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1   **Pilot testing of a sampling methodology for assessing seed attachment propensity and**

2   **transport rate in a soil matrix carried on boot soles and bike tires**

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15   **ABSTRACT**

16   Land managers of natural areas are under pressure to balance demands for increased recreation

17   access with protection of the natural resource. Unintended dispersal of seeds by visitors to natural

18   areas has high potential for weedy plant invasions, with initial seed attachment an important step in

19   the dispersal process. Although walking and mountain biking are popular nature-based recreation

20   activities there are few studies quantifying propensity for seed attachment and transport rate on

21 boot soles and none for bike tires. Attachment and transport rate can potentially be affected by a

22 wide range of factors for which field testing can be time-consuming and expensive. We pilot tested a

23 sampling methodology for measuring seed attachment and transport rate in a soil matrix carried on

24 boot soles and bike tires traversing a known quantity and density of a seed analog (beads) over

25 different distances and soil conditions. We found % attachment rate on boot soles was much lower

26 overall than previously reported but that boot soles had a higher propensity for seed attachment

27 than bike tires in almost all conditions. We believe our methodology offers a cost-effective option

28 for researchers seeking to manipulate and test effects of different influencing factors on these two

29 dispersal vectors.

30 **Keywords:**   weeds; seed attachment; human-mediated dispersal; tourism impacts

31 **1.   Introduction**

32 Invasive alien species of plants (weeds), together with animals, fungi and microbes are widely

33 recognised as posing a major threat to global biodiversity, second only to habitat destruction in their

34 impact (Randall, 1996; Vilà *et al*, 2011; Wittenberg and Cock, 2001; World Conservation Union

35 [IUCN], 2000). Weeds have been shown to cause billions of dollars of annual economic loss in

36 agriculture and forestry (Pimentel et al, 2001; Pimentel, 2002; Williams et al, 2010). They have also

37 been shown to alter ecological processes, degrade ecosystem services and disrupt ecological

38 integrity (DiTomaso, 2000; Mack and D'Antonio, 1998; Pejchar and Mooney, 2009; Pimentel, 2002;

39 Williams *et al*, 2010). Dispersal of weeds can occur via a variety of diaspores, including as adult

40 individuals, ramets, bulbs or seeds, and can be mediated both by natural vectors, e.g., wind, rain,

41 flowing water, animals, by humans or a combination of these (Nathan, 2006; Ridley, 1930,

42 Wichmann et al, 2009). Studies have shown that dispersal of even small numbers of seeds, especially

43 over large distances, can cause disproportionally large changes in ecological patterns (Cain, Milligan

44 and Strand, 2000; Higgins, Nathan and Cain, 2003; Nathan, 2006).

45   One human activity with high potential for unintentional dispersal of weed seeds is tourism

46   (including recreation). People today, especially in economically developed countries, have increasing

47   time for leisure (Molitor, 2000) and international tourism has demonstrated rapid and almost

48   continual growth in recent decades, with over 1 billion international tourists recorded in 2012

49   (UNWTO, 2013). Risk of human-mediated dispersal of seeds by recreation may be especially

50   important in protected natural areas, where it may be one of only a few human activities allowed

51   (Newsome, Moore and Dowling, 2002; Worboys, DeLacy and Lockwood, 2005) and where

52   introduced seeds may develop into invasive environmental weeds. Research has shown an

53   association between weed presence and tourism infrastructure in natural areas, especially adjoining

54   roads and tracks (Pickering, Bear and Hill, 2007; Potito and Beatty, 2005, Spellerberg, 1998) and

55   increasing weed diversity with increasing tourist visitation (Usher, 1988).

