

# **Investigating the mycorrhizal colonisation of *Sedum spp.* in the UK**

**A report submitted to the Alpine Garden Society, 2019**

**Dr Heather Rumble**  
**University of Portsmouth**



**Mycorrhiza:** "Mycorrhizal fungi are terrestrial fungi that colonise plant roots, forming ecologically and agronomically important symbioses called mycorrhizae, which are among the most common symbioses in nature."<sup>1</sup>

## Key Findings

- More mycorrhizal fungi are present in domestic sedum than in wild sedum
- Mycorrhizal fungi may be more common in plants from some UK regions than in others
- Different species of sedum may vary in the amount of mycorrhizal fungi they support
- DNA analysis of mycorrhizal species would be extremely valuable in future studies, to understand why these differences in fungal abundance occur

## Introduction

### The value of sedum

Sedum, or stonecrops, are a popular garden plant. They are colourful but resistant to difficult growing conditions such as dry soils. These drought-resistant properties have also made them the most common species to be planted on green (vegetated) roofs, which are being installed on flat roofs in most cities in the northern hemisphere. However, sedum in the UK is a relatively rare wild plant and throughout the world little is known about the ecology of this family.

The different habitats that sedum grow and are grown in make them an ideal plant to study competitive interactions. In the wild sedum often grows in harsh environments, such as mountainsides and coastal cliffs, either in isolation or in competition with other low-growing hardy vegetation such as mosses. But in gardens and on green roofs environmental conditions vary greatly and individuals are often planted in high densities, experiencing competition with individual sedum plants, other garden species and ruderal plants. Studying the ways that sedum cope with these varying environmental conditions and competitive scenarios enables ecologists to understand these processes and apply this to plant ecology more widely.

In addition to understanding ecological processes, sedum is also a species of economic value. Understanding how to maximise their growth both in nurseries (for gardens) and on green roofs will benefit sedum growers and gardeners.

### Mycorrhizal fungi

Mycorrhizal fungi are fungi that live in the roots of plants. Almost all species of land plants can form relationships with these fungi<sup>2</sup> and it has been widely thought that the relationship between plants and mycorrhizae is a mutualist one; mycorrhizae supply key nutrients to plants in exchange for sugars produced by the plant. However, it is becoming increasingly evident that the relationship between plants and mycorrhizae is not always beneficial and



can even be parasitic<sup>3</sup>. Whether the fungi is beneficial or parasitic can vary depending on the species of fungi, the species of plant, the environmental conditions and even the mix of plant species present in a single area<sup>4</sup>. It is possible that the relationship between the same plant and same mycorrhizal fungi could even switch between these modes as conditions or competition changes.

Understanding this relationship is important for two reasons. The first is commercial. Many companies produce mycorrhizal fungi for the nursery and green roof industry to enable better plant growth. But there is a lack of robust evidence about which species are most beneficial and whether benefits will be long lasting. There is increasing evidence to suggest that mycorrhiza is less needed by plants that are living in low competition, but challenging environments, such as deserts<sup>5,6</sup> and that these fungi are more useful for helping plants to outcompete their neighbours (i.e. to plants that are in competitive environments). As sedum are usually found in low competition environments, a key question is whether these fungi are actually needed by the plant.

The second important reason to study this relationship is to enable industry to apply higher levels of responsibility in their application of these fungi. In the 21<sup>st</sup> century, it is thought that humans have become a key agent in moving these fungi around the world<sup>7</sup>, because we readily transport plants without considering that fungi are also present within the roots. This means that as well as increasing the prevalence of sedum in cities, we may also be changing the community of fungi that live in these areas and creating reservoirs of fungi that could spill over into nearby habitats. This has the potential to change the way that plant communities develop in ways that we do not fully understand.

## This study

There are several inventories describing which species of plants form mycorrhizal relationships. Sedum, however, have not been well studied within this field. Table 1 presents a sample of the records within the academic literature and their findings. Overall, previous research suggests that most sedum either does not form these relationships, or forms very weak relationships. However, research on green roofs tells quite a different story, with very high proportions of mycorrhiza found in a variety of sedum species; *Sedum acre* is the only species within these green roof studies that appears to be non-mycorrhizal.

