Guidelines for Seed Collection and Stratification of Common Juniper (*Juniperus communis* L.)

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Abstract

Common juniper (*Juniperus communis* L.) is the most widely distributed conifer in the Northern Hemisphere. In the United Kingdom, juniper is one of only three native conifer species. Juniper populations are declining, however, particularly in southern England. In some cases, nursery production is seen as a means of boosting these populations. This article, which provides practical guidelines for collecting and processing berries and for stratifying the seeds, is intended for nursery managers, conservation practitioners, and related professionals who are concerned with propagating, restoring, and managing juniper ecosystems.

Introduction

Common juniper (*Juniperus communis* L.) is the most widely distributed conifer in the Northern Hemisphere, occurring in North America, Europe, Asia, and parts of North Africa (Thomas and others 2007). It is sometimes split into several subspecies (Eckenwalder 2009) or varieties (Farjon 1998). In the United Kingdom, three subspecies exist, viz. *J. communis* L. subsp. *nana* (J. & C. Presl) Nyman, *J. communis* L. subsp. *communis,* and *J. communis* subsp. *hemisphaerica* (J. & C. Presl) Nyman (BSBI 2012). This article refers to *J. communis* L. subsp. *communis*, which is widely distributed in the United Kingdom, although the populations are declining, particularly in southern England (Dearnley and Duckett 1999, Long and Williams 2007, Thomas and others 2007, Ward 1973). The decline is largely due to the lack of natural regeneration, which is attributed to poor seed quality, seed predation, and a shortage of suitable habitat conditions for germination and seedling establishment (Verheyen and others 2009, Ward 2010). Therefore, juniper is a United Kingdom Biodiversity Action Plan priority species (UK Biodiversity Reporting and Information Group 2007). Because of this priority, juniper is the focus of several *in situ* and *ex situ* conservation efforts. In some cases, nursery production is seen as a means of sustaining struggling juniper populations. Propagation from seeds, however, is neither simple nor straightforward. Like many other trees, juniper produces a large proportion of empty seeds. Empty seed production has a negative effect on the cost and efficiency of nursery production. Therefore, the overall aim of this article is to provide practical guidelines for propagating common juniper (*Juniperus communis* L. subsp. *communis*) from seeds.

Berry and Seed Quality Before Berry Collection

Testing berry and seed quality is essential for determining whether to collect berries and, also, how many to collect. The cut test is a crude, destructive, but quick means for determining berry and seed quality.

1. Check the berries for signs of seed predation. Parasitized berries usually can be readily distinguished from healthy berries. In the United Kingdom, the two main seed predators are eriophyid mites (*Trisetacus quadrisetus*) and juniper seed chalcids (*Megastigmus bipunctata*), the presence of which results in characteristic exit holes in the berries (figures 1a and 1b). In addition, eriophyid mites (figure 1c) cause fluted seed coats, which are visible when the berries are cut along the equator (figure 1d).
2. Select 10 plump purple berries per bush (figure 2a). Avoid green, wrinkly purple, or brown berries (figures 2b, 2c, and 2d). Remember that you can improve the accuracy of the cut test by increasing the sample size.
3. Cut the berries (at the equator) in half using a sharp penknife (preferably against a firm surface such as a notebook).
4. Count the seeds and assign them to either the filled or empty category based on a visual assessment of the cut seeds with regards to color, texture, and degree of embryo development in the following descriptions:
    a. Filled seeds contain a well-developed, firm, off-white (sometimes brownish) embryo and megagametophyte and, therefore, are scored as probably viable (figures 3a, 3b, and 3c).
    b. Empty seeds are entirely empty, contain shrivelled contents, or are embryo-less and, therefore, are scored as nonviable (figures 3a and 3d).