56   A small but growing number of studies have shown capacity for unintentional human-mediated

57   dispersal of seeds by tourists, either attaching directly to hikers' clothing or equipment, embedded

58   in soil picked up by vehicles, or animal dung/feed (for comprehensive reviews see Pickering and

59   Mount, 2010; Ansong and Pickering, 2013 and 2014). The number of seeds dispersed by such vectors

60   can be large (e.g., ≈1300 on a walker's socks after only a five minute hike through roadside

61   vegetation: Mount and Pickering, 2009) and of high species richness (e.g., > 750 species collected

62   from various tourism-related vectors: Pickering and Mount, 2010), of which a high proportion have

63   typically been subsequently identified as national or international invasive species (Mount and

64   Pickering, 2009).

65   Despite such demonstrated potential, controlled experiments to quantify propensity for seed

66   attachment and/or dispersal by people while hiking, either attaching directly to clothing or

67   embedded in a soil matrix carried on boot soles, are scarce. We found only two studies that

68   experimentally tested direct seed attachment rates on human skin/clothing (boots, socks, laces &

69   trousers: Falinski, 1972; boots, socks, laces, trousers and bare legs: Mount and Pickering, 2009) and

70 only a single study of seed attachment in a soil matrix carried on boot soles: Wichmann et al, 2009).

71 We also found only four studies that experimentally tested dispersal of seeds attaching directly to

72 clothing (trousers and shirts: Bullock and Primack, 1977; boots, socks, outer clothing and personal

73 luggage: Lee and Chown, 2009; trousers and socks: Ansong, Pickering and Arthur, 2015; Pickering,

74 Mount, Wichmann and Bullock, 2011) and a single study of seed dispersal via a soil matrix on boot

75 soles (Wichmann et al, 2009). Even within the few aforementioned experimental studies on seed

76 attachment on boots, relatively few factors affecting attachment rates appear to have been tested,

77 i.e. distance walked (Falinski, 1972), trousered vs bare leg (Mount and Pickering, 2009) and seed

78 species, individual walkers and boot types (Wichmann et al, 2009). Research on the effects of other

79 potentially important factors, for example seed size, mass and morphology, soil type and condition

80 (e.g., wet vs dry), appears to be scarce.

81 Alongside hiking, another recreation activity with high potential for weed seed introduction and/or

82 dispersal is off-road cycling ('biking') (Pickering, Hill, Newsome and Leung, 2010). Biking is

83 increasingly popular globally in backcountry/wilderness protected areas such as national parks

84 (Burgin and Hardiman, 2012; Hardiman and Burgin, 2013) and in open access peri-urban natural

85 areas (Chiu and Kriwoken, 2003) and its growth has led to increasing user group pressure for greater

86 access to natural areas. Although a small number of experimental studies have attempted to

87 measure biking's absolute and relative potential (e.g. vs hiking) for direct environmental degradation

88 of such factors as increased soil exposure, decreased vegetation cover and/or species richness (e.g.,

89 Newsome and Davies [2009]; Pickering, Rossi and Barros [2011]; Thurston and Reader [2001]), no

90 published studies to date have experimentally tested seed attachment or dispersal propensity on

91 mountain bike tires, either in absolute terms or relative to boot soles.

92 The propensity for attachment and dispersal of seeds in a soil matrix on boot soles or bike tires is

93 likely to differ for many reasons. Some key variables include: (i) available surface area of soles vs.

94 tires (tires larger than boots [Thurston and Reader, 2001]); (ii) ground contact pattern (boots:

95   discrete steps and equal distance covered by each boot; tires: continuous contact and different

96   ground contact distance covered by front and rear tires); (iii) ground contact pressure (biker higher

97   than walker [Thurston and Reader, 2001]); (iv) different tread patterns and depth of soles/tires; (v)

98   distance covered (bike riders typically travel faster and further than walkers for a given time/effort);

99   (vi) soil type and; (vii) soil condition (e.g. moisture content). The number and density of seeds

100 available for attachment, along with differences in their size, morphology, weight and surface

101 adhesion qualities, also potentially affect their attachment and/or dispersal rate. Field testing of

102 such multiple variables is typically time-consuming and expensive. Researchers therefore need a

103 sampling methodology that allows control of such variables while still representing 'real world'

104 behaviour. This study sought to fill an existing knowledge gap by testing a potential sampling

105 methodology for experimentally testing the absolute and relative propensity for seed attachment

106 and transport in a soil matrix (a) on boot soles and bike tires (b) in wet or dry soil (c) over different

107 distances travelled.