The current study aims to find out which species in the UK form mycorrhizal relationships and whether this differs in different areas of the UK, different habitats and whether different sedum species vary in their relationships. An analysis of roots from sedum individuals grown in wild environments was undertaken. This data was added to data collected by Scannell<sup>8</sup> in 2017, who studied green roof, nursery and garden sedum plants; these samples are termed as having been collected from “domestic” (as opposed to wild) sedum in this report.

This study will form the basis for a larger project on sedum/mycorrhiza interactions that aims to explore the biogeography of mycorrhiza in sedum plants and seeks to develop new ways to improve sedum plant growth for application in nurseries and on green roofs.

Table 1. Percentage colonisation of sedum species by mycorrhiza's in the literature. Bold entries denote papers investigating sedum growing on green roofs.

Species	% colonisation by mycorrhiza	Authority
<i>S. acre</i>	<b>Absent</b> <5%	Harley & Harley, 1987 <sup>9</sup> ; <b>Lundholm &amp; Kernaghan, 2014</b> <sup>10</sup> Ernst et al., 1980 <sup>11</sup>
<i>S. album</i>	Absent <b>20-50%</b>	Harley & Harley, 1987 <sup>9</sup> <b>Rumble et al., 2018</b> <sup>12</sup>
<i>S. alfredii</i>	20-50% >50%	Hu et al., 2013a <sup>13</sup> ; Hu et al., 2013b <sup>14</sup> Hu et al., 2013b <sup>14</sup>
<i>S. anglicum</i>	Absent	Harley & Harley, 1987 <sup>9</sup>
<i>S. dasyphyllum</i>	Absent	Harley & Harley, 1987 <sup>9</sup>
<i>S. forsterianum</i>	Absent	Harley & Harley, 1987 <sup>9</sup>
<i>S. kamtschatikum</i>	<b>&gt;50%</b>	<b>Rumble &amp; Gange, 2013</b> <sup>15</sup>
<i>S. lanceolatum</i>	Absent	Cripps & Eddington, 2005 <sup>16</sup>
<i>S. reflexum</i>	Absent <b>10-20%</b>	Harley & Harley, 1987 <sup>9</sup> <b>Rumble et al., 2018</b> <sup>12</sup>
<i>S. rhodanthum</i>	Absent	Cripps & Eddington, 2005 <sup>16</sup>
<i>S. rosea</i>	Present (no count)	Harley & Harley, 1987 <sup>9</sup>
<i>S. sexangulare</i>	Absent	Harley & Harley, 1987 <sup>9</sup>
<i>S. spurium</i>	Absent <b>10-20%</b> <b>&gt;50%</b>	Harley & Harley, 1987 <sup>9</sup> <b>Rumble et al., 2018</b> <sup>12</sup> <b>Lundholm &amp; Kernaghan, 2014</b> <sup>10</sup> ; <b>Rumble &amp; Gange 2017</b> <sup>17</sup>
<i>S. telephium</i>	Absent	Harley & Harley, 1987 <sup>9</sup>
<i>S. tenellum</i>	Absent	Onipchenko & Zobel, 2000 <sup>18</sup>
<i>S. villosum</i>	Absent	Harley & Harley, 1987 <sup>9</sup>



## Key terms

This study talks about three key structures within a mycorrhiza:

**Hyphae:** This is the main body of the fungus and is seen throughout the roots either in coils or in lines moving between and inside the plant root cells. The hyphae are the equivalent of a plant's stem, allowing the fungus to grow through plants and soil and transporting nutrients between different areas of the fungus.

**Vesicles:** These are the storage areas of the fungus. They form within plant root cells and are usually globular in shape.

**Arbuscules:** These are areas of nutrient exchange for the fungus and are densely branched to give them a high surface area. As a result, they look like a small tree within a root cell. They only have a lifespan of a few days, compared to hyphae and vesicles, which can live in roots for many years.

