habitat. Furthermore, establishment of a new twinflower patch can provide improved connectivity across the landscape and could potentially become an additional source of seed for further colonisation in the future. Although we already have some knowledge of the conditions that twinflower requires, an improved understanding of the ecological characteristics of what constitutes an appropriate twinflower site would help to guide the choice of release sites.

5.2. Habitat requirements for release sites

Habitat should resemble that described as NVC type W18 *Pinus sylvestris* – *Hylocomium splendens* woodland, in particular W18c *Luzula pilosa* sub-community (preferred), or W18b *Vaccinium myrtillus* – *V. vitis-idaea* sub-community, or W18a *Erica cinerea* – *Goodyera repens* sub-community (see Rodwell, J S (ed.) (1991) British Plant Communities. Volume 1. Woodlands and scrub).

The following provide a set of criteria for selection of release sites based on those applied in the Cairngorms area where transplants have been successfully established:

Slope/ aspect	Avoid exposed sites on south-facing aspects (S, SE, SW) and steep slopes (>35º) as these are more likely to dry out in summer.
Soil	Soils should be free-draining (not wet) and mildly acidic.
Canopy	Scots pine (*Pinus sylvestris*) should be the dominant canopy tree, with up to 20% birch (*Betula* spp.) and/or rowan (*Sorbus aucuparia*). A low frequency of European larch (*Larix decidua*) is acceptable in plantation sites. Canopy cover should ideally be 60-80% with a stand basal area between 30 and 50 m^2/ha. Periodic light thinning may be required to maintain these conditions in plantation sites through time.
Understory	Avoid areas where regenerating rowan and birch are forming dense thickets in the understory. Areas with a dense juniper understory (*Juniperus communis*) are also unsuitable, but open cover (up to 25%) is acceptable.
Field layer	The field layer should be predominantly short (≤15 cm), open and mossy. Dwarf-shrubs – combined cover of blaeberry (*Vaccinium myrtillus)* and cowberry (*V. vitis-idaea*) preferably ≤25%, and not exceeding 40%. Combined cover of heather (*Calluna vulgaris)* and bell heather (*Erica cinerea*) ≤10%. Grasses – up to 50% cover of wavy hair-grass (*Deschampsia flexuosa*) can be present, but <<10% cover of broadleaved grasses such as sweet vernal grass (*Anthoxanthum odoratum*) and *Agrostis* spp. Moss-layer – not deeper than 5 cm on average. *Hylocomium splendens* and *Pleurozium schreberi* should be the most abundant species. Avoid areas dominated by *Rhytidiadelphus triquetrus*.
Positive indicators	The presence of at least two of – heath bedstraw (*Galium saxatile*), wood sorrel (*Oxalis acetosella*), chickweed-wintergreen (*Lysimachia europaea*), common cow-wheat (*Melampyrum pratense*), hairy wood-rush (*Luzula pilosa*) – is essential. One or more of – creeping lady's-tresses (*Goodyera repens*), common wintergreen (*Pyrola minor*), intermediate wintergreen (*P. media*), serrated wintergreen (*Orthilia secunda*) – is desirable.
Negative indicators/ undesirable species	Avoid sites with >10% cover of sphagnum moss (*Sphagnum* spp.) or *Polytrichum commune*, and/or with frequent tufted hair-grass (*Deschampsia cespitosa*), purple moor-grass (*Molinia caerulea*) and/or cross-leaved heath (*Erica tetralix*) which indicates the sites are too wet. Bracken (*Pteridium aquilinum*), gorse (*Ulex europaea*), broom (*Cytisus scoparius*) should be absent from the vicinity of the site. Non-native conifers, including Norway spruce (*Picea abies*) and Sitka spruce (*P. sitchensis*), and invasive *Rhododendron ponticum*, which cause excessive shading, should not be present at or near the site as either established specimens or regenerating seedlings.
Browsing/ disturbance	Browsing levels should be light to moderate. Some localised disturbance / bare ground (e.g. from small mammals and/or large herbivores) is desirable to provide sites for future seedling establishment. Planting in exclosures is not recommended.
Management	Long-term favourable management should be secured – including continuity of a stable woodland canopy (i.e. avoiding heavy thinning / clearfell) over a minimum 1 ha area surrounding the transplant site. Similar suitable areas of habitat, 0.25 ha to 5 ha in size should be present across the wider surrounding woodland.

5.3. Numbers of donor populations required for transplantation into each release site

A minimum of 6 unrelated clones need to be established at each release site to ensure that a significant proportion of the genetic diversity within the total Scottish population is represented in the release site. This can be achieved by transplanting material from 5 different donor sites to add to the one clone already present at a reinforced site and 6 different donor sites into a reintroduced site. This prescription assumes that the 5 or 6 transplanted clones survive and thrive to reach flowering stage and contribute to the gene pool of future seedling recruits. It would be advisable to review at the 5- and 10-year monitoring stage to determine whether additional clones should be introduced to ensure that 6 or more clones survive and reach flowering stage. This is only worth doing if at least some of the clones are already thriving (suggesting the site is suitable).

5.4. Choice of suitable donor sites for translocation to each release site

5.4.1. Ensuring ecological suitability of translocated clones

Analysis of the climate space occupied by twinflower patches in Scotland indicates that the species occupies a wide climatic envelope with potential for local climatic adaptation. The main differences between sites are associated with elevation and the degree of oceanicity/continentality (first and second axes respectively in Fig. 4a and shown on a map of Scotland Fig. 4b).