108 **2.   Methods**

109 **2.1   Procedure**

110 We constructed a circular, prefabricated track measuring 0.75m wide with 50mm sidewalls and

111 external radius of 2.75m and internal radius 2.0m, giving a track centre line circumference of 14.92m

112 and surface area of 11.18m^2.The track was designed to simulate the width of a typical outdoor trail

113 and allow for a normal walking and cycling movement. Testing of different track widths and

114 circumferences showed that this was the smallest size in which a typical bike could be ridden in a

115 'normal' fashion (i.e. without the riders' feet or hands touching the ground or a wall for balance

116 support).

117 In real world conditions, the number and/or density of seeds available for attachment and dispersal

118 is likely to be highly variable and affected by many external factors; definition of what is a 'realistic'

119 and 'biologically-relevant' number and/or density is therefore situation-specific. To provide a

120 benchmark, however, we designed our seed/soil density to be comparable to that used in the

121 experiment by Wichmann *et al*. (2009). The aims and sampling methodologies of the two

122 experiments were very different, however. In Wichmann *et a*l's (2009) study, the researchers'

123 primary focus was on measuring seed dispersal rate carried in a soil matrix in boot soles over

124 distance, and their sampling protocol aimed to maximise initial seed attachment. They used 500g

125 (volume unspecified, probably ~ 0.5 litre) of a 'sandy silty loam' soil, oven dried at 30^0C, spread

126 evenly in a tray (400mm x 250mmm; soil depth unspecified), wetted with 50ml of water using a

127 plant mister and stirred (moisture level unspecified). A walker then placed both shoe-clad feet in the

128 tray and took 20 steps on the spot to pick up soil. The walker then stepped into a second tray

129 (unspecified; assumed to be of same dimensions as Tray 1) containing 100 evenly spread seeds,

130 either *Brassica oleracea* [wild cabbage] or *Brassica nigra* [black mustard], again taking 20 steps on

131 the spot. Assuming Tray 1 was filled to a soil depth of 20mm and Tray 1 and Tray 2 were of

132 equivalent dimensions, this would suggest a soil area of 100,000mm^2 and density of seeds

133 100/100,000mm^2 = 0.001 seeds/mm^2, although the actual density of seeds exposed to the boot soles

134 was probably much higher than this: 'probably artificially high' (Wichmann et al, 2009, p. 525, 530).

135 The number of seeds attaching was calculated by subtracting the number left in the tray from 100,

136 yielding the pickup rate (Wichmann *et al*, 2009, p. 524).

137 We used:

138 (1) 240 litres of soil spread evenly on the sampling track to an approximate depth of 20mm (0.02m

139 depth x 11.18m^2 area = 0.2236m^3). We used a commercially-obtained loam-based soil ("J. Arthur

140 Bower's Topsoil" TM: William Sinclair Horticulture Limited, 2008).

141 (2) 11,180 'seeds' (11.18m^2 area x 0.001 seeds/mm^2 = 11,180), i.e. 50 'seeds'/litre of soil (vs at least

142 200 seeds/litre of soil in Wichmann et al [2009]). Wichmann *et al* (2009) used a *Brassica*-species

143 seed, artificially coloured to aid on-ground identification. As artificially colouring the much larger

144 quantity of seeds we used was impractical, we used synthetic "seed beads" ('Size 11 Japanese Toho'

145 TM: Product code 11R43F; Beads Direct, 2013), purchased in a bright blue colour. The beads were

146 roughly spherical in shape and sampling measurements showed a mean maximum diameter 2.1mm

147 (SE = 0.07mm) and mean minimum diameter 1.6mm (SE = 0.09mm), making them comparable in size

148 and shape to the *Brassica* spp. employed by Wichmann *et al* (2009). The beads were sprinkled

149 evenly over the soil surface and mixed in by light raking before each sampling replicate.

