Seed viability analysis of a mature *Juniperus communis* Juniper population in the Chiltern Hills

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Summary

Great Britain has only three native conifers and one of these, *Juniperus communis*, is in serious decline. Understanding what has led to this decline is paramount. We examined seed viability in a mature stand of *Juniperus communis* ssp. *communis* on a steeply sloped mature chalk grassland SSSI site in the Chiltern Hills in Oxfordshire, England. The population is suffering from failed natural regeneration with the stand now of mature and even age. This study examined whether the lack of natural regeneration could be due to the mature Juniper stands having poor seed viability (<10%). The number of seeds per cone, seed damage and seed viability were assessed for 50 randomly selected cones from 50 individual bushes. The findings indicated the number of seeds per cone were low compared to that expected within a typical mixed age stand, based on previous studies. There was no evidence to suggest damage from insects had influenced the number of seeds and the level of seed viability was considered sufficient to support natural regeneration. These results indicate that in this population the seed viability level may not be the cause for failed regeneration, thus adding to the body of evidence which suggests that more conservation management and monitoring programmes in England to support seed germination are required to ensure the survival of *Juniperus communis*.

Introduction

J. communis is a dioecious, wind-pollinated gymnosperm (Jacquemart *et al.* 2021). Juniper is noted within the Natural Environment and Rural Communities (NERC) Act 2006, Section 41 (Species of principle importance in England) and without management intervention *J. communis* is predicted to become completely extinct across lowland England within fifty years (Wilkins and Duckworth 2011). In England the status of *J. communis* on the BSBI red list is Near Threatened (NR) and the species has suffered a 20% decline in Area of Occupancy (AOO) and a 24% decline in Extent of Occurrence (EOO) (Stroh *et al.* 2014).

The species has already been lost across nine lowland vice-counties including Bedfordshire, North Devon, North Somerset, North Essex and South Essex. Within VC23, *J. communis* is considered scarce. There are twelve sites recorded within Oxfordshire since the year 2000 where Juniper is present, ten within VC23 and two within VC22 (Erskine *et al.* 2018). A number of site extinctions have already been noted in Oxfordshire (Wilkins and Duckworth 2011). There are four sites recorded prior to the year 2000 which upon checking were not re-found, corresponding to two site loses in VC22 and two in VC23 (Erskine *et al*. 2018).

Many of the issues associated with management of *J. communis* stem from the unusual traits of the plant. Unlike many other conifers they are compact and stout with permanent juvenile foliage (adult foliage is not found in this species), they possess fleshy fruit-like scaled cones, and of particular note, they appear to be a pioneer species requiring mostly bare ground for germination (Ward and Shellswell 2017). Southern England populations, which grow primarily on chalk such as the Chiltern Hills, are contracting and the extent to which these unusual traits or others are contributing to this decline is unknown.

A range of contextual factors are considered relevant to the decline, including the influence of habitat loss and lack of appropriate land management. Additional factors may also include inbreeding depression, population sex ratio shifts and genetic variation issues (Merwe *et al.* 2000). These factors could provide focus for future research efforts. Juniper genetic variation has been studied specifically including three sites on the Chilterns and the level of variation was found to be very high within the studied populations at Bacombe Hill, Shirburn Hill and Bald Hill. The site at Bacombe Hill, Buckinghamshire, was found to be the only site studied that contained two distinct Juniper migrant groups which are believed to have existed side by side without gene exchange for generations (Merwe *et al*. 2000). It remains, however, that many populations on the Chilterns comprise aging stands that lack recruitment. This may indicate that such stands produce non-viable seeds and/or specific conditions are not being provided to support germination success.

In this study we sought to identify the seed viability rate of mature populations. Understanding this has ramifications for the effort and strategy required to manage and propagate the species. This current study examined the seed viability for a mature population of *J. communis* in Oxfordshire, which has not successfully naturally re-generated within the last 30 years. If the stands are not producing viable seeds, then strategies encouraging natural regeneration from that seed become less relevant. We hypothesised that lack of regeneration might be related to the degree of seed viability, if the seed viability is less than 10% (Wilkins and Duckworth, 2011). This would suggest additional propagation programmes are an essential requirement as part of conservation management for this particular population. If seed viability is over the 10% threshold, then the seed quality within the population may be considered acceptable for regeneration, and other management strategies such as turf scrapes to create bare ground for germination *in situ* may be preferred.