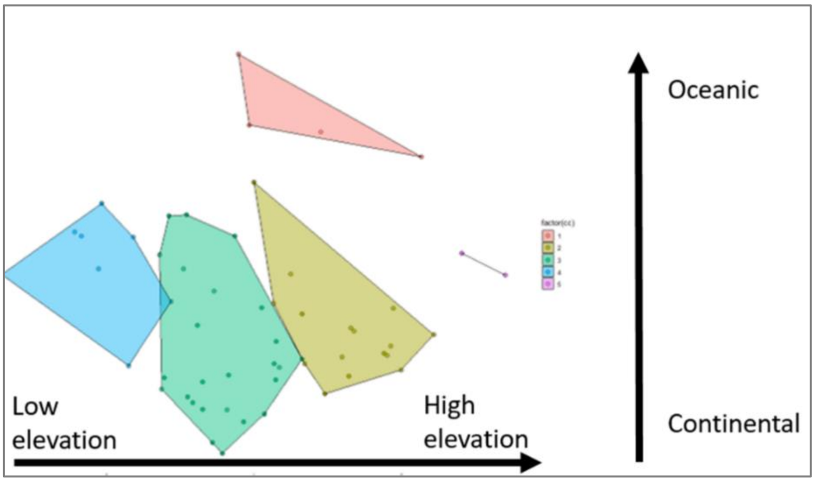

Figure 4a. Position of twinflower sites in climate space based on analysis of 10 climate variables.

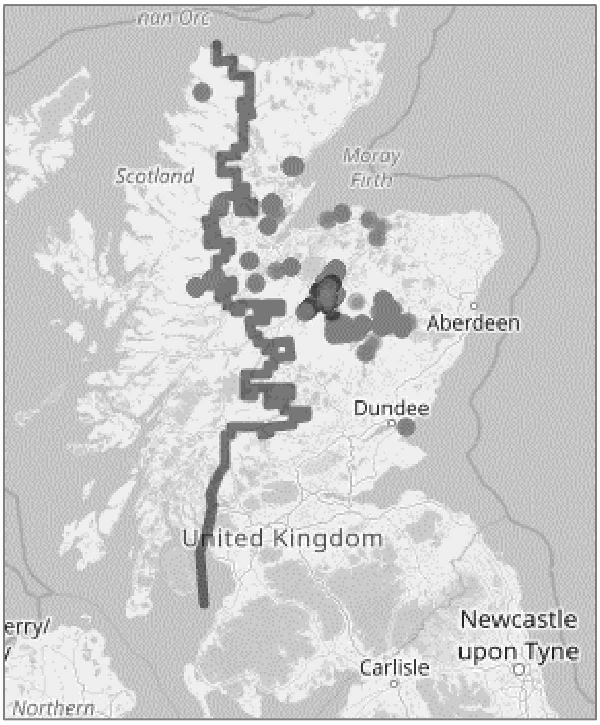

Figure 4b. Location of existing twinflower sites (grey dots) and boundary (grey line) of the two bioclimatic zones for twinflower in Scotland

This analysis suggests some simple best practice rules that can be applied to reduce the risk that transplants are ecologically unsuited to their release site:

Firstly -

> As long as sufficient material is available within a climatic region, transplantation should be avoided between sites from the north-west with more oceanic climates and those from the remainder of the range with more continental climates.

Secondly, and where possible -

> Within continental and oceanic groups of populations, donor and release sites should differ in elevation by no more than ±150 m.

The first step in choosing suitable donor sites should therefore be to identify candidate donor sites occurring within the same climatic zone and differing no more than 150 m in elevation from the release site.

5.4.2. Ensuring that transplants are unrelated

Further filtering of candidate donor sites (identified in 5.4.1 above) should be undertaken to ensure that translocated plants are not closely related either to each other or to individuals that are being reinforced at the release site. Genetic studies indicate that this can be done by ensuring that the donor sites involved in translocation to a release site are more than 1.5 km distant both from one another and from any site that is being reinforced.

5.5. Source of planting material – direct or *ex situ*

Twinflower material for translocation can either be collected directly from natural populations or may be material raised *ex situ* in nurseries before translocation.

5.5.1. Direct transplants

When direct transplantation is chosen, the only viable option is to use cuttings. What little seed is set in natural populations will, in the majority of cases, be inbred (resulting from selfing due to a leaky self-incompatibility system or near-sib mating) and plants derived from such seed will be of low fitness. Moreover, little is known about conditions necessary for establishment of seed, other than that bare areas arising from disturbance may be necessary.

An advantage of using cuttings taken directly from plants in wild patches (direct transplants) is that they are likely to be colonised by appropriate mycorrhizae, endophytes etc. and this may enhance their probability of establishment. However, material of this kind could potentially transfer native pathogens that may not be present at the release site and for this reason, only cuttings with roots that have not yet penetrated the soil at the donor site are recommended as the source of direct translocation material. There is considerable evidence from existing translocation programmes that directly transplanted twinflower establishes successfully from cuttings and this is the method of choice for translocation programmes when resources are scarce. A major limitation of this strategy is that removal of large numbers of cuttings from existing twinflower sites may increase the likelihood that donor patches are either lost or become insufficiently robust to act as donors in the future. As a rough guide, no more than 5% of shoots should be removed annually from any one clonal patch. Where donor patches are small, and removal of 5% of shoots would not yield sufficient material for direct transplanting, bulking-up *ex-situ* is the recommended course of action.