150 The sampling track was set up indoors on the University of Kent's Canterbury campus and sampling

151 was undertaken on the 4th, 6th and 7th September, 2013.

152 **2.2   Design**

153 The experiment was a 2×2×2 factorial design with factors Vector ("boot" vs "bike"), Soil Condition

154 ("moist" vs "wet") and Traversal Distance ("short" vs "long"). For operational reasons (e.g., "wet"

155 and "moist" could not be randomised), testing followed a systematic sampling order: boot, moist,

156 short; boot, moist, long; bike, moist, short; bike, moist, long; boot, wet, short; boot, wet, long; bike,

157 wet, short; bike, wet, long. The complete sequence was replicated 7 times.

158 The Vector "Boot" comprised one pair of newly-purchased general purpose wellington boots

159 ("Traditional Green PVC Wellington Boot", British size 8, heel/sole tread depth 10mm/5mm; Briers,

160 2011). "Bike" was a "hybrid" road/off road bicycle with side-pull caliper brakes and new tires (Claud

161 Butler "Urban 2000' 18" frame with Meghna "Explorer" 700mm x 38mm tires, with a tread depth

162 2mm).

163 Soil condition (MEA, 2013) was measured at the beginning, middle and end of each testing day,

164 using a Lutron soil moisture meter PMS-714 (Lutron, undated). "Moist" soil ranged between 18.7%-

165 21.6% during testing. After completion of moist testing, water was mist sprayed incrementally and

166 evenly onto the soil from a handheld garden sprayer and "wet" soil was >50% (moisture meter

167 maximum reading) throughout testing.

168 The Traversal Distance "short" test comprised one complete circuit of the track (≈15m) and a "long"

169 test comprised 10 circuits (≈150 m). Walking circuits were standardised to 25 discrete paces/circuit

170 (both feet combined). The same team member completed all walks and rides in an anticlockwise

171 direction.

172 On completion of each designated walk/ride distance, the walker stepped/bike was lifted carefully

173 into a sorting tray measuring 2300mm x 500mm x 50mm with a bright white base. Then during a

174 timed 10 minute period all the soil and beads adhering to boots/tires were carefully brushed off. The

175 beads were found (facilitated by their bright blue colour) and counted by team members using LED

176 head torches and magnifying glasses. After counting, beads were cleaned and, together with the soil

177 from the sorting tray, sprinkled evenly back around the track and the soil was raked over before

178 commencing the next test.

179 **2.3   Analyses**

180 As the outcome variable, the number of beads attaching, is a non-negative count, data were

181 analysed using (i) one-way ANOVA for testing bead attachment rate between left vs right boot soles

182 and front vs rear bike tires; and (ii) count models (Hilbe, 2011; Ridout, Demétrio and Hinde, 1998).

183 for testing main and interaction effects of the three factors: Vector (Boots; Tires), Soil Condition

184 (Moist; Wet) and Traversal Distance (Short; Long); replicate number was also included in the analysis

185 as a blocking factor, but was not significant. Poisson and negative binomial count models were

186 considered. For several of the 8 treatment combinations, variation between replicate counts was

187 much greater than would be expected if counts followed a Poisson distribution. Due to this over-

188 dispersion, a negative binomial model was used for analyses of the three factors. Analyses were

189 conducted in R, version 3.1.1 (R Core team, 2014). Results were accepted as significant at or below

190 the 5 % probability level.

