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Testing the capacity of clothing to act as a vector for non-native seed in protected areas

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Abstract

Although humans are a major mechanism for short and long distance seed dispersal, there is limited research testing clothing as a vector. The effect of different types of material (sports versus hiking socks), or different items of clothing (boots, socks, laces versus legs) or the same item (socks) worn in different places on seed composition were assessed in Kosciuszko National Park, Australia. Data was analyzed using Repeated Measures ANOVA, independent and paired t-tests, Multidimensional Scaling Ordinations and Analysis of Similarity. A total of 24776 seeds from 70 taxa were collected from the 207 pieces of clothing sampled, with seed identified from 31 native and 19 non-native species. Socks worn off-track collected more native seeds while those worn on roadsides collected more non-native seeds. Sports socks collected a greater diversity of seeds and more native seeds than hiking socks. Boots, uncovered socks and laces collect more seeds than covered socks and laces, resulting in 17% fewer seeds collected when wearing trousers. With seeds from over 179 species (134 recognized weeds) collected on clothing in this, and nine other studies, it is clear that clothing contributes to unintended human mediated seed dispersal, including for many invasive species.

Keywords: weeds; exotics, human mediated seed dispersal; invasion; alpine plants, protected area management

1. Introduction

Anthropogenic mass movement of species is one of the greatest environmental challenges faced by conservation organizations (WRI, 1992; IUCN, 2000). Human activities are an important mechanism for long distance dispersal of plants and animals including invasive species (WRI, 1992; IUCN, 2000; Groves et al., 2005; Nathan, 2006; Wichmann et al., 2009). Human mediated dispersal of plants can be deliberate for agricultural and ornamental purposes (Groves et al., 2005; Benvenuti, 2007), or accidental such as through agricultural seed contamination in: soil (Rejmanek, 2000; Benvenuti, 2007), garden waste (Groves et al., 2005; Hulme, 2006), on equipment or even on clothing (Table 1). Both deliberate and accidental introductions have dramatically increased the scale and rate at which plants are dispersed, including many invasive species (Richard and Hamilton, 1997; Mark and Lonsdale, 2001; Groves et al., 2005; Wichmann et al., 2009).

Limiting human mediated dispersal of non-native plants is important, particularly when they are invasive. Invasive plants can adversely affect flora and fauna, prevent the recruitment of native plants, alter hydrology and nutrient content of soils, change fire regimes, and affect fauna that use the plants for food and habitat (Csurhes and Edwards, 1998; Williams and West, 2000; Mayers and Bazely, 2003). Although there is renewed interest in understanding the invasive process, there is still limited research on the initial dispersal of species, particularly empirical studies (Puth and Post, 2005). For example out of 873 recent articles examining the process of invasion of exotic species, only 15 were empirical studies examining the initial dispersal of species in terrestrial systems.

The initial step in unintended human mediated seed dispersal is seed attachment. Although seeds are commonly observed on socks, laces, boots and trousers there is limited empirical data on clothing as a seed vector (Table 1). The authors have only found nine published empirical studies, and only three involved statistical testing of hypothesis. Seven studies examined seed attached to shoes and/or boots, four examined socks, two examined laces and four examined seed on trousers. Only two of the studies examined dispersal (Bullock and Primack, 1977; Wichmann et al., 2009). Based on the nine studies 139 species of plants have been identified that have seeds

that can attach to clothing and hence have the potential to be dispersed by humans over long distances.

Although these studies established that clothing can be an important mechanism for human mediated seed dispersal, research is required to quantify the amount and composition of seeds that can collect on clothing, and determine if factors such as the location the clothing is worn, the type of material it is made of, and if different clothing items, affect the amount and type of seeds collected. For example, the surface area of the item, the location (such as height) of the item and the adhesive quality of the item are all likely to effect seed attachment to clothing (Bullock and Primack, 1977; Whiman et al., 2005). Different combinations of clothing may affect what seeds attach so that someone wearing shorts, socks, shoelaces and boots may collect different seed than if they were wearing trousers which covered the socks, shoelaces and the top of boots. There are also likely to be differences in the seeds collected when hiking through disturbed areas such as roadsides and car parks where there are many non-native plant species, to the seeds collected when hiking in intact native vegetation away from roads and tracks. As hikes can commence from roadsides and car parks there is the risk that people may carry non-native seeds from these areas into the natural vegetation in protected areas.

The objectives of this study were to experimentally test aspects of seed attachment to clothing as part of assessing the risk of human mediated seed dispersal including for non-native plants. Three field experiments were conducted in a popular national park with high conservation values, Kosciuszko National Park, to determine: (1) What seeds are collected on clothing (species and number, native vs non-native); (2) If there are differences in the seeds collected on socks depending on where someone walks; (3) If there are differences in the seeds collected on different types of socks; (4) If there are differences in the seeds collected on different items of clothing (boots, socks, laces and trouser leg) and (5) if wearing trousers reduces the amount of seeds collected.

2. Methods

2.1. Study area

The potential for seed to attach to clothing was assessed in Kosciuszko National Park, in the Australian Alps bioregion of southeast Australia. The Park is a major tourist destination with around three million visitors a year (DEC, 2006). The alpine area around continental Australia's highest mountain, Mt Kosciuszko (~100 km²) is very popular for hiking with 79% of the around 100 000 people who visit the area during the snow free period, going hiking (Johnston and Growcock, 2005). Although most hikers remain on the formal trail system, hiking off trail is permitted and is popular to iconic destinations such as glacial lakes and for back country camping (Johnston and Growcock, 2005).

The unique combination of climate and geology in the Park contributes to a high degree of diversity and endemism in the flora. In the alpine zone there are 212 species of vascular plants of which 33 species are rare, and 21 endemic. This is among the highest values for endemism in mountainous regions in the world. Across the whole of the Park there are 852 native vascular species (Costin et al., 2000; DEC, 2006).

Non-native plants are increasing in the Park having been introduced and spread through grazing of cattle and sheep, soil conservation work, mining, the Snowy Mountains Hydroelectric Scheme and activities and infrastructure associated with tourism such as the ski resorts. Approximately two thirds of the 330 non-native plant species in the Park are found in the alpine and subalpine zones (DEC, 2006). The distributions of these species are strongly associated with visitor infrastructure, with a high cover and diversity of non-native species found along roadsides in the Park (Johnston and Pickering, 2001; McDougall et al., 2005; DEC, 2006; Pickering and Hill, 2007a, b).