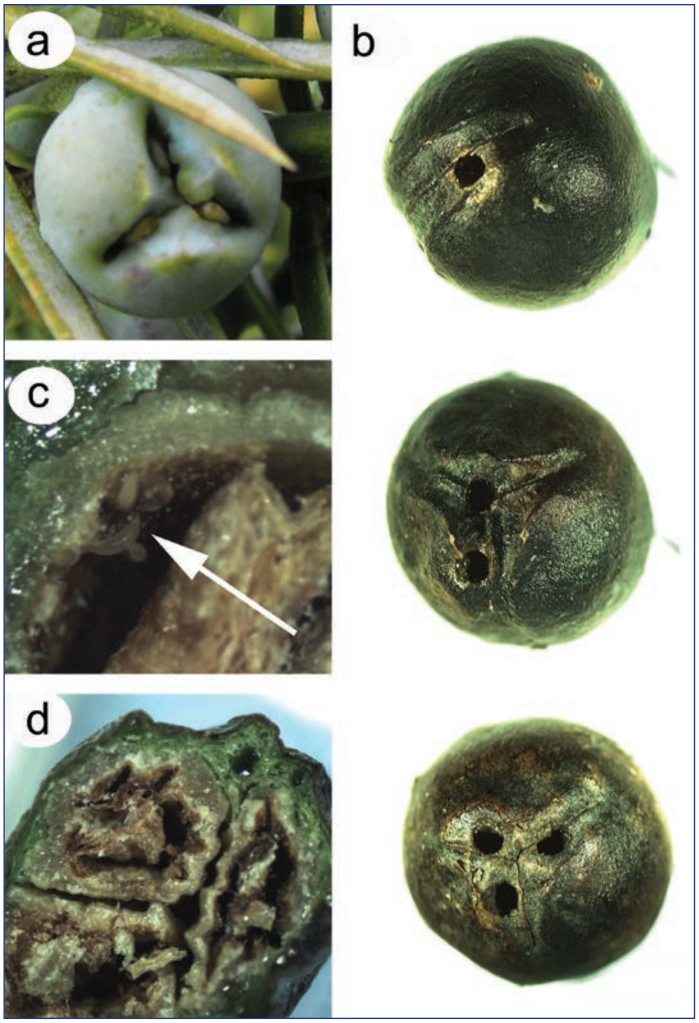

Figure 1. When collecting juniper berries, avoid parasitized berries with characteristic exit holes caused by (a) eriophyid mites or (b) juniper seed chalcids. The (c) eriophyid mites also cause (d) the seed coats to become fluted, which is visible when the berries are cut along the equator. (Photos by Shelagh McCartan, Forest Research, Forestry Commission, 2009)

A hand lens is sometimes useful. If less than 20 to 30 percent are filled seeds, then sample another bush. The age of the bushes also affects seed quality. In a small trial, we found that older bushes had only 4-percent filled seed compared with 70-percent filled seed in younger bushes (table 1). Using younger bushes may be particularly important for restoration management of juniper populations.

In addition, seed quality can vary significantly among populations and within the same population among years. In

Figure 2. When collecting, pick (a) plump purple berries and avoid (b) green, (c) wrinkly purple, and (d) brown berries, because using plump purple berries reduces the amount of processing and improves the seed-lot quality. (Photo by George Gate, Forest Research, Forestry Commission, 2009).