For an in-depth resource on analysing mycorrhizas, see: <https://mycorrhizas.info>

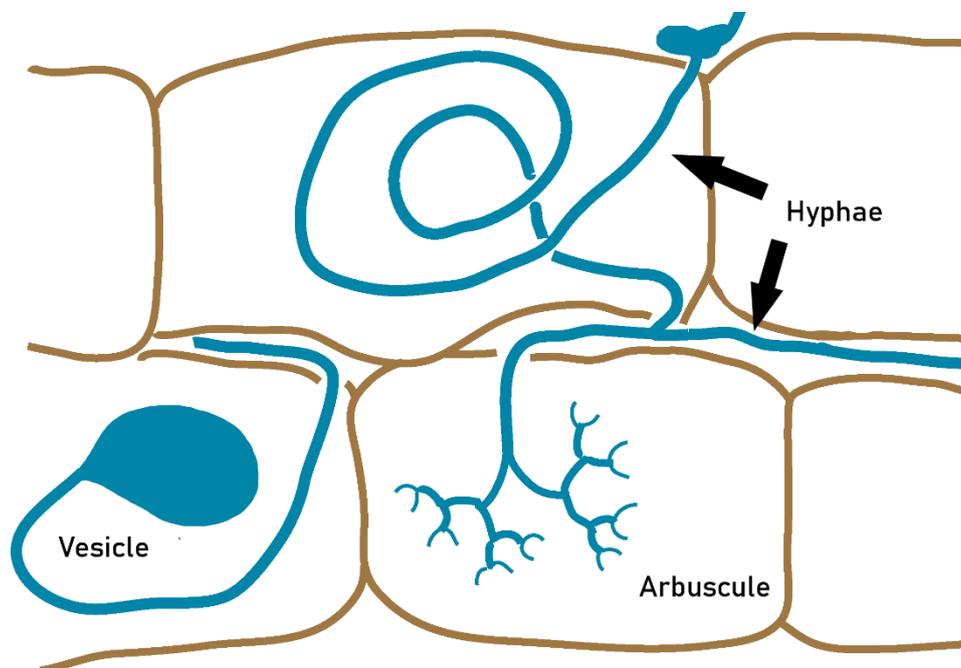


Fig 1. Diagram of a magnified plant root. Brown structures are the boundaries of plant root cells. Blue structures are mycorrhizae.

Adapted by the author from an original diagram by Ingrid Kottke, CC BY-SA 4.0.

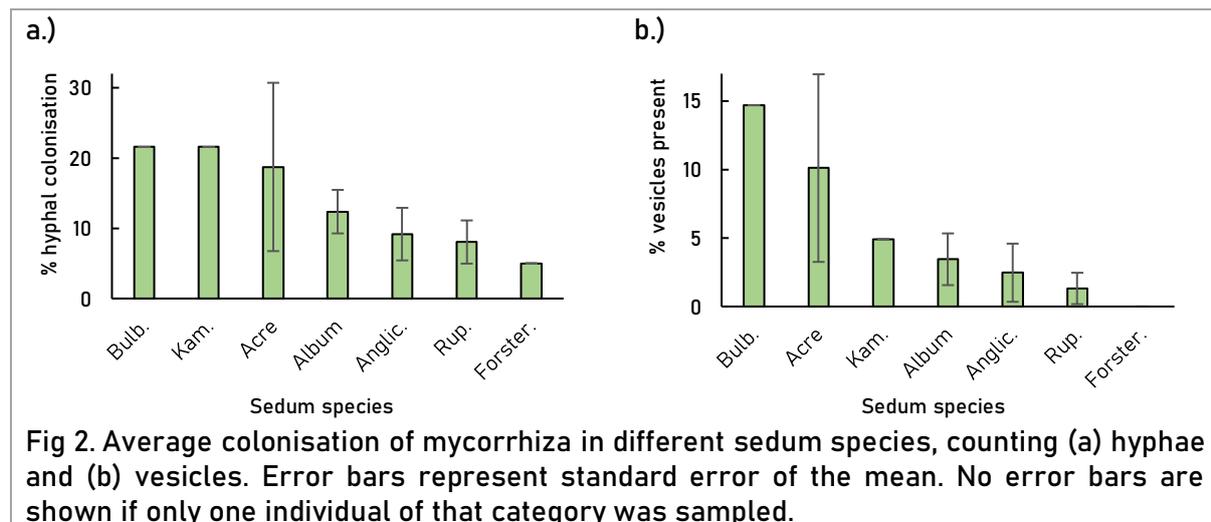


## Results and Discussion

### Detailed analysis of wild sedum

Thirty-one root samples were collected, comprising of six species: *Sedum acre*, *S. album*, *S. anglicum*, *S. rupestre*, *S. forsterianum*, *S. kamschaticum* and *S. bulbiferum*. For all species, at least one individual was colonised by fungal hyphae. Overall 0 to 51% of the root sections analysed contained fungi. Twenty-one individuals contained more than 5% fungi. All species except *S. forsterianum* contained vesicles and these were present in 1 to 20% of root sections. Arbuscules were rare, only present in four individuals of *S. anglicum*, *S. rupestre* and *S. kamschaticum*, with a prevalence between 1 and 4%.