Three experiments examining clothing as a seed vector were conducted in the Park between late January and mid February 2008 when many plant species in the subalpine and alpine zones of the Park are seeding (Fig. 1). The term "seed" is used here as a general descriptor to represent the dehiscent or indehiscent diaspores produced by a plant species, and hence for some species may refer to fruit. It did not include vegetative propagules. All experiments were conducted when vegetation and soils were relatively dry e.g. no mud was collected on boots. The location and style of hiking in the experiments matched those of general hikers in the region. The number and

species of seed on clothing items were assessed after experimental exposure of clothing to vegetation by removing seed directly from the socks, laces, and trousers and from loose material obtained from the leg and boots using tweezers and a dissecting microscope. Fragments of seed and empty infructescences were excluded from the count. Seed were identified using seed reference collections compiled from seeding species in the field in areas adjacent to, but not trampled, in the experiments, and floras such as Costin et al., (2000), and PlantNET (BGT, 2008). Nomenclature, family, origin (Europe, North and Central America, Africa, Asia, Middle East, Mediterranean or Australia), growth form (graminoid, herb or shrub) for all species was recorded. Although large numbers of seed from the native herb *Acaena* were collected it was not possible to consistently tell the seed of the common *Acaena novae-zelandiae* (Synonym *Acaena anserinifolia*) from the rare *Acaena* sp. A. as they only differ by 2 mm in the length of spines on the seed, and spines had often fallen off seed that was attached to clothing. Therefore all *Acaena* seed were assumed to be from the more common species, which occurs on roadside and in natural vegetation in the Park.

2.2. Experiment one: Effect of location of seed collected on socks

Seeds on socks worn only on roadsides, only in natural vegetation and all day in both roadside and natural vegetation were compared (Fig. 1). At the beginning of each day, for nine days, a hiker put on a new pair of cotton/nylon blend sport socks (Slazenger® sport sock) and hiked through roadside vegetation for five minutes. One sock was removed and bagged to give a measure of 'Roadside' seeds that could be collected at the start of the hike. The sock was replaced and the researcher then hiked through natural vegetation off track for the rest of the day. She then removed and bagged the sock worn 'All Day' in roadside and natural vegetation, and the sock only worn in 'Natural' vegetation.

The number of seeds and species richness (total, native and non-native species) per sock, were compared among the Roadside, Natural vegetation and All Day socks using a Repeated Measures ANOVA with the nine days as the repeated measure in the statistical package SPSS for Windows (version 15.0, 2007). Differences in the composition of seeds were also examined using ordinations performed in the multivariate statistical package PRIMER 6. This type of

ordination has produced reliable, simple and statistically significant analyses of a wide range of ecological community data and is commonly used to analyse composition data (Clarke 1993; Clarke et al., 2006). The data was log transformed and then a Bray Curtis dissimilarity matrix calculated. A Two-Way Analysis of Similarity (ANOSIM) without replication was performed on the matrix to test if there were significant differences in composition among All Day, Roadside and Natural vegetation socks with day as a block. ANOSIM is a non-parametric permutation procedure applied to the rank dissimilarity matrix that is analogous to Analysis of Variance (Clark, 1993). Variation in composition was graphically represented using non-metric multidimensional scaling (MDS) ordination, with the closeness of fit of the MDS model to the original similarity matrix represented by a stress value, with the smaller the stress value the better the visual representation of the matrix. The SIMPER function in PRIMER 6 was used to determine the level of similarity amongst the All Day, Roadside and Natural vegetation socks.

2.3. Experiment two: Sock types

There are a wide variety of socks that can be worn by people when hiking. They vary in material blends, thickness and height. Two common types of socks that are available in shops in the local area to Kosciuszko National Park are a thick, wool/nylon blend sock (Explorer® sock) typical of hiking socks, and a thinner, cotton/nylon blend sock (Slazenger® sport sock) typical of sports style socks. The Explorer sock was slightly longer than the sports sock extending 32.5 cm from heel to top of the sock, while for the sports sock the distance was 27.5 cm. These different materials and sizes of the socks might affect which seeds attach to socks.

Ten volunteers were randomly assigned one of the sock types to wear during an approximately five hour hike off the formal track system, mostly through tall alpine herbfield, which is the most common native vegetation alliance in the alpine zone (Costin et al., 2000, Fig. 1). On completion of the hike the socks were carefully removed and placed in bags (one sock per bag).

The dependent variables number of seeds (total, *Acaena novae-zelandiae*, other natives and all non-native seeds) and species richness (total, native and non-native) per sock per person (e.g average of two socks per person) were compared using One-Way Analysis of Variance in SPSS. The data was log transformed and then a normalized Bray Curtis similarity matrix calculated in

PRIMER 6. A nested ANOSIM was performed on the matrix using ‘individual’ nested within sock type. The variation between the sock types and the socks on an individual was graphically represented using MDS ordination. To determine the level of similarity, the SIMPER function was used between sock types and to calculate similarity values for the right and left sock per person.

2.4. Experiment three: Clothing comparison

To compare the ability of clothing items to collect seeds a double split plot design was used where all treatments were applied to a person at the same time. Firstly a person (plot) was ‘split’ into two legs where one leg was covered with a trouser and the other was left uncovered (bare leg). Each leg was then ‘split’ into the four clothing items of boots, shoelaces, socks, and the rest of the leg (Fig. 2).

The experiment was conducted in roadside vegetation along the Kosciuszko Road between the Charlotte Pass car park and Perisher Valley (Fig. 1). Prior to trampling, twenty replicate 50 m by 0.5 m areas were randomly assigned along the road. Within each area, a researcher hiked 50 m in one direction, then after turning and stepping 20 cm to the side, walking back to the start. This gave a total of 20 randomly selected replicate 100 m hikes through roadside vegetation. A new outfit was used for each hike, with the trouser randomly assigned to a leg (Fig. 2). An outfit consisted of one trouser leg that covered the leg from just below the knee (surface area = 2080 cm²) made from 100% drill cotton; a pair of Slazenger® sport socks (surface area = 818 cm² per sock); two 60 cm long synthetic flat laces (surface area = 207 cm²) each tied into a bow and attached to the top of each shoe tongue with a safety pin; and a pair of Jackeroo® Hiking Boots (surface area = 1714 cm²) with suede leather uppers and rubber treads (approx. 4 mm deep, Fig. 2). On completion of the hikes, all clothing items were carefully removed. The socks, trouser leg and shoelaces were placed into separate bags. The upper and tread of each boot and the bare leg was brushed down onto large sheets of paper and any plant material dislodged was bagged. The tread and material on the side of the boots and bare leg (unshaven, female) were visual examined to make sure no foreign material remained.

To determine if wearing trousers effects seed collection, the number of seeds and species richness were compared using paired t-tests between the covered and uncovered legs. The number of seeds and seed richness were also compared among clothing items on each leg. The different sizes of the clothing items was also taken into account by dividing the number of seeds collected by an item by the exposed surface area of that item. First a One-Way Repeated Measures ANOVA was used to test for any interaction between legs and items of clothing on a leg. Where there was a significant interaction, Repeated Measures ANOVA were performed separately on the clothing items on the trouser (covered) leg and then repeated for the clothing items on the bare (uncovered) leg. Simple contrasts were used to determine which items were statistically different to the others. To determine if each clothing pair (ie covered sock and uncovered sock) differed, a series of paired t-tests were performed between the covered and uncovered clothing items.