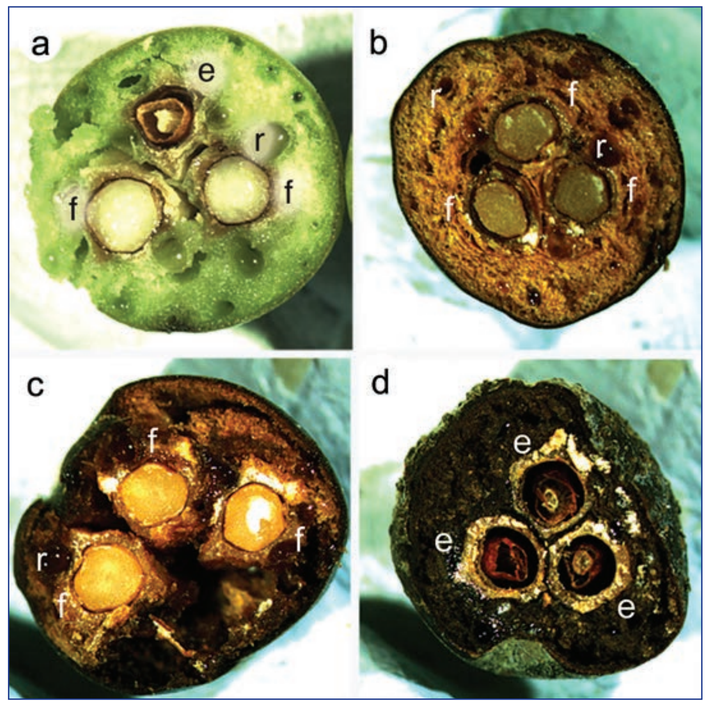

Figure 3. When determining seed quality, cut the berries along the equator and assess whether seeds are filled (see f in a, b, and c) or empty (see e in a and d). Also note the resin vesicles (r), which appear (a) green in immature berries and (b and c) amber in mature berries. (Photos by Shelagh McCartan, Forest Research, Forestry Commission, 2009 and 2012)

Table 1. Cut test results for plump purple berries collected from two populations in Aston Rowant, England. The young bushes were originally cuttings taken from the old bushes on an adjacent site. (Data source: OS grid reference SU7299, collected 2011)

Population	Total number of seeds*	Number of empty seeds	Number of filled seeds
Young (~10 years)	116	35	81
Old (~75 or more years)	106	102	4

OS = Ordnance Survey.
*Total number of seeds extracted from 40 berries.

2008, we found that berries from Harkerside Moor had only a 4-percent filled seed compared with 100 percent in Moughton Scarr, although both populations are found in the Yorkshire Dales National Park (table 2). Yet, in 2009, berries from Harkerside Moor had a 63-percent filled seed. Therefore, if seed quality is poor in a particular population, try again the following year.

Berry Collection

Collecting juniper berries is a labor-intensive process. The key to successful berry collection is timing; too early and the berries are not ripe, but too late and the birds will have eaten them. The optimum time in the United Kingdom is usually between late September and late October.

1. Get permission from the landowner.
2. Get outfitted properly using the following protective gear.
    a. Stout walking or Wellington boots appropriate for slippery or uneven terrain.
    b. Waterproof or thick trousers for going through overgrown bramble (*Rubus fruticosus* L.), bracken (*Pteridium aquilinum* [L.] Kuhn), and sloe (*Prunus spinosa* L.).
    c. Snug-fitting gloves (for instance, disposable latex gloves) to protect your hands from the prickly foliage.
3. Do a cut-test (described previously) on a small sample of berries to determine whether to collect the berries. If less than 20 to 30 percent are filled seeds or have signs of seed predation, then try another bush. Do not waste time collecting berries if the bushes show signs of heavy seed predation.
4. Collect plump purple berries (figure 2a) in heavy-duty polythene bags (to prevent rips from the prickly foliage). Juniper populations have 2- and 3-year reproductive cycles; berries appear to mature more rapidly in warmer climates than cooler ones (Ward 2010). Therefore, female cones and berries of two different generations usually occur simultaneously on a bush. We found that different color berries had different proportions of filled and empty seeds, ranging from 4-percent filled seed in brown berries to 77-percent in plump purple berries (table 3). So avoid green berries (figure 2b), which are still immature. Also avoid wrinkly purple (figure 2c) or brown berries (figure 2d), which often contain empty seeds. This strategy reduces the amount of processing and improves the overall quality of the seed lot.
5. Store berries in a loosely tied polythene bag (to allow for ventilation) in a refrigerator until required for processing. Remember to label the bag with collection details (collector's name, site location, and date).

One person can harvest about 7 oz (200 g) of berries in 1 hour from a good crop, possibly even more, depending on access to the bushes. It can take two people between 3 and 4 hours to collect a similar amount from a poor crop. In general, collect berries from at least 20 bushes (and also different growth forms) to maintain the genetic diversity of the population (Broome 2003). Also remember to leave some berries on the bushes for the birds.