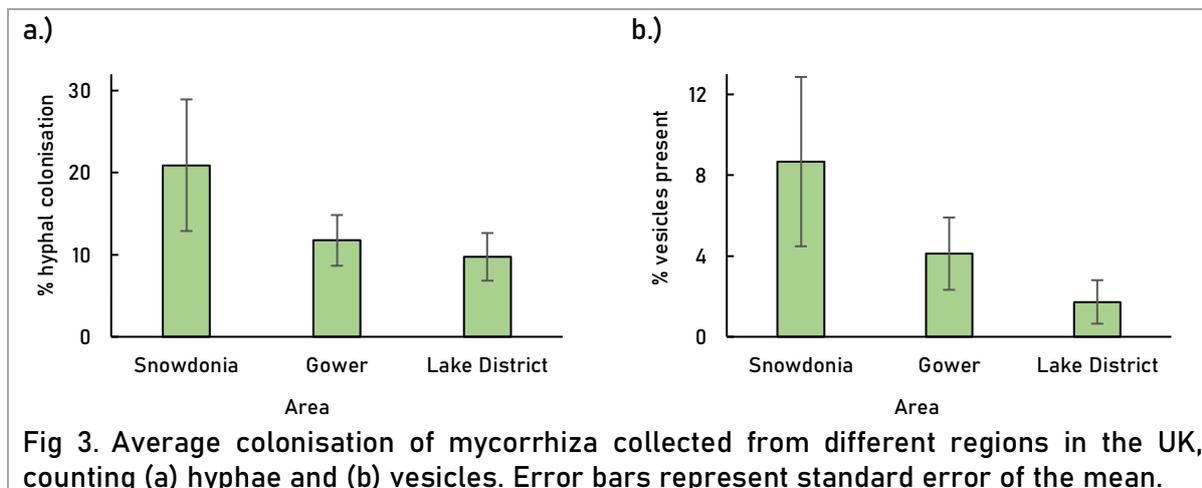
There is a suggestion that some species may be more mycorrhizal than others (Fig 2a.), but more data would be needed to verify this, because the range of colonisation varied so much within each species. This meant that no significant statistical difference was present. There were also no differences between the proportion of vesicles present (Fig 2b.), though again there may have been an overall trend denoting that some species may have had more vesicles than others. Some studies suggest that vesicle number can be an indicator of mycorrhizal species<sup>19</sup>. The results suggest that if this is the case, one species of sedum could form relationships with several different species of mycorrhiza, rather than the plant/mycorrhiza relationship being highly specific.