The composition of seeds per leg and per item were compared using separate ordinations in PRIMER. As a normalized Bray Curtis similarity matrixes can not be calculated for items that did not collect any seed, only the covered and uncovered boot and sock data were analyzed. Two other replicates (covered sock 1 and 6) were also removed from the analysis for the same reason. The data were log transformed and then Bray Curtis dissimilarity matrix calculated. To determine if items of clothing varied in composition, a Two-Way ANOSIM without an interaction was performed. The first factor was item of clothing (covered boot, covered sock, uncovered boot and uncovered sock) with transect treated as a block. To determine if the trouser and bare legs varied in composition, a One-Way ANOSIM was completed with leg (trouser and bare) as the factor. The SIMPER function was used to determine the level of similarity for the 'per leg' and 'per item' data sets.

3. Results

3.1. Total diversity and number of seed collected

Combining the three experiments a total of 24776 seeds were collected from 70 taxa of which 50 were identified to species level (Appendix A). There were seed from 31 native species, 19 non-native species, seed from two native genera (*Poa* species A. and an *Epilobium* sp.) that could not be identified to species level and 17 morphotaxa (Appendix A). Most seeds (69%, 17034 seeds)

came from just one species, the native herb *Acaena novae-zelandiae* with seeds found on 55 out of the 207 separate pieces of clothing assessed across the three experiments. The next most common species was the exotic grass *Festuca nigrescens* with 3677 seeds collected over 131 pieces of clothing. Of the seven species that were collected in all three experiments, three were native (*Acaena novae-zelandiae*, *Austrodanthonia alpicola* and *Poa fawcettia*) and four were non-native (*Festuca nigrescens*, *Actosella vulgaris*, *Dactylis glomerata* and *Poa pratensis*).

3.2. Experiment one: Effect of location on seed collected on socks

Over six hundred seeds were collected on socks worn for five minutes along the Roadside (648 ± 283 per sock). There were over 500 seeds attached to socks worn All Day (510 ± 189), significantly more than on socks only worn in natural vegetation (169 ± 70 , Tables 2 and 3). The All Day and Natural vegetation socks had similar amounts of native seeds excluding *Acaena novae-zelandiae* (69 ± 31 and 55 ± 25 respectively); which were significantly more than was Roadside socks (4 ± 2 native seed, Tables 2 and 3). The Roadside and All Day socks collected around 65 non-native seeds on average, significantly more than socks only worn in natural vegetation (Tables 2 and 3). Natural vegetation and All Day socks collected seeds from more native species than Roadside socks, but similar numbers of non-native species, around four species per sock (Tables 2 and 3). There was no significant difference in the composition of seeds among the socks for all seeds (Two-Way ANOSIM without replication, $Rho = 0.167$, $P = 0.093$, Fig. 3), or when *Acaena novae-zelandiae* seeds were removed from the analysis (Two-Way ANOSIM without replication, Global $Rho = 0.139$, $P = 0.162$).

3.3. Experiment two: Effect of the type of sock on seed collection

A total of 2037 seeds from 43 species were collected on socks worn by ten people after a one day hike off track (Appendix A). Although *Acaena novae-zelandiae* was still the most common species (570 seeds), fewer seeds were collected in this experiment (29 ± 123 per sock, Table 4) than on the socks worn in natural vegetation in experiment one (78 ± 28 per sock, Table 2). Sport socks collected more native seeds (92 ± 19 seeds, excluding *Acaena novae-zelandiae*) than Hiking socks (42 ± 9) (Table 4). Sports socks also collected seeds from a greater range of species

than Hiking socks (12.5 ± 1.1 vs 8.6 ± 0.6) although there was no significant difference when total diversity was separated into native and non-native components (Table 4).

There was a significant difference in species composition between the Hiking and Sport socks (Fig. 4, Sock type, $Rho = 0.73$, $P = 0.001$) in the nested ANOSIM. There was also considerable variation in the composition of seeds between legs with low similarity (SIMPER) values for seeds composition within a person. For the Hiking sock the similarity between left and right socks per person varied from 47% to 79%, with an average similarity of 65%. For the Sport sock values ranged from 57% to 73% with an average similarity of 62%. However, there was no significant consistent difference between the seeds collected on the left and right leg ($Rho = 0.226$, $p = 0.063$, Fig. 4).

3.4. Experiment three: Effect of clothing item on seed collected

A total of 10794 seeds were collected along the 20 roadside 100 m walks over the 160 pieces of clothing sampled (Appendix A). Of the fourteen species identified, only three were native (*Poa fawcettiae*, *Austrodanthonia alpicola* and *Acaena novae-zelandiae*) with 66% of all seeds from *Acaena novae-zelandiae*. The non-native grasses *Festuca nigrescens* (2692 seeds), was the most frequently collected species (96 times) followed by *Festuca elatior* (43 times).

There was significantly more seeds collected on a bare leg than on a leg covered by a trouser (Paired t-test, d.f. = 19, $t = 5.264$, $P = 0.001$). Therefore wearing trousers reduced the number of seeds collected from 295 ± 147 on a bare leg to 245 ± 184 on a covered leg (Table 5). There was no significant difference in species richness for the trousered and bare leg (Paired t-test, d.f. = 19, $t = 0.295$, $P = 0.772$) with an average of 3.6 ± 0.4 species on the trousered leg and 3.7 ± 0.3 on the bare leg.

Due to significant interactions between legs and items of clothing on a leg, further analysis of the clothing items was performed using separate Repeated Measures ANOVA for the covered and uncovered legs (Table 6). On the trousered and bare leg there were significant differences among clothing items in the number of seeds whether adjusted for the surface area of the item or not (Table 6). The uncovered boot, uncovered sock, covered boot and trouser collected the most seeds (Tables 5 and 7). Covering the sock with a trouser reduced the number seeds collected

from around 157 ± 76 to 10 ± 6 per sock. It also resulted in fewer seeds on the lace, with 66 ± 50 on the uncovered lace vs 30 ± 30 on the covered lace. The trouser material collected many seeds (146 ± 127), more than boots (60 ± 26 to 71 ± 24 seeds per boot). The number of seeds collected on the laces and trouser material were particularly variable with standard errors nearly as large as means (Table 5). There was a large variation in the number of seeds collected among hikes with the total number of seeds on a trouser leg varying between 7 and 3716 seeds. Likewise for the bare leg, the amount of seed collected varied from 10 to 3056 seeds.

Species richness differed among clothing items on the trouser leg and on the bare leg (Table 6). Richness per item ranged from 3.3 ± 0.3 on the covered boot to just 0.3 ± 0.1 on the bare leg (Table 5). Covered socks, uncovered boots and covered boots had similar species richness while seeds from fewer species were found on the covered sock and lace than the uncovered sock and lace (Tables 5 and 7). Therefore, wearing trousers reduced species richness on socks and laces but overall there was higher species richness on the leg with a trouser as the trouser material itself collecting seeds from a greater range of species (Tables 5 and 7).

When the composition of seeds were compared between the trouser and bare legs there was a significant difference (log data per leg, ANOSIM, $Rho = 0.111$, $P = 0.008$, Fig. 5a). The average similarity in composition between legs covered with a trouser and legs left uncovered was 48% although there was considerable variation among transects with average similarity in composition 49% for trouser legs and 54% for bare legs.