5.5.2. Transplants from *ex situ* populations

Where resources allow, *ex situ* collections of twinflower can be established to create clone banks in a nursery situation. Clone banks can play a very valuable role in bulking-up material collected in the wild for use in translocation programmes. The tendency of twinflower to produce prolific numbers of branches bearing their own roots means that clone banks can furnish a continuous source of cuttings. This is particularly important for clones that, in the wild, are small in size or difficult to access, such as many of those in the north-west region. Clone banks can also act to conserve clones that are threatened in the wild and provide the opportunity to transfer cuttings of groups of these clones to more favourable sites. To maintain clonal integrity, clones in the clone bank should be spatially separated as far as possible, invading shoots trimmed back, and all flowers removed.

Alternatively, and once an *ex situ* clone bank has been established, a 'seed orchard' can be created by positioning clones in close proximity and encouraging them to flower and cross-pollinate (natural cross-pollination by insects has be shown to produce seed in nursery situations). Progeny raised from seed produced by these orchards may be superior to individuals raised from cuttings of the parents because they will be cleared of potentially debilitating viruses that have accumulated in long lived clones and will possess novel genotypes not already present in the natural population. Note that use of seedlings raised from these seed orchards for reintroduction will require an amended transplant design to that suggested for transplantation of cuttings (SECTION 6.2) – e.g. plant out 25 seedlings (grown in cultivation for at least 1 year) in a grid with 4 m spacing between neighbours (over a 20 x 20 m area), assuming ~50% might survive.

To ensure that the sexually derived offspring from a seed orchard are ecologically suited to the release site, the parental genotypes included in the 'seed orchard' should be from the same geographic and altitudinal zone (SECTION 5.4.1) as the release site. Furthermore, they should be sourced from sites which are more than 1.5 km apart from one another to reduce possibility of offspring suffering from inbreeding depression (SECTION 5.4.2).

5.5.3. Biosecurity considerations

A major caveat to the use of all translocated material (cuttings (either directly transplanted from plants in the wild or derived from plants raised in the nursery) or whole plants derived by vegetative propagation or seedlings raised *ex situ* in translocation programmes) is that very strict biosecurity must be practiced throughout. Recent experience with restoration programmes for juniper has shown that planting of stock raised in commercial nurseries is associated with the introduction into native juniper populations of the exotic pathogen *Phytophthora austrocedr*i followed by widespread death of plants at the release site. Ongoing surveys of commercial nurseries are revealing high levels of *Phytophthora* contamination across nurseries. Diseases such as *Pythium* and *Phytophthoras* are soil borne, can be harboured by a wide range of plant species and are not visible above the ground such that movement of soil between sites in the wild as well as compost on nursery grown stock transferred to sites in the wild has the potential to introduce pathogens that threaten both twinflower and other plants that form part of the ecosystem.

In the light of these findings, biosecurity protocols are being formulated for restoration planting. In the meantime, the following advice should be followed:

i. Footwear and tools should be cleaned thoroughly when moving between wild sites or between wild sites and nurseries. Collected cutting material from the wild must be healthy and free from soil.
ii. Direct transfer of rooted cuttings <u>whose roots have not yet penetrated the soil</u> from donor to release site represents the method of least biosecurity risk and is the preferred option for reinforcement sites.
iii. Particular precautions need to be taken when nursery grown plants are transferred to the reintroduction/reinforcement sites. These include: never raising plants in commercial nurseries and finding suitable isolated locations away from garden situations but as near to the release sites as possible for establishing a small nursery; plants should be grown in commercially available sterile compost, using brand new pots or if pots are reused they must be sterilised for 24hrs in 10% bleach before use; plants should be placed on free draining benches which are far enough from the ground to avoid splash from the ground (1 metre) ; plants should be watered with mains tap or bore hole water as other sources can introduce diseases such as Phytophthoras; weed control must be practised, monitor plants and incinerate any plants showing signs of ill health.
iv. Transfer of potting medium (soil) in which cuttings or seedlings have been grown is a potent way of unintentionally introducing pests /pathogens into the release site. One way to

minimise this risk, is to either refresh the potting medium just before planting out, or plant bare root plants from the nursery or plant cuttings from nursery raised stock plants.

6. Practical protocols

The following protocols are based on experience gained by Andy Scobie during his work on the Cairngorms Rare Plants Project (CRPP). They have proved effective in establishing both clone banks and the multi-clonal populations required for twinflower restoration. It is recommended that they form the model for new restoration schemes, with appropriate modifications dependant on local circumstances.

6.1. Establishing a clone bank

Where it is desirable to establish *ex situ* collections of material, twinflower can be readily propagated from cuttings taken in the wild. These are best collected during the spring and early summer growing season (April to July). Twinflower shoots can be harvested and cut into sections c.15 cm long) behind nodes where roots have started to develop, then placed into pots (or multi-celled trays) containing a suitable growth medium. Rooting powder is not required.

A mix of multi-purpose compost and seed and cutting compost has been shown to be successful. Addition of sharp sand is used to ensure compost is free draining. The following mix is recommended: 3 parts multi-purpose peat-free compost, 2 parts seed and cutting compost and 1 part sharp sand.