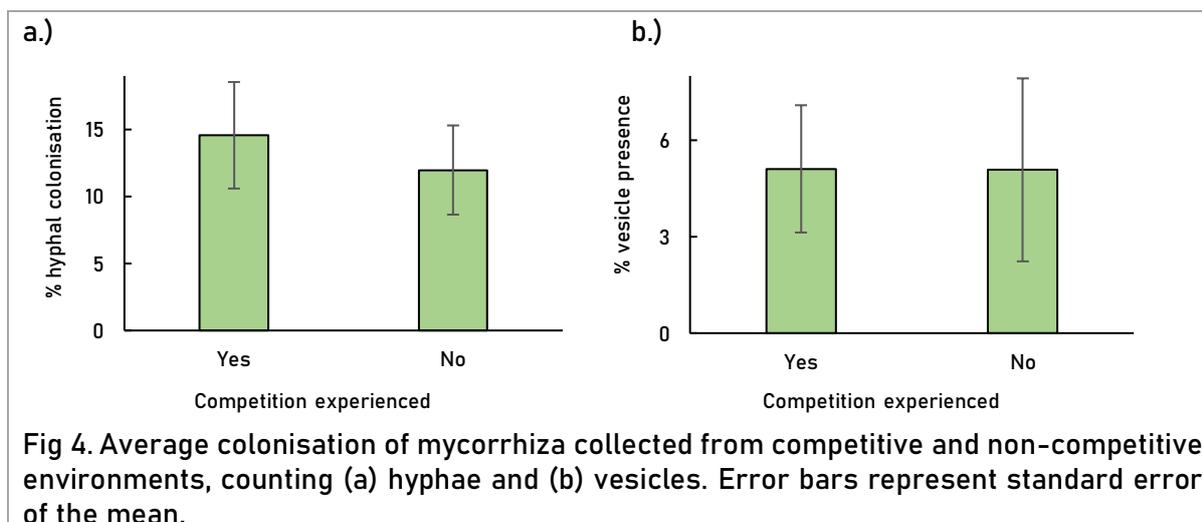
In terms of geographical regions, there was a suggestion that mycorrhizal colonisation was highest in species collected from Snowdonia than on the Gower or in the Lake District (Fig 3a.). However, again, this was not statistically significant due to high variability within each dataset, so more data would need to be collected to understand geographical variability. The pattern was more pronounced when analysing vesicles (Fig 3b.), but this was still not statistically significant. There are a wide range of factors that could enable one geographical area to be “more mycorrhizal” than others, including climate, plant diversity, density of gardens and human footfall (humans can transport mycorrhizal spores on their shoes). These factors could mean that different mycorrhizal species are more (or less) prevalent in



some areas than others, or that the environment is generally better for mycorrhiza. Identifying the mycorrhizal species living within these plants would enable us to investigate this phenomenon more robustly.



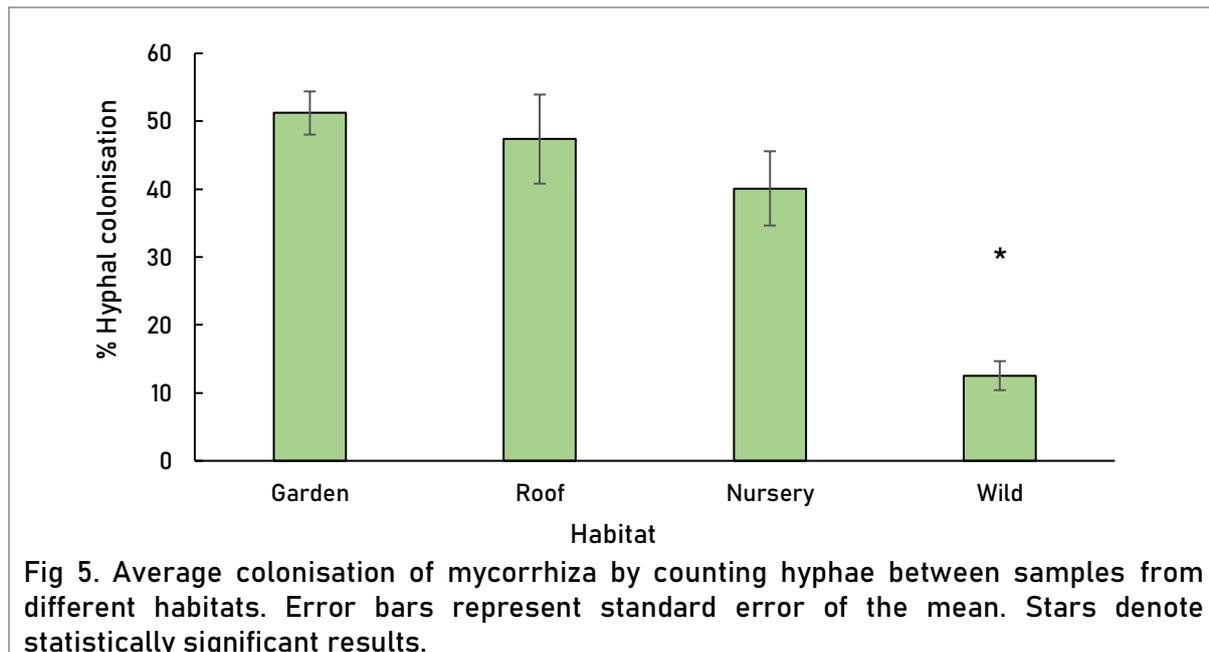
There was very little evidence to suggest that mycorrhizal colonisation differs in wild individuals of sedum depending on competition level (Fig 4.). Some authors suggest that mycorrhiza has evolved to mediate competition between plants and that plants growing in low competition environments may not, therefore, form functional associations with mycorrhiza<sup>5</sup>. Evidence from the current study suggests this is not the case for sedum, which harboured high levels of both hyphae and vesicles.