Covering boots and socks with a trouser resulted in differences in species composition compared to leaving the boots and socks uncovered (Nested ANOSIM $Rho = 0.427$, $P = 0.001$, Fig. 5b). The similarity in the composition of seeds between individual covered and uncovered socks and boots was low. Uncovered boots and uncovered socks had the highest average similarity of 53% with uncovered and covered socks having the least similarity in the composition (30%).

4. Discussion

These results demonstrate that people can accidentally collect and transport large numbers of native and non-native seeds on common clothing items such as boots, socks, laces and trousers.

This supports results from previous studies of clothing as seed vectors (Table 1). Combining the nine previous studies and the current study, seeds from 179 species have been found on clothing and equipment (Table 1), of which 134 occur in a major weed databases (Clayton et al., 2008), and 43 of which are considered international invasive species (Weber, 2003). In addition to identifying 40 new species that have seed that attach to clothing, the current research quantified the number and species richness of seeds that can be collected per item of clothing and demonstrated that where someone walks and what they wear is important. The study also demonstrates the highly variable nature of seed attachment to clothing, with differences in composition occurring between socks on the same individual as well as among people undertaking the same hike. Similar high variability among samples was found by Whinam et al. (2005) with one out of the 64 people examined carrying 309 seed/fruit out of the 981 collected while 20 people had no seed/fruit on their clothing or equipment.

4.1. Large numbers of seeds attached to clothing

People can collect large numbers of seeds when hiking, with ~1300 seeds per person on socks after a five minute hike in roadside vegetation and ~1020 seeds on socks at the end of a days hike. Most of the seed was from the widespread native herb *Acaena novae-zelandiae*, although some of the seed may have come from the rarer *Acaena* sp. A. Both species are well adapted to adhering to clothing with seeds in globular heads with long spines (PlantNet, 2008). Both have been collected from roadsides in the Australian Alps, indicating that they are capable of establishing in disturbed habitats (McDougall, 2001; Mallen-Cooper and Pickering, 2008). Therefore these species' normal ranges may have increased as a result of adhering to clothing and hence being transported along roadsides and tracks as well as into natural vegetation.

Non-native seeds were mostly collected from socks, laces, boots and trousers worn on short hikes on roadside vegetation at the car park at Charlotte Pass and along the Kosciuszko Road. Visitors regularly use these areas to access popular hiking tracks (Johnston and Growcock, 2005) and these roadsides have a high diversity and cover of non-native plant species (Hill and Pickering, 2006; Pickering and Hill, 2007a; Mallen-Cooper and Pickering, 2008). This may lead to the introduction and establishment of potential environmental weeds further along walking

tracks and into the natural plant communities (Carey and Watkinson, 1993; Dickens et al., 2004; Hill and Pickering, 2006).

4.2. Seeds from a wide range of species attached to clothing

A total of 50 species in the current study and 179 over all the studies (Table 1) of plants have seed that adheres to clothing. Additional research on clothing as a seed vector is likely to identify far more species, as there appears to be no studies of seed attaching to clothing from South and North America, Africa or Asia (Table 1). Few species are common among studies, with only 20 of the 179 species, reported in two or more studies. The annual grass *Poa annua* was the most commonly recorded species, with seed on clothing in the current and four other studies. A native of Europe, this species is invasive in parts of North America (USDA, 2008) and Australia (PlantNET, 2008). *Hypochaeris radicata* and *Plantago lanceolata* were common to four studies each. Both are perennial herbs native to Europe but are internationally invasive (Weber 2003). The five species recorded in three studies (*Bidens pilosa*, *Lolium perenne*, *Agrostis capillaries*, *Anthoxanthum odoratum* and *Poa pratensis*) are also all invasive species and have been introduced to Australia, where they occur on roadsides as weeds (Johnston and Pickering, 2001; PlantNET, 2008; Weber 2003).

4.3. Where clothing is worn effects the number and diversity of seeds collected

There were differences in the seeds collected on clothing depending on where, and for how long someone hiked. More non-native seeds were present on socks after five minutes of hiking in roadside vegetation than at the end of a days hiking in natural vegetation. This is likely to reflect the dominance of the roadside vegetation by non-native species. As the duration of exposure differed between the hikes on the roadside and in natural vegetation, the current study has not quantified the capacity of seed in the two habitats to attach to socks after the same length of exposure. What it does show, however, is that native seed may be carried out of a Park at the end of a days walk, and that non-native seed may be carried into the Park from roadsides at the start of a days walk. Transporting seeds from one location to another is important as it both removes seeds from the vegetation where it was collected and introduces it to a new location. In the new location, any seeds deposited may be able to germinate and establish, with non-native seeds

particularly likely to benefit from distances to the existing vegetation associated with hiking (Hansen and Clevenger, 2005; Hill and Pickering, 2006; Pickering and Hill, 2007a,b).

4.4. The amount and type of seed collected varies among clothing items

What someone wears can alter the number and type of seeds collected. In the current research the 87 socks sampled collected 17333 seeds from 50 species. The type of socks worn can affect which seeds are collected. Sports socks, which have a more open weave than the thicker, taller Hiking socks, collected more native seeds and seed from a greater range of species. Wearing trousers can reduce the number of seeds collected on socks by 94%, and by 55% on laces. However, as the drill cotton trouser legs collected large numbers of seeds, wearing trousers only reduced the total number of seeds collected by around 17%. When hiking in shorts, boots and socks collected equal amount of seeds, more than laces, which collect more than bare legs. When wearing trouser, the trouser material collect more seeds than boots, which collect more seeds than the partly covered shoelaces, which in turn collected more seeds than the covered socks. Changing what is worn is therefore one way of reducing the potential for human mediated seed dispersal. Although not tested here, it is possible that wearing gaiters and/or replacing laces with other types of fastenings on footwear, such as clips, could reduce the amount of seed adhering to clothing.

4.5. Characteristics of plants can affect their capacity to be collected on clothing

There are a number of factors that affect whether or not seeds are collected by clothing. Firstly the species must be present and seeding. Therefore, the number of seeds and diversity collected on clothing is governed by the temporal and spatial availability of the species. On the roadside transects 18 out of 32 species that were seeding were never collected on clothing, while five species were always collected if seeds were present. This highlights that collecting seeds on clothing is a selective dispersal mechanism that is likely to sort amongst species based on characteristics such as seed morphology, number of seeds and the height of the seeds relative to clothing items.

As the current experiments were conducted at the end of a wet (good) growing season, at the peak time of seed maturation and release (author. obs.), hiking at other times of the year or in

other locations may result in fewer seeds collecting per clothing item. For example, a preliminary study conducted in a conservation reserve in the subtropics of Australia, for example, only collected 103 seeds from 13 species on the same four clothing items (e.g. socks, boots, laces and trousers) after 800 m of hiking through a disturbed grassland in a conservation reserve (unpublished data).