Grow cuttings outside, not in a glasshouse, and keep compost moist but avoid over-watering. Use shade netting in summer to block out direct sunlight.

Once a cutting has rooted and established in an individual pot, it can either be directly planted out at the release site or maintained and 'bulked-up' in cultivation so that further cuttings can be taken from it.

Cuttings should be grown for at least a year in cultivation before being planted out (Fig. 5).

Due to the creeping habit of twinflower, planting out in old fish boxes, or large trays of a similar size, is a good way to encourage spread and rooting at the nodes, enabling the harvesting of additional cuttings.

Figure 5. Tray of twinflower cuttings - first growing season in cultivation.

6.2. Translocation protocols

6.2.1. Selection and design of release sites for reinforcement

Suitable release sites for reinforcement should support an existing twinflower patch that is persisting well vegetatively, is flowering, but is producing little or no seed each year due to reproductive isolation from neighbouring patches. Habitat conditions should be favourable for twinflower, both within the local area of each patch and in areas across the wider surrounding woodland (SECTION 5.2).

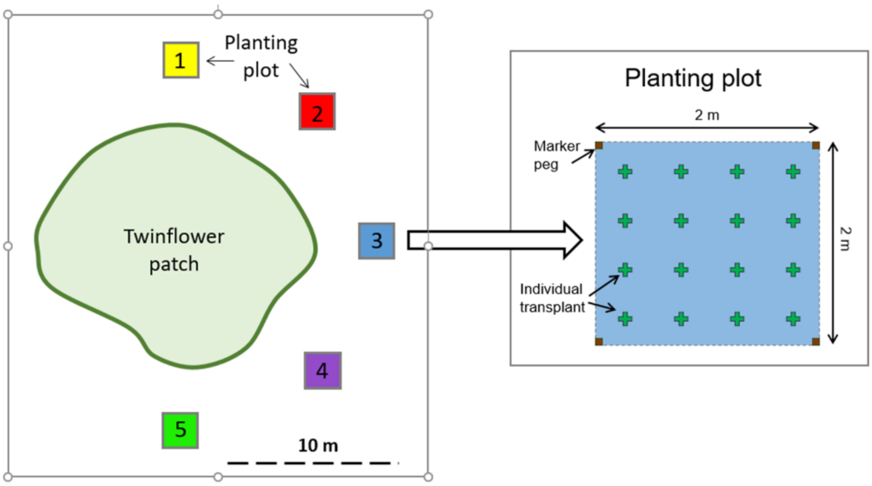

Figure 6. Recommended planting design for reinforcement of twinflower. Each planting plot consists of 16 cuttings of a single unique clone.

A minimum of six 2 x 2 m reinforcement planting plots, each to accommodate 16 transplants of one of the six clones being translocated, should be set up around the existing patch (Fig. 6). Plots should be established within 25 m of the natural twinflower patch, and not more than 10 m apart from their neighbouring planting plots. The exact positioning of plots can be varied at each site depending on the location of suitable micro-sites for planting. The aim is to establish each clone independently but in close enough proximity to their fellows and the natural patch to permit cross-pollination following successful establishment.

6.2.2. Selection and design of release sites for reintroduction

Release sites for reintroductions should be selected on the basis that although twinflower is currently absent, suitable habitat is present over an area of approximately 1 ha, sufficient to accommodate the planting plot design shown in Fig. 7 and to allow future spread following successful restoration of seed production (SECTION 5.2). Planting plots 2 x 2 m in size, each to accommodate 16 transplants of one of the six clones being translocated, should be aligned in north /south orientation and laid out in the arrangement shown in Fig.7. The aim is to establish each clone independently but in close enough proximity to the other five to permit cross-pollination between them (if establishment is successful).

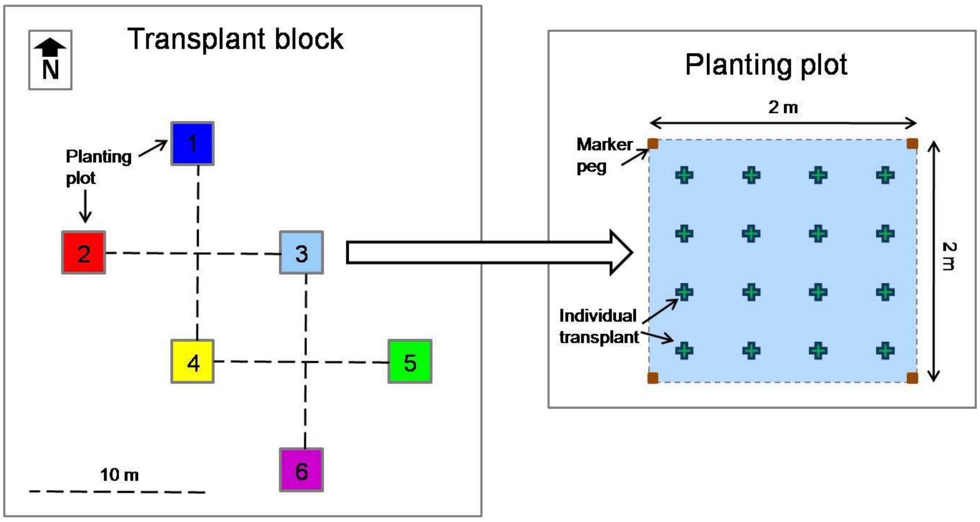

Figure 7. Recommended planting design for reintroducing twinflower. Each of the six planting plots consists of 16 cuttings of a single unique clone.