Methodology

Site Information

The research site is a SSSI under private ownership in Southern Oxfordshire, England, situated on the chalk at the South West end of the Chiltern Hills. The site is comprised primarily of steep chalk slopes which occupy the sides of a winding natural coombe, with extremely dry soil. Until recently, the site has received little management for decades. However, low numbers of rabbits and deer help to maintain a reasonably botanically diverse sward. The site currently supports a population of around 200 *J. communis* shrubs, all at a mature lifecycle phase.

Cone selection process and storage

At the research site seed collection for conservation takes place under licence and as part of this process a sample of 50 female cones was obtained. The sample area for the cones was approximately half of the total site area and contained a total of around 100 *J. communis* bushes which included male and female plants. The male and female bushes were well mixed and distributed within the area and were present in a sex ratio of around 1:1, which was also representative for the whole site. From the sample area, several hundred cones were collected via brushing foliage by hand with gloves into a communal collection tray. Invertebrates and debris were removed by hand. Individual containers of fifty cones each were randomly filled from the collection tray by dipping into the communally mixed cones. A random one of these containers was available for this study. The cone sizes were therefore representative of the entire cone collection.

All the bushes were in the mature age phase (over 40 years old). The cones collected were any previous year cones of a blue/black colour that were visibly available and which fell upon brushing. Any new current year (green) cones were excluded. Collection was undertaken in November 2021 and cones stored in a standard refrigerator at +4ºC. Stored in this manner, the seeds deteriorate only very slowly over a period of years (Wilkins and Duckworth, 2011). Cones collected were variable in size ranging from 6mm to 11mm in diameter (Figure 1).

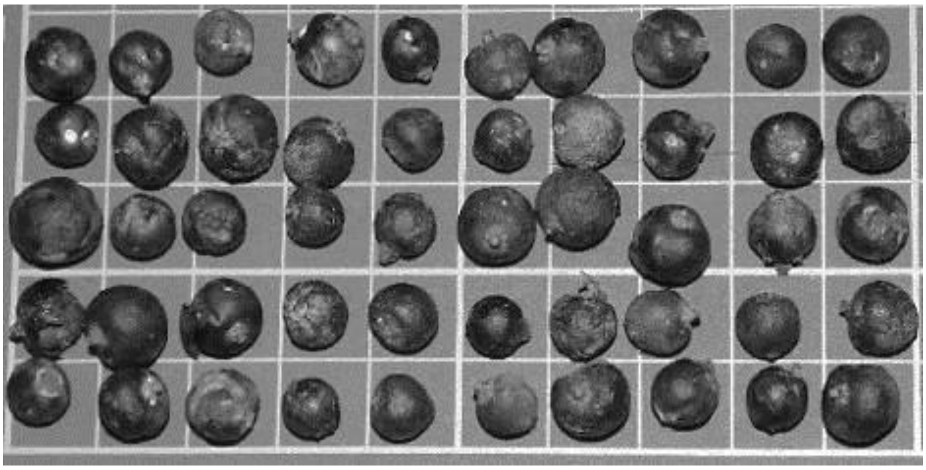

Figure 1. Collected Juniperus *communis* cones placed on a 10mm x 10mm grid for scale

Seed viability assessment

Before the seeds were tested for viability they needed to be removed from the cones. To access the seeds the outer fleshy cones scales were removed with a scalpel. Nitrile gloves were worn as the cones are particularly resinous and sticky to touch. Using the scalpel, a longitudinal incision was made to expose the seeds (Figure 2a). The seeds were then washed and wiped clean to remove any pithy material. Seeds were tallied at this point and assessed for any damage from disease or parasite infestation (Green et al. 2012, Ward and Shellswell 2017). Two measures of seed viability were compared: the non-destructive float-test, and the destructive cut-test.

The float-test involved placing the seeds in a container of water and observing after 30 minutes whether they float or sink. Viable seeds will have developed embryonic components making the seed heavier than seeds where development has failed. Each seed was placed one by one into the water container. The cut-test requires opening the seed to examine the intactness of the embryonic tissue. For the cut-test the protocol by Wilkins and Duckworth (2011) was followed, except that, rather than an equilateral cut across the entire cone, each seed was removed, cleaned and separately cut to ensure consistency in the position of seed cutting. This involved cutting each seed at the point at which the seed begins to slope to the tip (Figure 2 b,c).

Figure 2. Extraction and dissection of *Juniperus communis* seeds

a. Cone cut in half exposing three seeds	b. Cut seed viable (filled)	c. Cut seed non-viable (not filled)

Results

Seed viability varied according to the method of assessment. On first inspection none of the seeds appeared to have been eaten by insects or mites or were diseased; this was confirmed when the seeds were opened. Out of 50 cones, 43 (86%) contained three seeds and seven contained two seeds only (14%).