4.6. Limitation of the current research

The current research was designed to assess the number and type of seeds that could be collected on clothing worn by people hiking in a protected area, and did not look directly at seed dispersal. Only two out of nine studies of clothing as a seed vector examined dispersal (Bullock and Primack, 1977; Wichmann et al., 2009). They found that seed could be dispersed over long distances when attached to clothing, with seed still present on soil in the tread of shoes at 5 km (Wichmann et al., 2009) and attached to trouser material at 2.4 km (Bullock and Primack, 1977). Research assessing dispersal distances for more than the five species so far examined is required. Experiments comparing attachment among species that standardizes exposure and that measured dispersal kernels could identify plant traits that facilitate short and long distance dispersal by humans (Wichmann et al., 2009). This could include comparing the long distance dispersal potential of clothing between invasive and non invasive species. For example, dispersal distances could be examined for species such as the native *Acaena novae-zelandiae* and the invasive species *Anthoxanthum odoratum*, *Festuca nigrescens*, *Actosella vulgaris*, *Dactylis glomerata* and *Poa pratensis* that were found in the current study to have large amounts of seed attached to clothes. This type of research would assist in our understanding of invasion, as seed dispersal is a crucial, but under researched stage of the invasion process (Puth and Post, 2005), and humans are an important, but poorly understood mechanism for long distance dispersal (Nathan, 2006; Wichmann et al., 2009).

Research that assesses what seeds people may introduce into a park on their clothing when they first arrive would also compliment the studies already completed on dung (Whinam et al., 1994; Weaver and Adams, 1996; Campbell and Gibson, 2001; Cosyns and Hoffmann, 2005; Couvreur et al., 2004; Wells and Lauenroth, 2007; Gower, 2008) and vehicles (Schmidt, 1989; Lonsdale and Lane, 1994; Hodkinson and Thompson, 1997; Zwaenepoel et al., 2006; von der Lippe and

Kowarik, 2007). Other areas for further research would be to examine the potential for mountain bike riding, quad bikes and horse riding (coats) used for tourism to act as vectors for seeds in protected areas.

4.7. Management implications

Organizations, involved in the control of non-native plants need to consider the implications of the large number and diversity of seeds that can be carried on clothing. As roadside vegetation is often dominated by non-native species, as peoples clothing (Table 1, Appendix A) and vehicles can collect many of these species (Schmidt, 1989; Lonsdale and Lane, 1994; Hodgkinson and Thompson, 1997; Zwaenepoel et al., 2006; von der Lippe and Kowarik, 2007), strategies to reduce the number of seeds present on roadsides are important. For example, the common practices of spraying and/or mowing roadsides prior to seeding can not only reduce the seed bank on the roadside, but can reduce the potential for people and vehicles to collect and transfer seeds.

People can change what they wear, where they go and what they do in ways to reduce their potential as seed vectors. This includes checking if seeds are already on clothing prior to travel to new destinations. Covering socks with trousers or gaiters and replacing laces is also likely to reduce the number of seeds collected, while changing the type of sock worn could reduce the range of seeds collected even when socks are uncovered. Whilst in areas particularly at risk from non-native plants such as protected area, people should avoid walking or parking on roadsides, etc, particularly when plants are seeding. Education about not picking seeds off clothing when sitting down to lunch, or at the end of a hike, may also prevent the establishment of species in places that may already be disturbed and hence more suitable for non-native plants. Basically a precautionary principle needs to be applied to clothing as a seed vector as the current research has clearly demonstrated that large numbers of seed from a wide range of species can collect on clothing.

Conclusions

A large amount of seed from a range of species can attach to clothing including socks, shoes, laces and trouser legs after even a short hike through roadside vegetation in a protected area.

This includes seed from a range of non-native invasive species. Different types and combinations of clothing can alter the amount and type of seed dispersed, although there is high variability even among the seed on the left and right sock of a hiker. Further research is required on unintended human seed dispersal including in a wider range of ecosystems/continents, on dispersal as well as attachment, and that identifies plant traits that may facilitate adhesion dispersal on humans.

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Table 1.
 Details of the current and nine other published studies of clothing as a seed vector.

Source	Country	Type of clothing					Design	Methods used				Diversity collected		
		Shoes	Socks	Laces	Trousers	Other		Rep.	# per item/area	Manip.	Statistics	Fixed # species	# taxa	# species
Woodruffe-Peacock 1918	England	Yes				Yes	Obs.	No	No	No	No	No	20	20
Healy 1943	New Zealand		Yes		Yes		Obs.	No	Yes	No	No	No	33	1
Clifford 1956	England	Yes					Obs.	Yes	No	No	No	No	42	39
Falinski 1972	Poland	Yes	Yes	Yes	Yes		Exper.	Yes	No	Yes	Yes	No	32	30
Bullock and Primack 1977	Costa Rica				Yes	Yes	Exper.	Yes	Yes	Yes	Yes	Yes	3	2
Wace 1985	Australia	Yes	Yes		Yes		Obs.	Yes	No	No	No	No	38	24
Higashino et al. 1983	Hawaii	Yes					Exper.	Yes	No	No	No	No	16	12
Whinam et al. 2005	Australia	Yes	Yes			Yes	Exper.	Yes	No	No	No	No	81	32
Wichmann et al. 2009	England	Yes					Exper.	Yes	Yes	Yes	Yes	Yes	2	2
This study	Australia	Yes	Yes	Yes	Yes		Exper.	Yes	Yes	Yes	Yes	No	70	50
Total														179

Table 2

The number of seeds and species richness (mean \pm standard error) of seed on socks worn for five minutes hiking on the Roadside, worn only in the Natural vegetation over a days hike, or worn in All Day (roadside and natural vegetation) in Kosciuszko National Park, Australia. N = 9. The number of seeds data was natural log transformed prior to analysis. Bold P values are significant at $P < 0.05$.

	All Day	Roadside	Natural	d.f.	F	P
Number of seeds						
Total over all socks	4590	5877	1524			
All seeds per sock	510 \pm 189	648 \pm 283	169 \pm 70	2	14.487	0.003
<i>Acaena</i> sp. per sock	374 \pm 176	582 \pm 289	78 \pm 28	2	0.924	0.417
Other native per sock	69 \pm 31	4 \pm 2	55 \pm 25	2	13.600	0.004
Non-native seed per sock	68 \pm 43	61 \pm 29	33 \pm 22	2	4.666	0.025
Species richness						
Sum over all socks	31	20	36			
All species per sock	10.9 \pm 1.2	6.3 \pm 0.7	10.7 \pm 1.8	2	6.422	0.009
Native species per sock	5.8 \pm 0.8	1.4 \pm 0.4	5.1 \pm 0.9	2	15.077	<0.001
Non-native species per sock	4.1 \pm 0.5	3.8 \pm 0.7	3.7 \pm 0.8	2	0.248	0.783

Table 3

Contrasts from significant Repeated Measures ANOVA comparing number of seed and species richness of seed on socks worn for five minutes hiking on the Roadside, worn only in the Natural vegetation over a days hike, or worn in All Day (roadside and natural vegetation) in Kosciuszko National Park, Australia. Bold P values are significant at $P < 0.05$.