There is currently a debate among mycorrhizal ecologists about what constitutes a mycorrhizal relationship. Some authors<sup>20</sup> argue that arbuscules are necessary for a relationship to be functional, because these structures indicate an exchange of nutrients between the plant and the fungus. Others<sup>21</sup> argue that this is an oversimplification of the evidence, with emerging evidence that other structures of the mycorrhiza (such as the hyphae) are able to transfer nutrients as well<sup>22</sup>. In the current study and in previous studies by the author, arbuscules have been found in very low numbers in sedum. Future studies should examine sedum at different life stages to determine if fungi in younger plants have more arbuscules, which could be the case given their short lifespan<sup>23</sup>.

## Analysis by habitat

Twenty-eight samples, collected by Scannell<sup>8</sup> were added to the wild samples, totalling 59 samples overall. This included 32 wild specimens and 27 domestic samples: Seven collected from green roofs, six collected from gardens and 14 collected from garden centres and nurseries. There were statistically significant differences in mycorrhizal colonisation levels between the different habitats, with wild sedum harbouring less mycorrhiza than the other three habitats<sup>a</sup>. The range of colonisation in domestic samples was between 10 and 80%.



In Rumble & Gange (2013)<sup>15</sup> we remarked on the high levels of mycorrhizal colonisation in green roof sedum when compared with literature records, with the few records that exist about sedum suggesting very low or no colonisation. As investigating mycorrhizal colonisation was not the primary aim of Rumble & Gange (2013)<sup>15</sup>, we did not know if this was a recurring pattern. The current finding confirms that a range of sedum harbour fungi within their roots and that there is an apparent split between wild and domestic individuals. This finding is intriguing and significant, justifying further research that could be both applicable and gain significant insight into mycorrhizal ecology and biogeography.

The first question is why this pattern occurs. Is biogeography a key driver? For example, is the high prevalence of mycorrhiza in domestic samples due to plants being grown in high population densities, where sources of inocula are greater? Or is the species of mycorrhiza in domestic species different to wild species and, perhaps, better at colonisation? These questions have key implications for landscape ecology, because if we routinely plant sedum

<sup>a</sup>  $\chi^2(3) = 34.02, p < 0.001$ . Mean rank scores were 18.28 for wild plants, 45.64 for roof plants, 40.36 for nursery plants and 50.08 for garden plants.



in high volumes in cities, which is likely with higher adoption of green roofs, we could alter the ecology of nearby habitats by producing successful source populations, or reservoirs. Whether this would be a positive or negative change is unknown but should not be overlooked.

This is a key question for the mycorrhizal inoculum industry: is the relationship between sedum and mycorrhiza positive, neutral or negative, and does this vary between domestic and wild individuals? Positive plant growth is a key driver for the development of inocula, but this should not be at the cost of local ecology. Thus, development of new inocula needs to go hand in hand with an understanding of the impact different mycorrhizal species have on plant growth within the wider environment.

## Conclusions and next steps

The confirmation that domestic sedum is more highly colonised by mycorrhiza than wild species is a key finding that justifies future research, both to enable a better understanding of ecological processes as well as to aid industry in developing better inoculants for sedum growers. It has not been possible, with a small sample size, to elucidate why this pattern occurs, but the project is a successful proof of concept for this type of study. Data collected will be used to justify a larger version of this project, which aims not only to quantify mycorrhizal colonisation between species, habitats and geographical areas, but will also identify mycorrhizal species using DNA analysis. It also aims to determine the nature of the relationship between these organisms, to determine if the relationship is positive, negative or neutral, enabling a better understanding of mycorrhizal colonisation in sedum as well as in other plants.