6.2.3. Setting up planting plots

Individual 2 x 2 m planting plots should be permanently marked at their corners with sturdy wooden pegs hammered securely into the ground. To prepare plots for planting, approximately 50% of the moss-layer should be raked out to create some bare ground to aid establishment of the transplants. A four by four grid should then be established with plant labels within the plot to create 16 planting locations (Fig. 8).

Figure 8. Single clone planting plot marked with pegs, and position of each of the 16 transplants of the single clone within the plot (white labels).

6.2.4. Transplant units

Transplant units, whether direct transplants or from *ex situ* clone banks (SECTION 5.5), should comprise a section of shoot, up to 40 cm in length, with at least one set of well-developed roots and several side shoots (Fig. 9).

These can be collected from the donor patch or clone bank by gently prising up sections of shoot by hand, following back from the growing tip, and pulling upwards gently so as not to damage the roots of the cutting whilst causing minimum damage to the remaining plant.

Once collected, cuttings should be stored in labelled clip-top plastic bags and kept damp e.g. with moist cotton wool, to prevent them from drying out. Cuttings should be stored in cool conditions and planted at the appropriate release sites within three days of collection.

Figure 9. Rooted twinflower stolon for use as a direct transplant unit.

6.2.5. Planting a planting plot

Transplanting is best done either in early spring (April to mid-May), or late summer/early autumn (Sept to-end of October) to avoid planting out during the main summer period when drought can be a risk, especially with direct transplants.

At each pre-marked position, stolons from the appropriate clone should be planted by using a trowel to cut a shallow slot in the soil, 3 to 4 cm deep, the same length as the shoot. The roots should then be carefully placed in the slot and the shoot section covered with soil at several points along its length in order to hold it in place (Fig.10).

Figure 10. Twinflower stolon shortly after transplanting into prepared plot.

6.2.6. Documentation of transplants

It is important that each translocation conducted should be fully documented in a standard format that is readily accessible and interpretable. The documentation should provide details of release sites, donor patches, planting design, and baseline monitoring data from the transplant site. For a reinforcement plot, baseline monitoring would typically comprise: GPS grid references for the natural twinflower patch at the donor site and planting plot locations in the release site; a sketch map showing the extent of the twinflower patch and location of the planting plots in relation to key features; measurements of the dimensions of the extant twinflower patch (maximum length x maximum breadth measured in metres), and the typical % cover of twinflower in the patch; information on slope, aspect, canopy, associated species, NVC community; fixed point photographs of the existing twinflower patch and the planting plots (providing a record of habitat conditions and cover of twinflower at time of planting). If any of these locations do not appear on the exisiting database, please inform the appropriate Vice County Recorder of the BSBI https://bsbi.org/local-botany

To assist with this recording programme and to standardise the information recorded, an example Excel spreadsheet for documenting a reinforcement programme established by Andy Scobie in the CRPP is included as Appendix 2. This format can be used as a standard reporting sheet for initial documentation of translocation programmes, and as part of the application to SNH for permission to transplant (section 9).

7. Monitoring of release sites

Key questions	**Monitoring measure**
Have the transplanted clones established and spread?	Area occupied by each clone % cover / density of shoots for each clone
Are they flowering?	Flowering density of each clone
Is there evidence of seed set?	Fruit set levels
Has seedling recruitment taken place and/or is there potential for to take place?	Searches for seedlings / new recruits Germination tests (in situ / ex situ)
Are there long-term changes in genetic structure?	Genetic sampling / paternity analysis

7.1. Protocol applied to reintroduction sites

For each planting plot in the transplant block, the following monitoring measures should be taken in at least years one, five and ten after their creation:

i) *Extent of area occupied by transplanted clone* – lay a tape through the plot in north-south and east-west directions, and measure the maximum outer dimensions of the patch or the group of transplants, to nearest 0.1 m.

ii) *Density of twinflower* – measured using a 0.5 x 0.5 m quadrat, with divisions every 10 cm (creating 36 intersections), count number of intersections touching a twinflower shoot (out of a total of 36 possible touches).

iii) *Density of flowering spikes* – count the total number of inflorescences in 0.5 x 0.5 m quadrats (as for density above)

iv) *Percentage fruit set* – examine each flower on every inflorescence for evidence of fruit set (see Fig. 11 for details), sampled in 0.5 x 0.5 m quadrats (as above). Record number of fruits out of total number of flowers. Calculate % fruit set value = total fruits/total flowers examined. Fruit set should be recorded between end of July and mid-August, allowing 6 weeks for potential fruits to develop following flowering.

v) *Cover of associated ground vegetation* – total percentage cover of: (a) dwarf-shrubs, (b) forbs, (c) graminoids, (d) moss, and (e) bare ground should be recorded for the plot as a whole (the total of these can therefore exceed 100%).

vi) *Vegetation height* – measured in 5 random places within the plot (to the nearest cm).

vii) *Tree canopy cover / tree basal area* – record % canopy cover and/or repeat basal area measurements at sites if these were taken at the outset; note occurrence (yes/no) of side-lighting (e.g. site is at edge of stand, adjacent to track).

viii) *Photographs* – photograph of each plot (always from south edge of plot).

ix) *Seedling recruitment* – if/when seed set had been recorded in the translocated population, systematic searches for seedlings should be undertaken within and around the site to note the presence and location of any seedling recruits.

x) *Germination tests / paternity analysis / genetic sampling* – these measures are more aspirational than essential but could be included (where resources allow) as part of a long-term assessment of the success of the translocation (e.g. at 20 year monitoring).