	All Day Sock		Roadside Sock	
	F	P	F	P
Number of seeds				
All seeds				
Roadside sock	0.905	0.369		
Natural vegetation sock	12.999	0.007	0.930	0.363
Native seed				
Roadside sock	29.381	0.001		
Natural vegetation sock	1.483	0.258	20.493	0.002
Non-native seeds				
Roadside sock	0.190	0.675		
Natural vegetation sock	4.932	0.057	10.446	0.012
Species richness				
Total species				
Roadside sock	26.000	0.001		
Natural vegetation sock	1.600	0.242	11.524	0.009
Native species				
Roadside sock	15.858	0.004		
Natural vegetation sock	0.029	0.868	5.930	0.041

Table 4

The number of seed and species richness (mean \pm standard error) of seed per sock per person collected on the Hiking socks and Sports socks over a five hour hike in alpine vegetation in Kosciuszko National Park, Australia. Values are average per sock per person, N = 5 people per sock type. The number of seed data was natural log transformed prior to analysis. Bold P values are significant at $P < 0.05$.

	Hiking	Sport	Total	d.f.	F	P
Number of seed						
Sum over all socks	796	1286	2 037			
All seed per sock	77 \pm 18	127 \pm 30	102 \pm 19	8	1.345	0.216
All seed without <i>Acaena</i>	43 \pm 9	104 \pm 25	73 \pm 16	8	2.634	0.030
<i>Acaena</i> sp. per sock	34 \pm 21	23 \pm 17	29 \pm 123	8	0.192	0.852
Other native per sock	42 \pm 9	92 \pm 19	66.5 \pm 13	8	2.490	0.037
Non-native seed per sock	2.0 \pm 0.6	2.6 \pm 0.5	2.3 \pm 0.4	8	2.00	0.081
Species richness						
Sum over all socks	29	35	43			
All seed per sock	8.6 \pm 0.6	12.5 \pm 1.1	10.6 \pm 0.9	8	2.961	0.018
Native seed per sock	8.4 \pm 0.9	10.8 \pm 0.9	9.6 \pm 0.7	8	1.897	0.094
Non-native seed per sock	2.0 \pm 0.6	2.6 \pm 0.5	2.3 \pm 0.4	8	0.739	0.481

Table 5

The number of seed and species richness of seed collected on the boots, socks, laces and leg (flesh or trouser material) for a bare leg and one covered by a trouser, worn whilst hiking for 100 m through roadside vegetation along the Kosciuszko Road. N = 20 replicate 100m hikes. There were a total of 32 species found seeding over all 20 hikes.

Item	Trouser Leg		Bare Leg	
	Total	Mean \pm SE	Total	Mean \pm SE
# seed per item				
Boot	1209	60.5 \pm 26.2	1427	71.4 \pm 23.6
Sock	202	10.1 \pm 5.8	3143	157.3 \pm 75.8
Lace	607	30.4 \pm 29.9	1314	65.7 \pm 50.1
Leg	2886	144.5 \pm 126.6	6	0.3 \pm 0.1
Total per leg	4904	245.4 \pm 184.3	5890	294.7 \pm 147.4
Species richness per item				
Boot	13	3.3 \pm 0.3	13	2.8 \pm 0.3
Sock	5	1.2 \pm 0.1	9	2.7 \pm 0.2
Lace	4	0.4 \pm 0.2	6	0.8 \pm 0.2
Leg	7	1.0 \pm 0.3	2	0.3 \pm 0.1
Total per leg	13	3.6 \pm 0.4	13	3.7 \pm 0.3

Table 6

Results from Repeated Measures ANOVA comparing the difference in (a) the number of seed (log transformed) (b) the number of seed adjusted for the surface area of the item and then log transformed) and (c) the species richness per item among the four items of clothing worn when hiking through roadside vegetation along the Kosciuszko Road. Bold P values are significant at $P < 0.05$. d.f. = 17.

	F	P
(a) # seed		
Trouser leg	34.378	< 0.001
Bare leg	65.520	< 0.001
(b) # seed adjusted for surface area		
Trouser leg	39.858	< 0.001
Bare leg	51.928	< 0.001
(c) Species richness		
Trouser leg	35.195	< 0.001
Bare leg	51.892	< 0.001

Table 7

Contrasts from Repeated Measures ANOVA and t-tests among clothing items. Statistical tests comparing the difference in (a) the number of seed (log transformed) (b) the number of seed adjusted for the surface area of the item and then log transformed and (c) the species richness per item among the four items of clothing worn when hiking through roadside vegetation along the Kosciuszko Road. Values in light grey are for contrasts from Repeated measures ANOVA among items on the trouser (covered) leg. Values in dark grey are for contrasts from Repeated measures ANOVA among items on the bare (uncovered) leg. Values on the diagonal are from paired t-tests for individual items between the covered and uncovered leg. Bold P values are significant at $P < 0.05$. d.f. = 17.

	Boot		Sock		Lace		Bare Leg	
	F	P	F	P	F	P	F	P
(a) # seed								
Boot	1.412	0.174	68.648	<0.001	126.980	<0.001	37.854	<0.001
Sock	4.477	0.048	10.734	<0.001	6.143	0.023	0.170	0.898
Lace	36.892	<0.001	41.512	<0.001	2.419	0.026	5.468	0.030
Trouser material	174.607	<0.001	132.888	<0.001	5.877	0.025	2.265	0.035
(b) # seed adjusted for surface area								
Boot	1.215	0.239	28.278	<0.001	16.103	0.001	157.377	<0.001
Sock	14.747	<0.001	7.245	0.001	22.664	<0.001	155.692	<0.001
Lace	55.212	<0.001	11.994	0.003	2.399	0.027	6.196	0.022
Trouser material	32.359	<0.001	6.992	0.016	3.902	0.063	2.607	0.017
(c) Species richness								
Boot	1.675	0.110	0.028	0.869	52.937	<0.001	116.893	<0.001
Sock	33.374	<0.001	4.807	<0.001	38.585	<0.001	138.568	<0.001
Lace	126.585	<0.001	16.800	<0.001	2.651	0.016	4.561	0.046
Trouser material	40.007	<0.001	2.654	0.120	7.969	0.011	2.333	0.031