191 **3.   Results**

192 Beads were only recorded attaching to boots and tires along with soil; no "bead-only" attachment

193 was recorded under any sampling parameter combination. We observed that boots predominantly

194 tended to pick up soil and beads in the heel treads, with soil tightly compacted and requiring beads

195 to be physically extracted by the researchers, with very few beads (estimated <5%) attaching to the

196 remainder of the soles. One-way ANOVA testing revealed no significant difference in bead

197 attachment quantity or % rate between left and right boots for all parameter combinations ($F_{1,54}$ =

198 1.49, P = 0.23). In contrast, bike tires showed a significant difference ($F_{1,54}$ = 15.30, P < 0.0003) in

199 bead attachment quantity and % attachment rate between front and rear tires, with attachment on

200 the front tyre at least an order of magnitude higher than the rear for all sampling parameter

201 combinations except "short traversal, moist soil" (zero bead attachment recorded on both tires for

202 all replicates, see Table 1 and 2).

203 The negative binomial model provided adequate fit for the data; that is predicted seed-counts did

204 not differ significantly from the observed data, $\chi^2(49)$ = 62.49, p < 0.093. Observed bead counts and

205 attachment rates are therefore reported here (Table 1 and 2). Model-parameters, fit-indices and

206 selection-criteria for the negative binomial model are reported, together with significance values for

207 each effect, in Table 3. The model's intercept represents an arbitrarily chosen baseline for

208 comparison, in this case the bike/long/moist condition. The log-coefficient for the intercept

209 represents the estimated number of seeds in that condition once exponentiated, so exp(0.81) = 2.25

210 seeds in the bike/long/moist condition. As previously mentioned, model-estimated and actual

211 number of seeds (2.9) did not significantly differ and, therefore, actual seed numbers are reported in

212 Table 1. Condition effects in the model are calculated by adding relevant coefficients for main- and

213 interaction-effects to the baseline before exponentiation. For example, to calculate the estimated

214 number of seeds in the boot/long/wet condition, we added estimates for the Intercept, Vector, Soil

215 Condition, and Vector x Soil Condition: exp(0.81+1.70+2.83+(-2.23)) = 22.42 seeds, actual seed

216 number = 24.7. Note that significant main effects should not be interpreted in the negative binomial

217 model in the presence of significant interactions as they may be misleading. Condition analyses

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218   showed that, whilst there were significant effects of each of the three experimental factors (Vector,

219   Soil Condition and Traversal Distance), all but one (Soil Condition x Traversal Distance) of the

220   interactions between these factors were also statistically significant (Table 3). Owing to the

221   complexity of these results and to avoid extensive statistical copy, results are summarised in the

222   following plain text. Consistently more beads attached over the long traversal distance than over the

223   short traversal distance; however the ratio of short to long was variable. More beads attached under

224   wet conditions than under moist conditions, although again the ratio of wet to moist was variable.

225   Generally, more beads attached to boots than to bike tires under the same conditions, but again the

226   ratio was variable and this pattern reversed under the long wet conditions (Table 1). In summary,

227   bead attachment was higher for longer traversals and under wet soil conditions. Bead attachment

228   was generally higher on boots than on tires, except when traversal distance was long and the soil

229   condition was wet. Mean % attachment rate of beads from total available (11,180) was very low

230   over all treatment combinations, ranging from 0.07% (SE = 0.02%) – 0.22% (SE = 0.03%) for boots

231   and 0.00% (SE = 0.00%) – 0.31% (SE = 0.04%) for tires (Table 1).

232   **4.   Discussion**

233   Our finding that bike tires had a lower propensity than boot soles to pick up beads under all

234   conditions tested except over 150m distance travelled in wet soil was initially surprising and

235   counter-intuitive, given the tires' larger overall surface area than the boot soles. However, the result

236   that the bike tires tended to pick up fewer beads than boot soles makes sense, as the tread depth of

237   the tires was shallower (2mm) than that of the boots (sole 5mm; heel 10mm) and hence the

238   beads/soil may not have adhered as tightly to the tires as they did to the bottom of the boot. This is

239   supported by the observation reported during testing that beads attaching to boot soles were

240   predominantly in the heel treads (see Results above). It may be that for shorter distances and/or

241   dryer soils the potentially deeper and narrower tread of the boot soles meant that more beads were

242   retained on boots, but that on a longer rider on wet soil, the greater surface area of the tyre

243 becomes more important, allowing soil to attach over a greater area resulting in more beads

244 attaching. Increasing the density of beads in the soil in a repeat experiment so there are fewer zeros

245 and low numbers attaching may assist in testing this hypothesis.