Quadrats for measures of twinflower density, flowering and fruit set should be sampled along transect lines. Space quadrats 1 m apart along the transects. You can use the same tapes as those for measuring extent, forming a N-S and E-W transect. Avoid recording quadrats that span the edge of the patch (Fig. 12).

Figure 11. Scoring system to be used for recording fruit set.

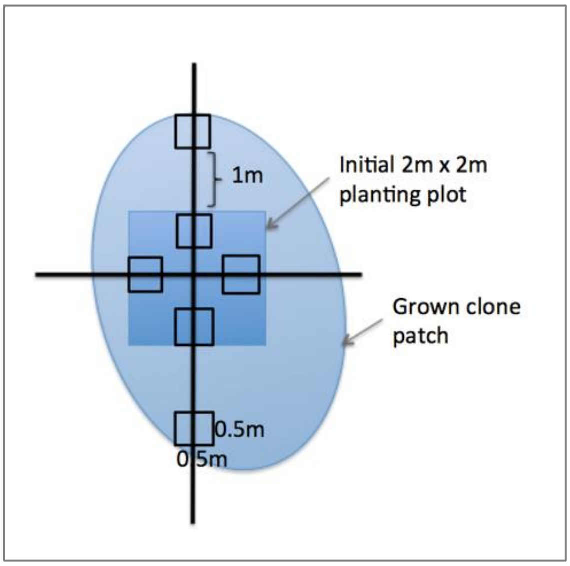

Figure 12. Schematic for layout of transects and quadrats for monitoring a planting plot within a transplant block.

7.2. Additional monitoring for reinforcement sites

In addition to the measurements made on transplant plots, measure the above variables for the natural patch at the reinforcement site. For larger patches (>10 x 10 m), spacing between quadrats along the transect lines could be increased to 2 m.

8. Managing sites for twinflower restoration

While the guidance here is focussed on restoration via translocation intervention, the maintenance of existing natural twinflower populations through appropriate habitat management is also of vital importance to maintain the existing genetic resource, and should form a key part of any conservation strategy. Many of the key principles in this section apply equally to the management of sites to ensure the survival of relict twinflower populations, and additional information can be found in the Plantlife Pinewood Management Guide.

8.1. Provision of suitable habitat to facilitate restoration

For robust, dynamic twinflower populations to be restored across the landscape, the first step is to establish, via translocation, multi-clonal populations with the potential for seed production at currently suitable sites; the second is to ensure that suitable habitat is provided at an appropriate spatial and temporal scale for seeding populations to colonize, both now and in the future.

While twinflower can survive in a broad range of woodland stand conditions, in order for translocations to succeed and for subsequent spread to occur, habitat conditions must be present which are suitable for twinflower to thrive – i.e. those which are optimal for vegetative growth, flowering, seed production and seedling recruitment.

8.2. Optimal habitat conditions for growth and spread

In Scotland, twinflower thrives best in woodland areas with moderate canopy shade (see SECTION 5.2 for description of habitat type) where canopy cover is in the region of 60-80% (basal area 30-50 m^2/ha), with occasional gaps to encourage flowering. Under this level of shade, important humidity is retained, and competition from light-demanding dwarf-shrubs such as blaeberry *Vaccinium myrtillus* and heather *Calluna vulgaris* is reduced, providing an open, mossy field-layer favourable for the growth of twinflower.

Management should aim to create and maintain an intimate patchwork of moderately shady areas of woodland of both sufficient size (0.25-5 ha) and frequency that they support the local spread of twinflower within and around existing sites and provide opportunities for colonization of new areas following long-distance dispersal of seed.

Browsing and disturbance levels are equally important considerations. While prolonged heavy grazing is ultimately damaging, a moderate level of browsing (by deer or livestock) can be beneficial for controlling dwarf-shrubs such as heather and blaeberry which can exclude twinflower, especially in situations where the woodland canopy is more open. Total absence of browsing animals is not beneficial and therefore the use of exclosures is not recommended.

Twinflower also requires open micro-sites (bare ground) to establish successfully from seed. At sites where natural disturbance levels are currently low (e.g. as a result of low numbers of large herbivores), intervention will be required to ensure the periodic provision of small patches of bare ground.

Ground disturbance created during thinning and timber extraction activities is one potential source of bare ground. Reinstatement of practices such as seasonal woodland cattle grazing (where appropriate) could help to address constraints imposed by unfavourable field-layer conditions and limited ground disturbance. Alternatives include brush-cutting and scarifying.

8.3. Site specific management considerations for twinflower restoration

Sites where twinflower occurs can be split into three broad habitat/management types: (i) semi-natural woodland managed for conservation/restoration of the Caledonian forest habitat; (ii) native Scots pine plantations managed for timber production; (iii) relict sites in open heathland situations. The first two are places where translocations could be established, the latter less so.