Seed viability determined by the float method suggested that just over half of the seeds were viable (Figure 3). This was more than double the number of viable seeds predicted by the cut method, where less than a quarter of the *J. communis* seeds were actually filled with embryonic tissue (Figure 3).

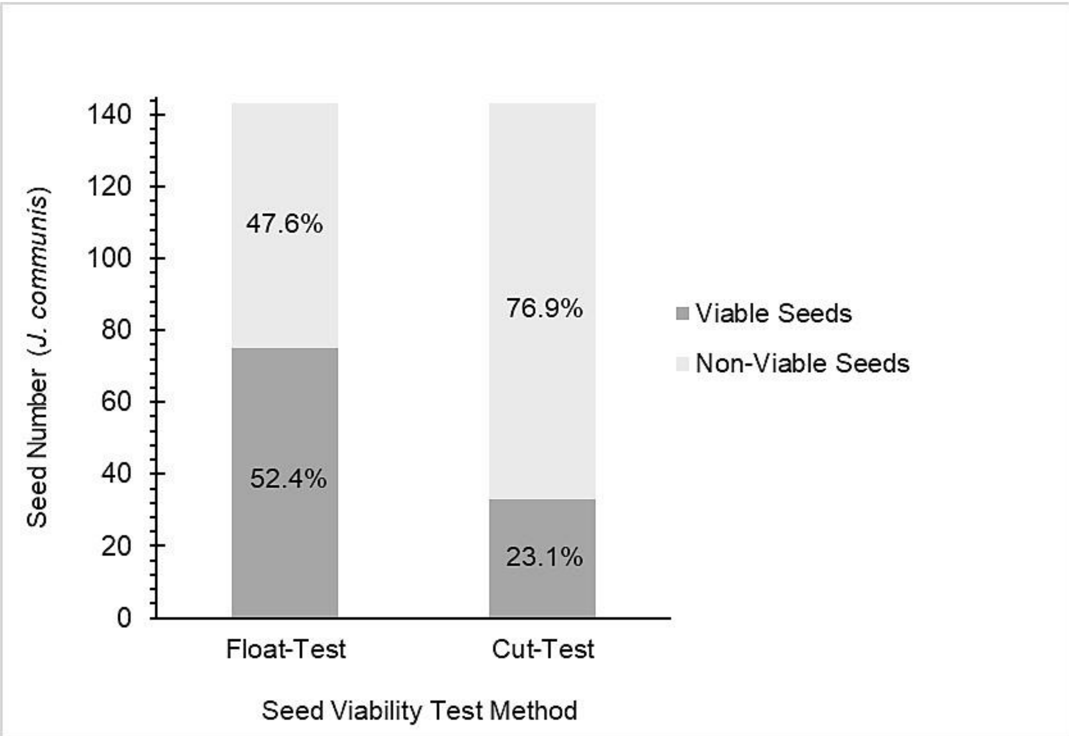

Figure 3. The proportion of viable (filled) and non-viable (unfilled) seeds of *J. communis* based on dissected seed samples via the seed cut-test

Using the cut-test 17 cones (34%) contained at least one viable seed, but only 7 cones (14%) had a full complement of seeds (corresponding to either two or three seeds depending on the total number of seeds in the cone) so that the average number of seeds where viable seeds were present was 1.94 per cone.

Discussion and Analysis

The percentage of viable seeds was above the recommended threshold (10%) suggesting that the lack of seed regeneration at this site is not likely to be related to seed viability. However, a study by Plantlife (2004) noted that the average number of seeds per cone is usually three but can range from one to five seeds and Broome (2003) indicated that *J. communis* cones normally have three to five seeds. No cone from this population had more than three seeds and over 10% of these only had two seeds. This could be related to the late maturity of the plants or indicate the plants are under stress such as lacking the nutritional support to bear more seeds. We are confident the lack of seed viability in the seeds present was not a direct consequence of damage by insects or mites.

In terms of assessing seed viability, the more destructive technique of cutting the seed was more accurate than using the float-test. The float-test relies on the assumption that only seeds that are filled with embryonic tissue will sink. Unfortunately, the float-test may be susceptible to errors due to variations in the seed surface thickness and moisture level, meaning some empty seeds may also sink. However, none of the viable seeds floated which means none of the viable seeds were missed. Thus, the float-test is a valid initial step to determining which seeds might be best to use for propagation with the caveat that not all of the sunk seeds will be viable. There is a further option of x-ray testing to determine whether embryonic tissue is present, however this requires access to specific resources and facilities. The mild x-ray doses utilised in this approach are known to not injure seeds (Simak, 1957).