Table 2. Cut test results for plump purple berries collected from three populations within the Yorkshire Dales National Park, England. (Data source: OS grid references SE01199818, SD79317135, and SD89299893, collected 2008)

Population (OS grid reference)	Total number of seeds*	Number of empty seeds	Number of filled seeds
Harkerside Moor (SE01199818)	28	27	1
Moughton Scarr (SD79317135)	21	0	21
Thwaitestones (SD89299893)	30	7	23

OS = Ordnance Survey.
*Total number of seeds extracted from 10 berries except for Moughton Scarr, where only 9 berries were used.

Table 3. Cut test results for different color berries collected from a population at Thwaitestones, England. The berries were collected on the same day from several bushes. (Data source: OS grid reference SD89299893, collected 2008)

Berry color	Total number of seeds*	Number of empty seeds	Number of filled seeds
Green (figure 2b)	29	17	12
Plump purple (figure 2a)	30	7	23
Wrinkly purple (figure 2c)	29	24	5
Brown (figure 2d)	28	28	1

OS = Ordnance Survey.
*Total number of seeds extracted from 10 berries.

Processing Berries

Processing berries is a sticky but worthwhile process, which removes potential chemical inhibitors, and thereby improves germination (Broome 2003). The process described in the following seven steps is suitable for small batches of berries (about 7 oz or 200 g):

1. Soak berries in water for 2 or more hours (to soften the flesh).
2. Macerate berries in a domestic blender (figure 4a). Use one or two short pulses of about 5 seconds on the lowest setting. Ensure that berries are just covered with water.
3. Screen pulp through stackable sieves with decreasing mesh sizes (for instance, 0.25 in [6.30 mm], 0.13 in [3.35 mm], and 0.09 in [2.24 mm]) under running water (figure 4b). This step is important for removing small pieces of pulp and, therefore, reduces time spent on step 4. If a large number of intact berries remain, repeat steps 2 and 3. Do not put naked seeds in the blender, because the blades will damage them.
4. Float off remaining pulp and empty seeds under running water. Use a large beaker or bucket (about 5.3 qts or 5.0 L) to allow sufficient depth for the filled and empty seeds to separate (figure 4c). Adjusting the flow rate is an art, so it is recommended to trap waste in a hand-held sieve in case of a mishap. As an alternative method, fill the beaker with water, stir the pulp vigorously, wait a few seconds to allow the filled seeds to settle (or sink), and then scoop out the floating waste with a tea strainer.
5. Spread filled seeds thinly on a tray and air-dry overnight. If you have a sufficient number of seeds, do a cut test (described previously) on a small sample of the 'sinkers' to confirm that the seeds are filled. A small proportion of empty seeds have very thick seed coats, which makes separation impossible.

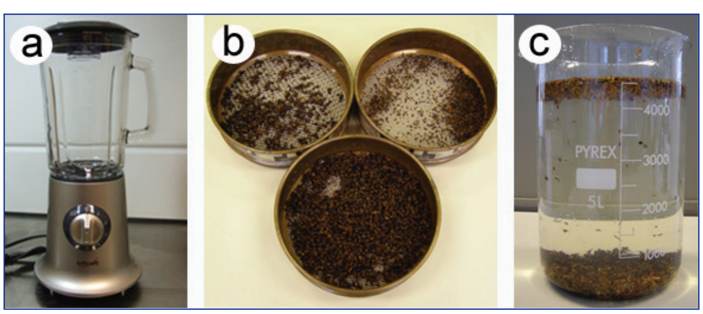

Figure 4. When processing, (a) macerate the berries in a domestic blender, (b) wash the pulp through a series of stackable sieves with decreasing mesh sizes, and then (c) float off the empty seeds in a large beaker. (Photos by Shelagh McCartan, Forest Research, Forestry Commission, 2008 and 2009)

6. Store the seeds in a loosely tied polythene bag (to allow for ventilation) in a refrigerator until required for propagation (up to 4 weeks). Do not store seeds for too long because they may deteriorate at high moisture content. For long-term storage, air-dry the seeds to low moisture content (about 10 to 15 percent fresh weight basis) and then store in an airtight, 500-gauge polythene bag in a refrigerator (39.2 °F [4.0 °C]) until needed.
7. Clean sticky hands, glassware, and sieves with methylated spirits.