8.3.1. Semi-natural Caledonian forest managed for conservation

In general, these sites are managed to promote natural regeneration and expansion of the Caledonian forest habitat by reducing browsing pressure from deer. The widespread recruitment of new generations of trees resulting from this management will, over time, create future woodland with a variable age structure, stem density and light regime, including moderately shady areas suitable for twinflower (Fig. 13).

Suitable habitat is therefore likely to become increasingly available for twinflower at these sites in the future. It may, however, take three to four decades before this new woodland becomes favourable for natural colonisation by twinflower (i.e. once tree regeneration has developed into the pole stage and started to self-thin).

Figure 13. Natural regeneration of Scots pine in Glen Feshie following reduction in browsing pressure.

Sub-optimal field-layer conditions in many existing open, mature Caledonian forest remnants pose a further constraint on locations where twinflower can be established at present (and an equal threat to the survival of relict populations persisting in these situations). This is due to the widespread dominance of luxuriant dwarf-shrubs in the field-layer, and a deep underlying moss layer, which have developed in response to the significant reduction in deer numbers and associated browsing and disturbance.

Experience suggests that mid-aged Scots pine plantations (40-70 years old), or similar stands resulting from natural regeneration, that occur within the existing semi-natural woodland matrix, are more likely to contain areas with sufficient canopy shade and short, open field-layer conditions favourable for twinflower at this point in time.

Establishing seeding populations of twinflower in currently suitable mid-aged plantation stands, which lie adjacent to areas where woodland is naturally regenerating, is a good strategy to ensure that twinflower is best placed to take advantage of this new habitat in the future. Locations such as this should be considered to be a high priority for twinflower restoration and should be managed to enhance their long-term suitability by:

i) Maintaining patches of moderately shady woodland (as described in SECTION 8.2 above) around twinflower sites and across the wider surrounding woodland, subjected to periodic light canopy thinning only (where required), for the lifetime of the current stand of canopy trees.

ii) Increase age class diversity across uniform stands by felling (or heavy thinning) an intimate patchwork of small coups through time to encourage recruitment of new generations of young trees – thereby ensuring the provision of areas of shady woodland habitat suitable for colonisation by twinflower in the future.

8.3.2. Scots pine plantations managed for timber production

As well as semi-natural woodland, twinflower is often associated with native Scots pine plantations which have a long history of management for timber production. Commercial woodlands could therefore play a key role in providing sites for twinflower restoration, both now and in the future. However, some specific management considerations in relation to thinning treatments and age class diversity will be required to optimise their suitability.

Twinflower populations rise and fall in response to cycles of thinning, felling and reestablishment of the woodland canopy (as illustrated in Figure 14), and can be pushed to extinction at the extremes – i.e. when subjected to too much or too little canopy shade, or too much competition from overtopping dwarf-shrubs.

Extensive, single age-class plantation stands, subjected to uniform thinning treatments, will therefore either be largely suitable or largely unsuitable for twinflower at any given point in time. Moving away from large-scale uniformity in age-class and thinning treatments is a good way to ensure that some suitable shady woodland habitat is always available somewhere in the plantation for twinflower and other specialist pinewood plants at any given time.

The following is recommended as a potential way forward for twinflower in plantation sites:

i) To prolong the suitability of woodland areas that currently support twinflower, or could potentially support twinflower following reintroduction, the canopy should be managed to maintain a moderate level of canopy shade (60-80% canopy cover, basal area 30-50 m^2/ha) for as long as possible. In practice this may mean periodic light thinning for the lifetime of the stand. In addition, there should be other coups (in the order of 0.25-5 ha in size) with a similar light regime across the wider woodland, at least some of them within 200 m of seeding twinflower populations.

ii) To ensure the continued provision of suitable areas for twinflower to disperse into and colonise through time, an intimate patchwork of different age classes needs to be present across the woodland. In practice this may mean breaking up large sub-compartments into a series of smaller ones (in the order of 5 ha in size), with adjacent coups being at least 30 years apart in age.

While clearly not desirable or practical to manage all woodlands, or indeed entire woodlands, in this way, targeting particular stands which currently support twinflower patches suitable for reinforcement and/or identifying new areas where stand conditions are currently suitable for twinflower reintroductions (and perhaps where efficiency of timber production may be less compromised), and using these as focal points may be a more realistic goal.

1. Initial establishment – heavy shade during thicket stage of tree establishment will largely exclude twinflower, pushing it out to the edges of tracks, clearings or gaps where light levels are more favourable.

2. Young to mid-age – as the stand develops into the pole-stage and the first and second thinnings are undertaken, the light regime becomes highly suitable for twinflower and it can spread back in from the edges, growing rapidly in the absence of competition from other more light-demanding dwarf-shrubs.

3. Mid-age to mature – as the stand ages it is subjected to further often heavier thinnings, twinflower responds to increased light levels (growing more vigorously, often with a higher incidence of flowering). At the same time, light demanding dwarf-shrubs establish and gradually become dominant in the field-layer, outcompeting twinflower which begins slowly to decline during this open phase of the woodland (when canopy cover is <50%).

4. Final felling – the final seed-tree felling, or clearfell, opening up the canopy completely, can expose already declining twinflower populations to further extremes of temperature and dryness and/or increased competition from overtopping dwarf-shrubs in the field-layer. Some patches may survive this stage, others may not.