## References

1. Koide, R. T. Nucleic Acid Isolation from Ecological Samples—Fungal Associations, Mycorrhizae. *Methods Enzymol.* **395**, 58–72 (2005).
2. van der Heijden, M. G. A., Martin, F. M., Selosse, M.-A. & Sanders, I. R. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytol.* **205**, 1406–1423 (2015).
3. Jin, L., Wang, Q., Wang, Q., Wang, X. & Gange, A. C. Mycorrhizal-induced growth depression in plants. *Symbiosis* **72**, 81–88 (2017).
4. Smith, S. E. & Smith, F. A. Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia* **104**, 1–13 (2012).
5. Olsson, P. A. & Tyler, G. Occurrence of non-mycorrhizal plant species in south Swedish rocky habitats is related to exchangeable soil phosphate. *J. Ecol.* **92**, 808–815 (2004).
6. Hempel, S. *et al.* Mycorrhizas in the Central European flora: relationships with plant life history traits and ecology. *Ecology* **94**, 1389–1399 (2013).
7. Davison, J. *et al.* Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science* **349**, 970–3 (2015).
8. Scannell, D. Assessing the success of Sedum plants on green roofs compared to ground-level habitats using mycorrhizal bioassay. (University of Portsmouth, 2017).
9. Harley, J. L. & Harley, E. L. A check-list of mycorrhiza in the British flora. *New Phytol.* **105**, 1–102 (1987).

10. John, J., Lundholm, J. & Kernaghan, G. Colonization of green roof plants by mycorrhizal and root endophytic fungi. *Ecol. Eng.* **71**, 651–659 (2014).
11. Ernst, W. H. O., van Duin, W. E. & Oolbekking, G. T. Vesicular-arbuscular mycorrhiza in dune vegetation. *Acta Bot. Neerl.* **33**, 151–160 (1984).
12. Rumble, H., Finch, P. & Gange, A. C. Green roof soil organisms: Anthropogenic assemblages or natural communities? *Appl. Soil Ecol.* **126**, 11–20 (2018).
13. Hu, J. *et al.* Arbuscular mycorrhizal fungi induce differential Cd and P acquisition by Alfred stonecrop (*Sedum alfredii* Hance) and upland kangkong (*Ipomoea aquatica* Forsk.) in an intercropping system. *Appl. Soil Ecol.* **63**, 29–35 (2013).
14. Hu, J. *et al.* Arbuscular mycorrhizal fungi enhance both absorption and stabilization of Cd by Alfred stonecrop (*Sedum alfredii* Hance) and perennial ryegrass (*Lolium perenne* L.) in a Cd-contaminated acidic soil. *Chemosphere* **93**, 1359–1365 (2013).
15. Rumble, H. & Gange, A. C. Soil microarthropod community dynamics in extensive green roofs. *Ecol. Eng.* **57**, 197–204 (2013).
16. Cripps, C. L. & Eddington, L. H. Distribution of mycorrhizal types among Alpine vascular plant families on the Beartooth Plateau, Rocky Mountains, U.S.A., in reference to large-scale patterns in Arctic-Alpine habitats. *Arctic, Antarct. Alp. Res.* **37**, 177–188 (2005).
17. Rumble, H. & Gange, A. C. Microbial inoculants as a soil remediation tool for extensive green roofs. *Ecol. Eng.* **102**, (2017).
18. Onipchenko, V. G. & Zobel, M. Mycorrhiza, vegetative mobility and responses to disturbance of alpine plants in the northwestern caucasus. *Folia Geobot.* **35**, 1–11 (2000).
19. Brundrett, M. Mycorrhizal associations: The web resource. Section 4. Arbuscular mycorrhizas. (2008). Available at: <https://mycorrhizas.info/vam.html#S6>. (Accessed: 5th August 2019)
20. Brundrett, M. & Tedersoo, L. Misdiagnosis of mycorrhizas and inappropriate recycling of data can lead to false conclusions. *New Phytol.* **221**, 18–24 (2018).
21. Bueno, C. G. *et al.* Misdiagnosis and uncritical use of plant mycorrhizal data are not the only elephants in the room. *New Phytol.* (2019).
22. Manjarrez, M., Christophersen, H. M., Smith, S. E. & Smith, F. A. Cortical colonisation is not an absolute requirement for phosphorus transfer to plants in arbuscular mycorrhizas formed by *Scutellospora calospora* in a tomato mutant: Evidence from physiology and gene expression. *Funct. Plant Biol.* **37**, 1132–1142 (2010).
23. Montero, H., Choi, J. & Paszkowski, U. Arbuscular mycorrhizal phenotyping: the dos and don'ts. *New Phytol.* **221**, 1182–1186 (2018).
24. Vierheilig, Coughlan, Wyss & Piche. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Appl. Environ. Microbiol.* **64**, 5004–5007 (1998).
25. McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L. & Swan, J. A. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol.* **115**, 495–501 (1990).