This process is very efficient at separating filled and empty seeds. However, you can modify the steps to fit your needs. Remember that a trade-off exists between efficiency (time and effort of processing) and gain (proportion of empty seeds removed from seed lot). For production purposes, note that seed size varies among populations, which influences the number of filled seeds per gram. We found that this seed count ranged from about 89 seeds per gram for Dalcataig to 124 for Harting Down (table 4).

Seed Stratification and Germination

Juniper seeds are dormant and require stratification to germinate (Johnsen and Alexander 1974). Stratification of deeply dormant seeds such as juniper often requires alternating warm and cold periods as described in the following steps.

1. Sow seeds in trays containing moistened potting medium (peat: grit [1:1 v/v]) and lightly cover with the same mixture. Do not sow seeds too densely because it can make pricking out the seedlings difficult.
2. Place trays in loosely tied polythene bags (to reduce water loss but allow for ventilation).
3. Transfer the trays to an incubator set at an alternating 50/59 °F (10/15 °C) (12/12 hr) or leave in a room with a similar temperature range for 2 weeks or more. We found that extending this warm phase enables a few more seeds to germinate but can delay seedling emergence by an equivalent length of time (figure 5).

Table 4. Average number of filled seeds per gram (0.04 oz)* for four populations in the United Kingdom. (Data source: OS grid references SU803185, NT229649, SE01199818, and NH367143, collected 2009)

Population (OS grid reference)	Average number of seeds per gram (0.04 oz)
Harting Down, England (SU803185)	124 ± 4
Pentlands, Scotland (NT229649)	106 ± 3
Harkerside Moor, England (SE01199818)	96 ± 3
Dalcataig, Scotland (NH367143)	89 ± 4

OS = Ordnance Survey.
*This calculation was based on the weight of 100 seeds (N = 8 for each population).

4. Transfer trays to a refrigerator (39.2 °F [4.0 °C]). After 18 to 20 weeks in the cold phase, the seedlings start emerging readily at temperatures between 39.2 and 59.0 °F (4.0 and 15.0 °C). Some seeds will not germinate even when they are filled.

If germination is good, then few benefits remain to repeating steps 3 and 4 because hardly any additional seeds will germinate in the next cycle. Significant differences may exist between populations, largely because of genotype and environmental factors (Tylkowski 2009). In some cases, seeds may require two or more growing seasons to break dormancy, particularly when they are grown under nursery or field conditions (Broome 2003, Tylkowski 2009). If germination is poor, then it may be quicker and more efficient to simply collect and process more berries the following year.

Pricking Out Seedlings

Even after stratification, seedlings emerge slowly and erratically over several weeks. Therefore, it is critical to check progress regularly.

1. When the cotyledons start unfolding, carefully lift the seedlings using a table fork or similar implement. Then, using a dibber, transplant them into plug-trays containing potting mixture (peat: grit [1:1 v/v]). Transplant sooner rather than later to minimize damage to the roots.
2. Place plug-trays in a mist-bed for a few weeks. Gradually reduce the humidity as the seedlings harden off.

For further information on reintroducing juniper seedlings to the natural habitat, see Wilkins and Duckworth (2011).

Conclusions

Common juniper has a long and complicated reproductive life cycle spanning 2 or 3 years. Usually female cones and berries of two or more generations occur simultaneously on a bush. Therefore, it is critical to pick only mature (plump purple) berries and to avoid immature (green) or unproductive (wrinkly purple or brown) berries. Cautious berry picking improves the overall quality of the seed lot and reduces the time and effort spent on processing. Unlike propagating many other conifers, processing junipers is complicated; extracting juniper seeds involves macerating, sieving, and floating berry pulp to separate filled and empty seeds. This extraction is a sticky, time-consuming but worthwhile process. Processing offers the following benefits:

- A higher proportion of filled seeds due to the removal of empty seeds.
- Faster germination of seeds due to the removal of chemical inhibitors in the berries.