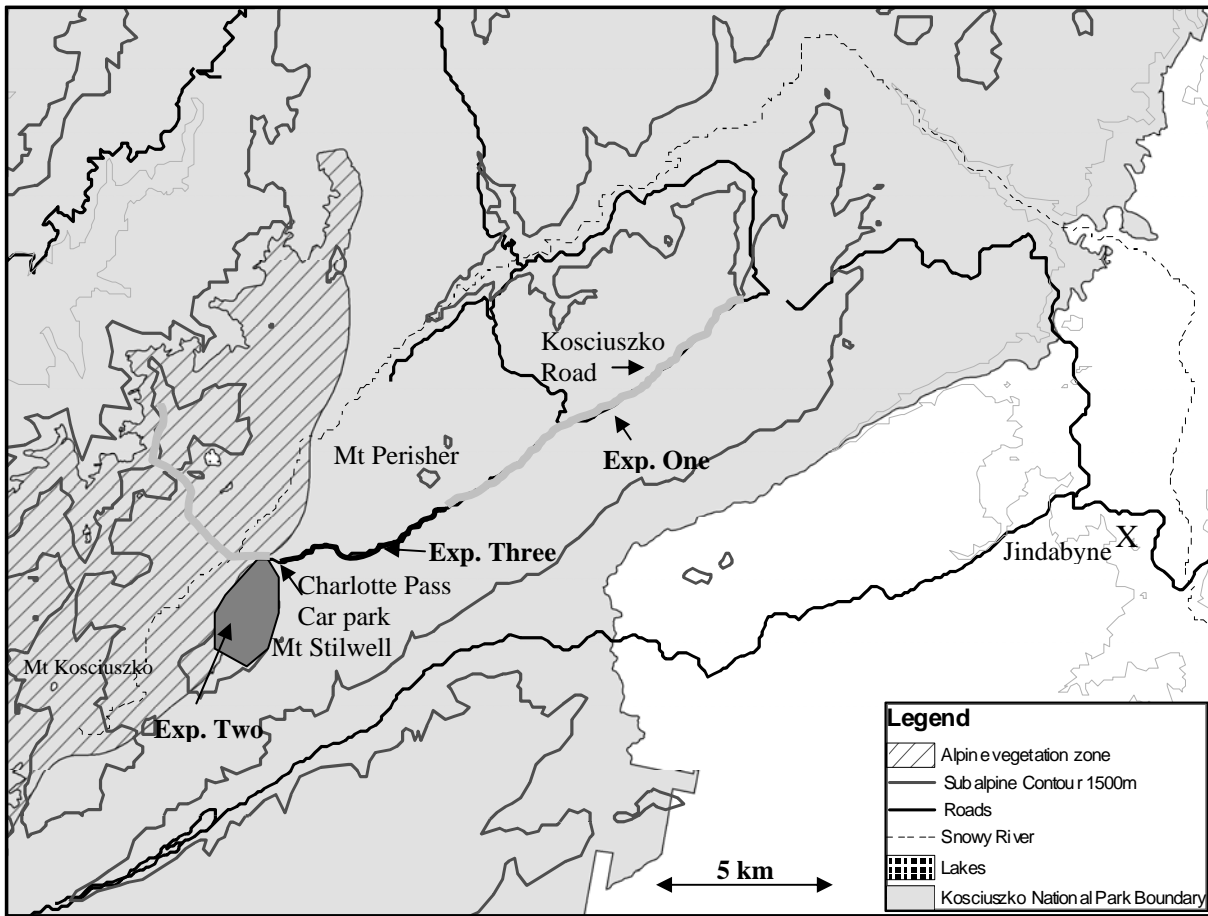


Fig 1. Location of seed collection experiments in alpine and subalpine zones of Kosciuszko National Park, Australia: Exp. 1 What seed do socks collect?, Exp. 2 Sock types, and Exp. 3 Clothing item comparison.



Fig. 2. Clothing worn to assess the seed collected on different items of clothing when hiking through roadside vegetation along Kosciuszko Road, Kosciuszko National Park, Australia

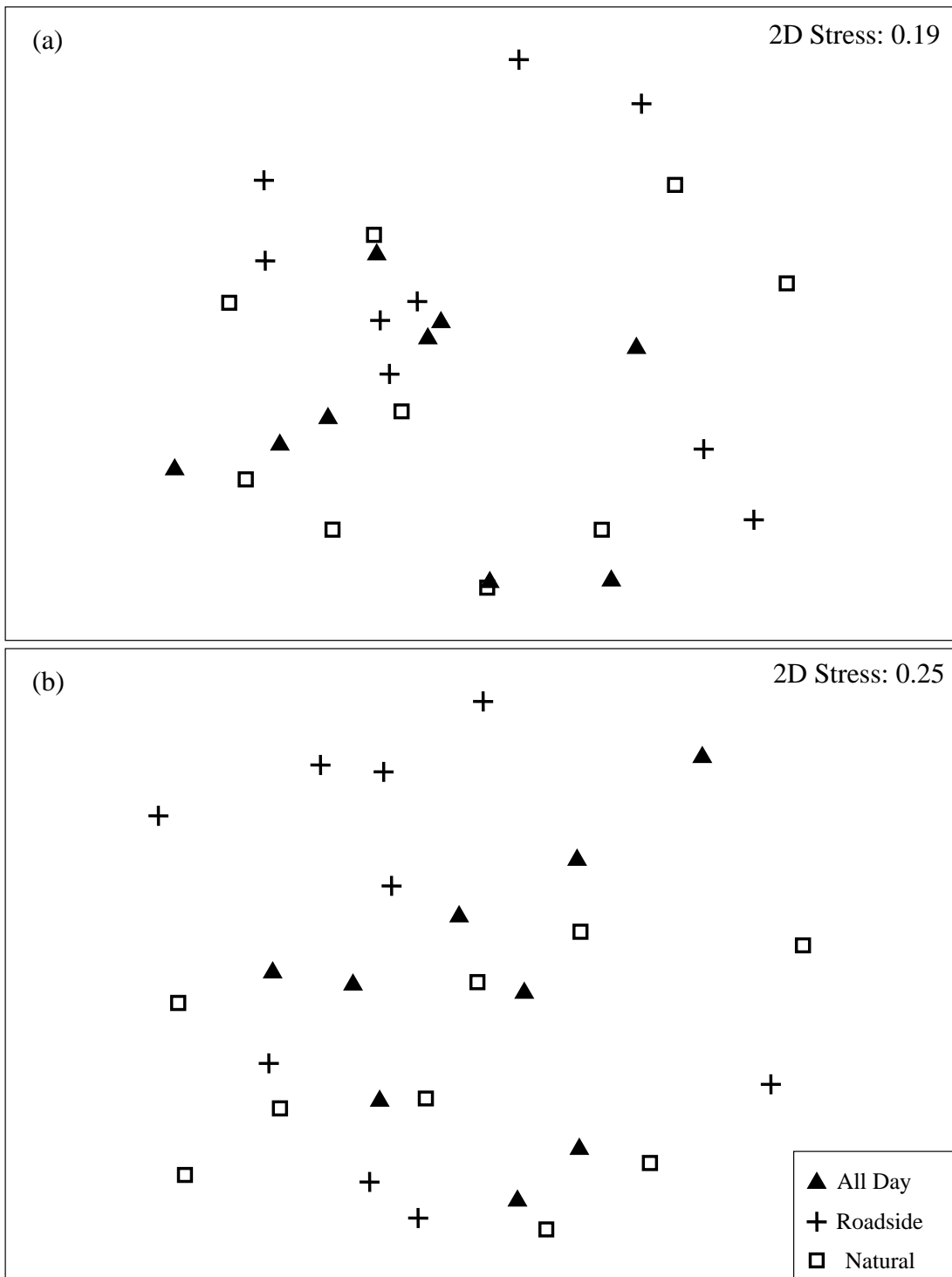


Fig. 3. Multidimensional scaling ordination plot of the composition of seed collected on the All Day sock, Roadside sock and Natural vegetation sock. (a) $\log(n + 1)$ of seed composition. (b) presence/absence of species on socks.