246 It must also be remembered that beads were only picked up along with soil in our experiment. It is

247 possible that in other circumstances, for example seeds growing on trackside vegetation and

248 possessing traits affecting attachment on walkers'/riders' clothing, for example differing

249 morphology, mass and infructescence height might affect attachment rate, as might walkers' and

250 riders' relative speed of travel along such tracks.

251 Our study gives the first published quantification of the propensity for attachment of a seed analog

252 on bike tires, both in absolute terms and comparative to boot soles. It provides a comparison with

253 the very small number of controlled experiments quantifying seed attachment rate on footwear,

254 either directly or in a soil matrix, for a measured sampling effort (e.g., compare Mount and Pickering,

255 2009; Wichmann et al, 2009). However, comparison of our results with previous studies must be

256 considered relative to the respective studies' very differing sampling protocols and to several

257 important caveats which we detail below.

258 Our "long" test distance (≈150m) was broadly comparable to that employed by Mount and Pickering

259 (2009; Experiment 3) who experimentally tested seed attachment on a single pair of boots worn by a

260 single walker over 100m (n =20). Their mean seed attachment quantity on boot uppers (excluding

261 laces) and soles combined (number attaching specifically to soles unreported) was 60.5 (SE = 26.2)

262 (trousered leg) and 71.4 (SE = 23.6) (bare leg). Our mean observed attachment quantity and

263 variability were substantially lower, both for boots (7.7 [SE = 1.82] – 24.7 [SE = 3.25]) and tires (0.00

264 [SE = 0.00] – 34.6 [SE = 4.42]) under both moist and wet soil conditions (Table 1). However, these

265 results are not directly comparable owing to very different sampling protocols employed: in the

266 Mount and Pickering (2009) study (i) their walker traversed Australian alpine roadside vegetation,

267 not a walking track; (ii) they measured direct seed attachment on the boots from plants and/or loose

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268   seed on the soil surface, not in the soil matrix; (iii) soil was "relatively dry" (moisture level not

269   reported) and no soil was collected on the boots and; (iv) seed quantity available for attachment was

270   unknown.

271   A key issue in all studies attempting to quantify seed attachment rates is 'what constitutes a realistic

272   soil seed density in natural areas?' As previously noted, our experiment employed beads of

273   comparable size, shape and density as the seeds used by Wichmann et al (2009). Our "short" walking

274   distance of 25 steps was also broadly comparable to their sampling protocol of 20 steps. However,

275   as their study was primarily focused on seed dispersal distance, their sampling protocol design was

276   designed to maximise seed attachment and their 20 steps were repeated 'on the spot' in each of

277   two small [0.4 x 0.25m2] trays containing (i) wetted soil (moisture % level not reported) and (ii) 100

278   seeds. They recorded high attachment rates, ranging from (Experiment 1: two seed species, one

279   walker and boot type) 4%-93% attachment, mean 52% and 42%, variability unreported and

280   (Experiment 2: one seed species, 10 walkers, mix of walking/Wellington boots) 26%-52%

281   attachment, mean % and variability unreported**].** The authors noted that their sampling protocol did

282   not match the "real situation" and that their recorded attachment rates were 'probably artificially

283   high' (Wichmann et al, 2009, p. 525, 530). In comparison, our observed attachment rates on boots in

284   the short distance test, under arguably more realistic "real world" conditions, were typically two

285   orders of magnitude lower, with means ranging 0.07% [SE = 0.02%] – 0.18% [SE = 0.03%].