Figure 14. Key stages of a Scots pine plantation and the impact upon the growth and survival of twinflower.

8.3.3. Relict sites in open heathland

These sites are unlikely to be suitable for translocations due to the sub-optimal nature of habitat conditions – twinflower very much persisting rather than thriving in these locations (Fig. 15). However, there is much value in ensuring the continued survival of twinflower at these sites in order to preserve the genetic diversity present within these populations and, where appropriate, to provide a source of material to be used in translocations at other sites.

At the very least, it is essential that patches in open heathland are protected from muirburn. Since twinflower has no underground structures (e.g. rhizomes) from which to regenerate, an intense fire removing all aboveground growth can be very damaging.

Figure 15. Twinflower can persist in open heathland situations on north facing slopes but these sites must be protected from muirburn.

A moderate level of browsing by deer or livestock can be beneficial, maintaining a degree of openness amongst the dwarf-shrubs for twinflower to persist (though prolonged heavy grazing is damaging and should be avoided).

Restoration of a native woodland canopy over the site (where appropriate) would be a valuable long-term management objective. Ideally this would be achieved via natural regeneration from existing seed sources. Care is required if trees are to be planted and/or fencing used to exclude browsing animals. Plant trees at wider spacing and/or leave some small gaps over areas currently occupied by twinflower to ensure that some can survive the thicket stage of development. Fence areas for as short a time as possible and consider some interim field-layer management, e.g. brush-cutting, if there is a risk that twinflower may be excluded altogether by overtopping dwarf-shrubs.

9. Translocation guidance, permissions and licencing

Before undertaking a translocation programme, you are advised to read The Scottish Code for Conservation Translocations.

The sequence of events should then be:

i) Making full use of information on twinflower resources that are found on the Twinflower Restoration portal (https://andrewrfr365.shinyapps.io/Latest/), decide on the nature of your proposed translocation programme (*reinforcement* or *reintroduction*) and identify potential donor and release sites using the guidance provided earlier in this publication.

ii) Obtain permission from the landowner(s) of the release and donor site(s) for the proposed translocation(s).

iii) Where release and/or donor locations fall within designated sites, consult local NatureScot Operations staff. Consent should be applied for by the landowner (or third party on behalf of landowner) to plant at release sites and/or collect from donor patches (where identified as an Operation Requiring Consent).

iv) Fill out a Scottish Code for Conservation Translocations – Translocation Project Form.

   You will need to include full details of the proposed release site(s) together with a list of provisional donor sites.

   To help with filling in the translocation form, a partially completed example for a reinforcement programme and associated data sheet are provided in Appendices 1 and 2. These relate to translocations carried out by Andy Scobie in the Cairngorms Rare Plants Project and indicate the level of detail required in completing the form.

v) Submit your Project Proposal form to the NatureScot Licensing Team at:

   NatureScot, Great Glen House, Leachkin Road, Inverness IV3 8NW.

   email: licensing@nature.scot

The NatureScot Licencing Team will inform you as to whether the translocation programme can proceed and will issue a licence if this is necessary.

10. Co-ordinating restoration of twinflower across Scotland

Twinflower restoration is likely to be most successful if the extensive database of extant clones and *ex situ* collections can be readily accessed by interested groups, and the efforts made by these different groups are co-ordinated. Collation of the data on establishment and monitoring of restoration sites will provide an overview of the scale and geographic range of restoration efforts and can be used to avoid duplication and encourage optimal placing of restoration sites. Analysis of centralised records of monitoring results will also facilitate modification and refinement of restoration guidance based on practical field experience.

To obtain these multiple benefits the dedicated Twinflower Restoration portal (https://andrewrfr365.shinyapps.io/Latest/) has been created. Here, existing information on the *in situ* and *ex situ* twinflower resource in Scotland can be found. The site is also set up to accept data from translocation programmes that includes initial documentation of *reinforcement* and *reintroduction* sites, and the later results from monitoring of these sites. All those undertaking a programme of twinflower restoration are strongly encouraged to deposit the documentation of their translocations and their monitoring results in the dedicated Twinflower Restoration portal to ensure that the maximum benefit is gained from their efforts within the entire twinflower restoration community.

11. Contacts for advice on twinflower restoration

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C. APPENDICES

Appendix 1. Example SNH Translocation Project Form for a translocation programme involving reinforcement

Appendix 2. Example Excel spreadsheet for documenting a translocation programme involving reinforcement

Glossary of terms – (mostly as defined in The Scottish Code for Conservation Translocations https://www.nature.scot/scottish-code-conservation-translocations)

Adaptation: changes in the genetic composition, behaviours /growth responses of individuals or populations that make them more suited to a particular set of environmental conditions

Conservation translocation: the intentional movement and release of a living organism where the primary objective is a conservation benefit

Donor site: the place where translocated organisms are taken from

Ex situ: individuals or populations housed away from their natural habitat (e.g. in zoos, botanic gardens, seedbanks, cryopreservation)

Native range: refers to the part of the natural range where a species or other taxonomic entity currently naturally occurs (e.g. the places it has reached without movement by humans and still occurs at)

Natural range: refers to the natural past or present distribution of a species or other taxonomic entity (e.g. the places it has reached without movement by humans); natural range includes all locations where a species is indigenous. Natural population that is now extinct. These populations form part of the natural range but not the native range

Release site: the place in which translocated organisms are released