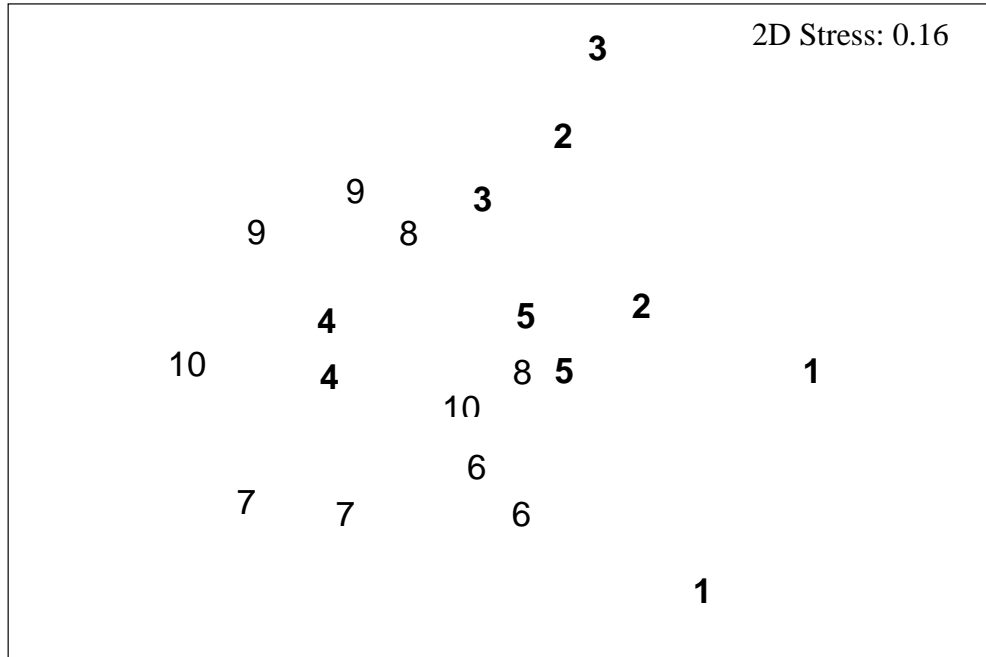


Fig. 4. Multi-dimensional scaling ordination plot of the composition of seeds collected ($\log(n + 1)$) on either Hiking (Bold, individuals 1-5) or Sports socks (unbold, individuals 6-10) when undertaking a five hour hike through Kosciuszko alpine area. Each number represents is an individual hiker, with each number shown twice, once for each sock the individual wore hiking e.g. the two bold number ones represent the composition of the left and right socks worn by the first of ten individuals who went hiking.

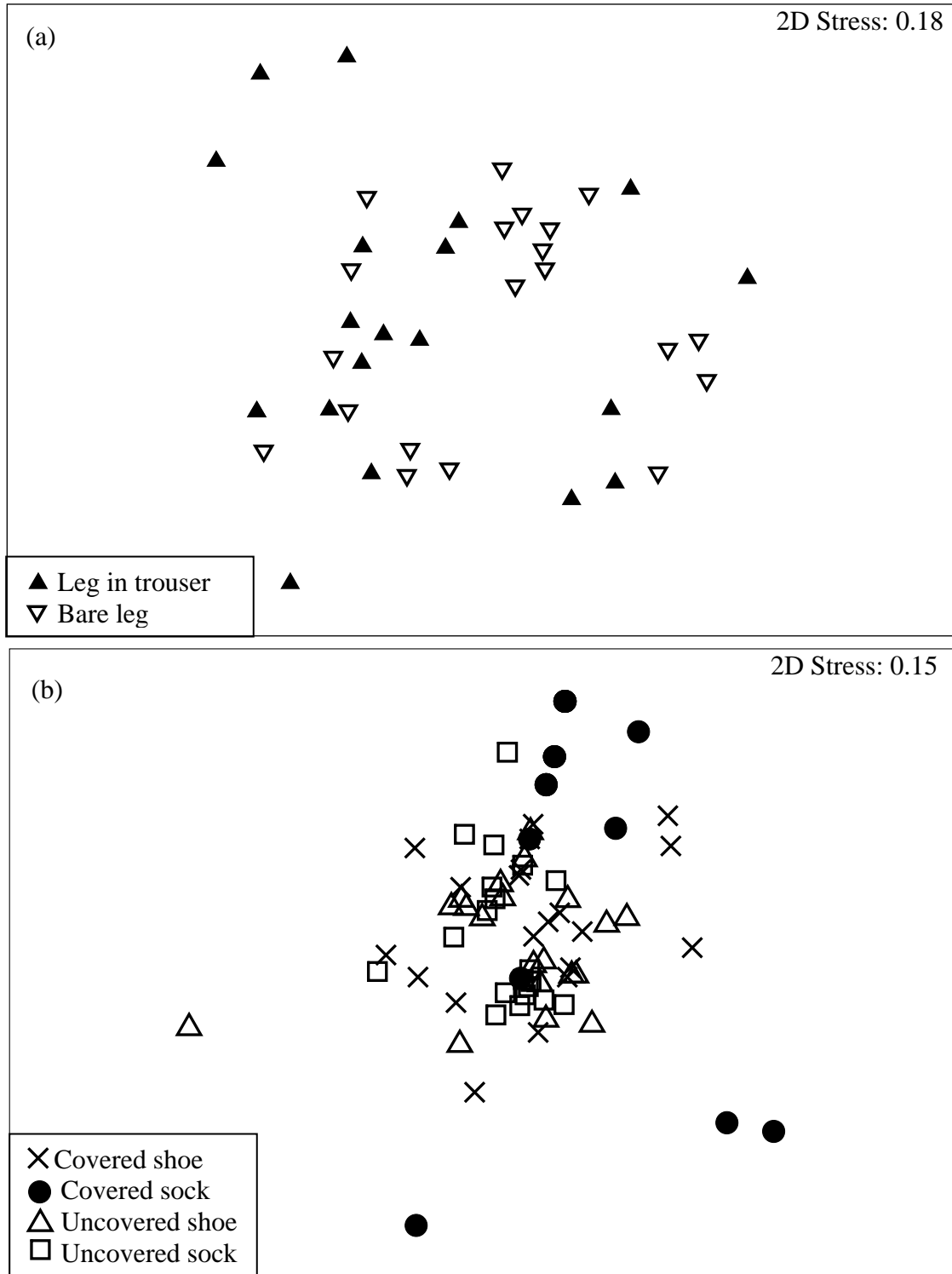


Fig. 5. Multidimensional scaling ordination plot showing the similarity in species composition ($\log(n + 1)$) collected on (a) the leg covered with a trouser and the bare leg (b) the shoes and socks covered by a trouser and the shoes and socks left uncovered on a leg wearing shorts worn whilst hiking through roadside vegetation along the Kosciuszko Road.