286   Attachment rates on bike tires over the same distance were lower still, with means ranging 0.00%

287   [SE = 0.00%] – 0.14% [SE = 0.05%) (Table 1).

288   **5.   Caveats and Conclusion**

289   Our study suggests potential benefits of a new methodology by which researchers might cost-

290   effectively manipulate and test the effects of different influencing factors on initial seed attachment

291   and transport rate in a soil matrix on boot soles and bike tires, both in absolute and comparable

292   quantities. However, our results are subject to the following important caveats.

293   Firstly, we were using plastic beads as an analog for seeds, not real seeds. However, seeds of

294   different species exist in a wide range of morphologies and adhesive qualities, masses and sizes and

295   we therefore argue that our beads can be considered as a representative analog of real seeds on all

296   three parameters except for the small hole centring the beads. The only two previous controlled

297   studies of direct seed attachment on boots that we found (eg Falinksi, 1972; Mount and Pickering,

298   2009) recorded such diversity, although neither was able to quantify attachment rate in proportion

299   to a known available seed quantity, unlike our study. Only one other controlled study (Wichmann et

300   al., 2009) has tested attachment propensity in a soil matrix on boot soles for pre-selected, specified

301   seed types (2: *Brassica oleracea* ssp. and *Brassica nigra*): as previously noted our beads were

302   specifically selected to be a comparable size and shape to seeds used in that study.

303   Secondly, although our use of the circular test track allowed us to simulate a realistic walking and

304   riding pattern and beads were available for attachment from on top of/within shallow surface soil,

305   similar to conditions likely to be the case in a natural environment, the methodology employed in

306   the "long" (≈150m) test distance, necessitating repeatedly walking/riding the same track, meant that

307   some beads might have become attached, detached and subsequently reattached on boot soles and

308   bike tires. Although we were unable to quantify this, we regularly observed soil dropping back onto

309   the track from both boots and bike tires during circuits. This was especially marked for the bike

310   under "wet" conditions, with soil (possibly containing beads) picked up on the tires often unable to

311   pass through the caliper brake pads and subsequently ejected back onto the track. This issue was

312   probably less likely to occur for boot soles because, as previously noted, boots predominantly

313   tended to pick up soil and beads in the heel treads, with soil tightly compacted and requiring beads

314   to be physically extracted by the researchers, with very few beads (estimated <5%) attaching to the

315   remainder of the soles. In defence of the sampling methodology, however, we argue:

316   (i)   This study is a pilot test of a potentially very flexible and cost-effective sampling methodology;

317   the possible occurrence and scale of the potential attach/detach/reattach issue would benefit

318   from further testing.

319   (ii)   The % of beads attaching from the available bead reservoir on a 'short'/single circuit was very

320   low overall (0.07%-0.18% boot soles; 0.00%-0.14% bike tires); this suggests that the probability

321   of the same individual beads re-attaching during multiple circuits is likely to be very low.

322   (iii) The 'short'/single circuit distance test is unaffected by this potential issue and estimates of seed

323   attachment over longer distances can therefore be arrived at via simple multiplication.

324   Thirdly, time and funding limits meant that our small-scale experiment used the same, single

325   walker/rider for all tests and only 1 pair of boots and 1 bike. Boots and bike tires obviously come in

326   a very wide variety of materials, sizes and tread patterns and these may affect seed attachment rate.

327   Different walking/riding behaviour of individuals may also have an effect. Wichmann et al (2009)

328   found seed attachment rate differed significantly among different walkers and shoe type (walking

329   boots vs Wellington boots), although not among different shoe sizes.