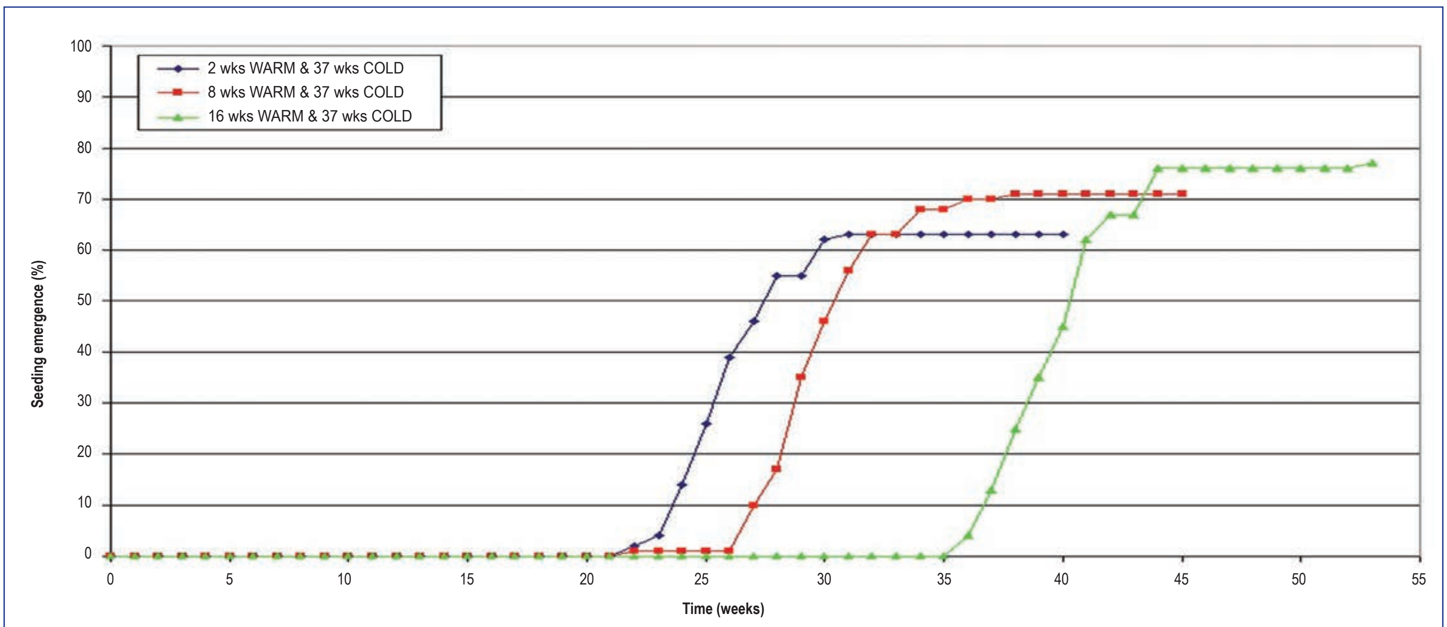

Figure 5. Seedling emergence of juniper after various lengths of warm (alternating 50/59 °F or 10/15 °C [12:12 hr]): cold (39.2 °F or 4.0 °C) stratification (seeds collected from Thwaitestones, England). Note: The seedlings readily emerged at 39.2 °F (4.0 °C). (Data source: SD89299893, collected 2008)

In addition, juniper seeds require stratification to break dormancy. Stratifying the seeds serves two purposes: (1) the warm phase allows the embryo to mature and/or inhibitors to leach out of the seed coat, and (2) the cold phase breaks dormancy (Johnsen and Alexander 1974, Pack 1921, Thomas and others 2007). Stratifying seeds offers the following benefits:

- A higher germination percentage.
- Faster and more uniform germination of seeds due to dormancy breakage.

We hope these guidelines are a significant step toward cost-effective propagation and *ex situ* conservation of common juniper.

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