The author would like to thank the Alpine Garden Society for funding this project. Thanks also to the Botanical Society of Britain and Ireland for their support in obtaining species maps for this study; Natural Resources Wales for their advice and The Climber's Club for permission to take root samples on their property.

Dr Heather Rumble

School of the Environment, Geography and Geosciences,  
University of Portsmouth, Buckingham Building,  
Lion Terrace, Portsmouth,  
PO9 3HE

[heather.rumble@port.ac.uk](mailto:heather.rumble@port.ac.uk)  
+44 (0)2392 846540



## Appendix I: Study Methods

### Study Locations

After preliminary analysis of soil acidity in the UK, as well as discussion with ecologists responsible for plant records in several UK national parks, three areas were chosen for this study: Snowdonia, the Gower Peninsula and the Lake District. Records of locations of *sedum spp.* were obtained from the Botanical Society of Britain and Ireland, with additional records for Snowdonia obtained from Cofnod. Lists of potential individuals for sampling were obtained based on accessibility of the site, the age of the record and the specific species of sedum, with lists of primary and backup individuals identified.

Forty-three percent of the records verified in Snowdonia contained the target species and 28% each for the Gower and Lake District. In the Gower, sedum was extremely abundant, so this did not greatly impact fieldwork. In the Lake District the low number of accurate records meant that two trips were necessary. Overall 53 records were investigated and 17 were present and sampled. An additional 26 plants were sampled that were in neither of the databases, totalling 43 sampled individuals for the project. Snowdonia samples were obtained in August 2018, Gower samples in September 2018 and Lake District samples in November 2018 and May 2019.

### Field Sampling

Roots were sampled from target plants non-destructively, by gently lifting root matter from the soil. Where roots were present hanging into free air, such as when plants were growing on walls, roots from both the soil and from the air were obtained. Depending on the size of the plant, samples varied from 5-6 small roots to 1cm<sup>3</sup> of root matter. A number of site details were noted: The site grid reference, the species of sedum, the overall terrain (e.g. mountains), the local habitat (e.g. mountain, village) and the micro-habitat (e.g. crack in wall) and whether the individual was growing alone or in competition. Photographs of each plant were also taken. Root samples were stored in paper envelopes and dried in the sun or by a radiator in the field. On return to the lab they were stored in 70% ethanol until ready to be analysed.

### Laboratory analysis

Roots were washed of ethanol and cleared and stained via the method of Vierheilig et al., (1998)<sup>24</sup>. Briefly, roots are boiled at 80°C in a solution of 10% KOH to remove tannins. The timing required varied depending on each individual; some samples required 40 minutes, whilst others needed in excess of 50 minutes. Roots were then washed and dried and boiled, again at 80°C, this time in a solution of 0.5% Quink Ink, 15% HCl and 84.5% water. This stage is required to dye fungal strands for observation under a microscope. Roots were then analysed under a light microscope at x200 magnification using the crosshair eyepiece method of McGonigle et al., (1990)<sup>25</sup>. Approximately 100 observations of each root sample was undertaken, noting whether samples contained fungal structures or not. In addition the type of fungal structure was recorded (hyphae, vesicle and arbuscule). A record of clearing success was also kept to aid in developing more accurate boiling times for each species in the future.

### Statistics

Counts of hyphae, vesicles and arbuscules were converted to a percentage of the total counts for that individual. Statistics were undertaken in IBM SPSS 25.0 for Windows. Sample groups were uneven and non-normal, so non-parametric analysis was undertaken: Kruskal-Wallis was used to analyse differences between sedum species, area sampled and habitat sampled from; Mann-Whitney U was used to analyse differences between competitive and non-competitive individuals.