330   For the above reasons, our results presented here are necessarily case-specific and cannot be

331   generalised more widely to define the absolute relative propensity for seed attachment and

332   transport rate in a soil matrix on boot soles and bike tires. We nevertheless suggest that the

333   methodology as trialled here shows significant promise for researchers to use it more

334   comprehensively to test the attachment rate of different seed types under a range of densities and

335   soil conditions across a variety of different compounds and sizes of boot soles and bike tires, in a

336   way that is cost-effective and that reflects real-world walker and biker behaviour.

337   **Disclosure statement**

338   No potential conflict of interest was reported by the authors.

339   **Acknowledgements**

Table 1. Summary of results showing absolute and comparative propensity for bead attachment (observed data) on boot soles and bike tires over seven replicated tests. Note: (i) Total number of beads attaching over all tests = 810; (ii) Total number of beads available for attaching per test = 11,180.

	Moist				Wet			
	Total # beads attaching	% of total beads attaching all tests	M # (SE) of total beads attaching	Mean % attachment of beads available (SE)	Total # beads attaching	% of total beads attaching all tests	M # (SE) of total beads attaching	Mean % attachment of beads available (SE)
Boot Short left	19				48			
Boot Short right	35				89			
Boot Short total	54	6.7	7.7 (1.82)	0.07 (0.02)	137	16.9	19.6 (3.78)	0.18 (0.03)
Boot Long left	39				88			
Boot Long right	37				85			
Boot Long total	76	9.4	10.9 (1.37)	0.10 (0.01)	173	13.2	24.7 (3.25)	0.22 (0.03)
Bike Short front	0				100			
Bike Short rear	0				7			
Bike Short total	0	0.00	0.0 (0.00)	0.00 (0.00)	107	21.4	15.3 (5.13)	0.14 (0.05)
Bike Long front	19				230			
Bike Long rear	2				12			
Bike Long total	21	2.6	2.9 (0.83)	0.03 (0.01)	242	29.9	34.6 (4.42)	0.31 (0.04)

Table 2. Summary of raw data showing actual number of beads attaching on boot soles and bike tires by treatment and replicate. Total number of beads available for attaching per test = 11,180.

			Boot soles				
Left moist short	Right moist short	Left moist long	Right moist long	Left wet short	Right wet short	Left wet long	Right wet long
0	0	3	5	3	4	7	8
2	2	12	6	3	14	7	14
4	3	6	3	18	11	20	22
3	5	4	4	0	11	16	10
2	7	4	9	1	18	12	8
6	9	6	5	3	15	14	8
2	9	4	5	20	16	12	15

			Bike tires				
Front moist short	Rear moist short	Front moist long	Rear moist long	Front wet short	Rear wet short	Front wet long	Rear wet long
0	0	1	0	10	5	39	0
0	0	6	0	16	0	42	0
0	0	1	0	17	1	34	1
0	0	4	0	43	0	20	3
0	0	2	0	6	0	28	6
0	0	3	2	7	0	17	0
0	0	2	0	1	1	50	2

Table 3. Negative binomial model showing results of the three-factor analysis. Reported are parameter estimates (log-coefficients and associated, robust standard errors), fit- and model selection indices (LL, AIC, BIC) and associated degrees of freedom (*df*). (** = significant at $P < .01$; *** = significant at $P < .001$).

	Log-coefficient (SE)	*z*	*P*
Intercept	0.81 (0.32)**	2.893	.004
Vector	1.70 (0.32)***	5.240	<.001
Soil Condition	2.83 (0.33)***	8.944	<.001
Traversal Distance	-1.59 (0.38)***	-4.125	<.001
Vector x Traversal Distance	0.99 (0.36)**	2.994	.003
Vector x Soil Condition	-2.23 (0.34)***	-5.994	<.001
Soil Condition x Traversal Distance	0.57 (0.31)	1.649	.099
α (dispersion parameter)	0.20		
Log-Likelihood (LL)	-165.56, *df* = 8		
Akaike information criterion (AIC)	347.11, *df* = 8		
Bayesian information criterion (BIC)	363.32, *df* = 8		
Residual deviance	62.49, *df* = 49		

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