Under the guidelines set by Wilkins and Duckworth (2011) the population would be designated as having ‘moderate’ natural regeneration capacity, suggesting that the focus should be on creating the conditions required to maximise germination and establishment at this site. Due to the range of factors that limit Juniper germination this may not be straightforward. Germination requires bare ground, minimal browsing, slow succession and medium to high light levels (Wilkins and Duckworth 2011). Other uncertainties include the suggestion that seeds are ‘deeply dormant’ and will only germinate after a sequence of seasonal temperature changes (Wilkins and Duckworth 2011). Drier and warmer conditions due to climate change are likely to impact self-propagation as higher temperatures and droughts can have a marked impact on recruitment success, as confirmed by trial plot observations on the Chilterns. Such shifts in climatic conditions may mean landscape level aspects become an increasing area of relevance for targeting conservation efforts.

The findings reported here refer only to *J. communis* ssp. *communis* Common Juniper which is predominantly present over sections of central Southern England (Thomas *et al.* 2007). It is not known if the other two subspecies *J. communis* ssp. *nana* Dwarf Juniper, which is found in more upland areas typically in Northern England and Scotland and *J. communis* ssp. *hemisphaerica*, a rare prostrate form found in Southern Cornwall (Ward and Shellswell 2017) differ in their germination traits.

Conclusions

The tested *J. communis* population seed viability rate has been found to be 23.1%, which places it over the 10% threshold. This indicates the seed viability within the population is acceptable to support natural regeneration if conservation interventions such as turf scrapes, grazing controls and regular monitoring and reporting are implemented.

Despite the encouraging findings, there is a caveat in that the visual assessment of the seed viability does not necessarily translate to germination success. Factors influencing germination success and seedling establishment are more likely to be core triggers of the decline of *J. communis* and may require further research. Anecdotal evidence for instance suggests the seeds need to have pre-treatment of acidic wash. Whether or not this is something achieved through exposure to particular soil conditions or requires a third party such as a bird is not clear. The potential ability of such a mature and seemingly dormant *J. communis* population to re-generate is encouraging, provided optimum conditions are created by appropriate long-term conservation management practices. In many respects these requirements reflect the necessary re-creation of post-glacial conditions, serving as a reminder of the pioneer nature of the species.

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References

Broome, A. 2003. *Growing Juniper: Propagation and Establishment Practices*, Forestry Commission, Edinburgh

Green, S., Hendry, S. J., MacAskill, G. A., Laue, B. E., Steele, H. 2012. Dieback and mortality of *Juniperus communis* in Britain associated with *Phytophthora austrocedrae*, *New Disease Reports*, 26: 2

Erskine, S. E., Killick, H. J., Lambrick, C. R., Lee, E. M. 2018. *Oxfordshire's threatened plants: A register of rare and scarce plants of the administrative county, and of vice-county 23*, Pisces Publications, Newbury

Jacquemart, A. L., Buyens, C., Delescaille, L.M., Van Rossum, F. 2021. Using genetic evaluation to guide conservation of remnant *Juniperus communis* (Cupressaceae) populations, *Plant Biology* 23: 193–204

Merwe, M. Van Der, Winfield M. O., Arnold G. M., Parker J. S. 2000. Spatial and Temporal Aspects of the Genetic Structure of Juniperus Communis Populations, *Molecular Ecology*, 9: 379–86.

Plantlife 2004. *Juniperus communis L. dossier*, Plantlife International

Simak, M. 1957. *The X-ray contrast method for seed testing: Scots Pine - Pinus sylvestris*.

Reports of the Swedish Institute of Experimental Forestry, 47: 1-22.

Stroh, P. A., Leach, S. J., August, T. A., Walker, K. J., Pearman, D. A., Rumsey, F. J., Harrower, C. A., Fay, M. F., Martin, J. P., Pankhurst, T., Preston, C. D., Taylor, I. 2014. *A Vascular Plant Red List for England*, BSBI, Bristol. Available via: https://bsbi.org/wp-content/uploads/dlm_uploads/England_Red_List_1.pdf

Thomas, P. A., El-Barghathi, M. and Polwart, A. 2007. Biological Flora of the British Isles: *Juniperus Communis* L., *Journal of Ecology*, 95: 1404–1440

Ward, L.K., Shellswell, C.H. 2017. *Looking after Juniper: Ecology, Conservation and Folklore*, Plantlife, Salisbury

Wilkins, T.C., Duckworth, J.C. 2011. *Breaking new ground for juniper – a management handbook for lowland England*, Plantlife, Salisbury, UK