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1 **Survival of *Phoma koolunga*, a causal agent of ascochyta blight, on field pea stubble**
2 **or as pseudosclerotia in soil**

3 **M. Khani[a,c], J. A. Davidson[a,b], M. R. Sosnowski[a,b] and E. S. Scott[a*]**

4 [a]School of Agriculture, Food and Wine, Waite Research Institute, The University of
5 Adelaide, South Australia, 5005, Australia; [b]South Australian Research and Development
6 Institute, GPO Box 397, Adelaide, South Australia, 5001, Australia; and [c]Darab College
7 of Agriculture and Natural Resources, Shiraz University, Iran
8 *Corresponding author: eileen.scott@adelaide.edu.au

9 **Abstract**

10 *Phoma koolunga* is a recently recognised pathogen in the ascochyta blight complex of
11 field pea (*Pisum sativum*). Unlike the other three ascochyta blight pathogens, survival of
12 *P. koolunga* is poorly understood. Survival of this fungus was examined on field pea
13 stubble and as pseudosclerotia on the surface of, and buried in, field soil. Pseudosclerotia
14 were formed in plates containing potato dextrose agar (PDA) mixed with sand or amended
15 with fluorocytocin. After one month, *P. koolunga* was recovered on amended PDA from
16 93% of stubble sections retrieved from the soil surface, 36% of buried stubble sections
17 and 100% of pseudosclerotia buried in field soil, pasteurised or not. The frequency of
18 recovery of *P. koolunga* decreased over time and the fungus was not recovered from
19 stubble on the soil surface at month 15, nor was it recovered from stubble buried in soil
20 at months 11 and later or from pseudosclerotia buried for 18 months. In a pot bioassay,
21 most ascochyta blight lesions developed on plants inoculated with stubble retrieved from
22 the soil surface after one month. Infectivity of the inoculum decreased over time. Disease
23 on plants inoculated with stubble that had been buried or left on the soil surface for up to
24 6 and 5 months, respectively, and pseudosclerotia retrieved at 14 months and later from

25 field soil did not differ from the non-inoculated control. These results suggest that field
26 pea stubble may play a role in survival of *P. koolunga*, especially if it remains on the soil
27 surface. In addition, pseudosclerotia are able to persist in soil and infect field pea plants
28 into the next season.

29 *Keywords:* Infested stubble, stubble burial, *Pisum sativum*, infectivity

30 **Introduction**

31 Ascochyta blight is a devastating foliar disease of field pea (*Pisum sativum* L.) in
32 Australia and around the world (Bretag & Ramsey, 2001). It is considered the most
33 important disease of field pea in Australia, responsible for 15% production losses
34 annually and up to 75% yield loss in individual crops (Bretag *et al.*, 2006; McMurray *et*
35 *al.*, 2011). This disease is usually referred to as the ascochyta blight complex, because it
36 is caused by a number of closely related fungal species which exist independently of each
37 other. Until 2009, three species were recognised as causal agents of ascochyta blight, *viz.*
38 *Didymella pinodes* (syn. *Peyronellea pinodes* and *Mycosphaerella pinodes*), *Phoma*
39 *medicaginis* var. *pinodella* and *Ascochyta pisi*. Davidson *et al.* (2009a) characterised
40 *Phoma koolunga* as a fourth fungal species which can cause ascochyta blight on field pea.

41 The application of foliar fungicides to control ascochyta blight on field pea is
42 usually uneconomic (Warkentin *et al.*, 2000; McMurray *et al.*, 2011) and resistant pea
43 genotypes are not available (Bretag *et al.*, 2006). Therefore, disease control relies on
44 cultural methods. Burying infested field pea stubble, rotations after field pea crops and a
45 delay in sowing seed to avoid the peak release of ascospores from infested stubble are
46 considered the most important practical methods to manage this disease (Bretag *et al.*,
47 2006; McDonald & Peck, 2009; Salam *et al.*, 2011).

48 Incidence and severity of ascochyta blight increase when field pea crops are sown
49 in the vicinity of field pea stubble infested with *D. pinodes* (Davidson & Ramsey, 2000;

50 Galloway & McLeod, 2001; Bretag *et al.*, 2006; Davidson *et al.*, 2013). This is attributed
51 to production of survival structures of the causal agents on the stubble (Dickinson &
52 Sheridan, 1968; Zhang *et al.*, 2005; Bretag *et al.*, 2006; McDonald & Peck, 2009;
53 Davidson *et al.*, 2013). The major pathogen of the ascochyta blight complex, *D. pinodes*,
54 can survive from 4 to 18 months as pseudothecia or sclerotia on field pea stubble, or as
55 chlamydospores in infested soil for at least 12 months (Zhang *et al.*, 2005; Bretag *et al.*,
56 2006). Moreover, Davidson *et al.* (1999) reported that after 4 months, stubble from
57 ascochyta blight-affected field peas incubated on the soil surface or underground was not
58 infectious, whereas McDonald and Peck (2009) showed that the amount of *D. pinodes*
59 inoculum on field pea stubble was high the year after a field pea crop and declined rapidly
60 after one season. Recently, Davidson *et al.* (2011) showed that in a bioassay, the severity
61 of ascochyta blight symptoms, including stem lesions and leaf spots, was positively
62 correlated with quantity of soil borne inoculum of these pathogens, including *P.*
63 *koolunga*.

64 The survival of *D. pinodes*, *P. medicaginis* var. *pinodella* and *A. pisi* on field pea
65 residues is well-documented, however, information about *P. koolunga* is lacking.
66 Although Davidson *et al*. (2009a) reported that pseudosclerotia of *P. koolunga* may be
67 present in culture, pseudosclerotia have not been described in any detail and their role in
68 the survival of *P. koolunga* is completely unknown. The objectives of this study were to
69 examine the survival of *P. koolunga* over time in infested field pea stubble buried in soil
70 or placed on the soil surface and to study the production of pseudosclerotia *in vitro* and
71 their survival in soil.

72 **Materials and methods**

73 **Stubble and pseudosclerotia – production and burial**

74 Field pea stubble that was heavily infested with *P. koolunga* was generated from plants
75 at Waite Campus, South Australia (SA) in November 2011. These plants had been
76 inoculated 3 weeks post-sowing with a mixed pycnidiospore suspension (5×10^5 spores
77 mL^{-1}) from *P. koolunga* isolates 139/03, 142/03, 81/06 and FT07026 until runoff. The
78 inoculation was repeated three times at 6-day intervals. The harvested stubble was stored
79 at 4°C until used. Stubble was placed in bags and buried using methods adapted from
80 Naseri *et al.* (2008). Briefly, basal parts of the stems from the crown to 40 cm above were
81 cut into 10-cm pieces and 104 lots, each comprising 15 stem pieces, were weighed
82 individually and placed in 15 cm^2 plastic mesh bags (mesh pore size 1 mm^2). Each bag
83 was placed on, or buried 5-10 cm below, the soil surface in 20-L pots outdoors at the
84 Waite Campus in February 2012. This soil was collected from a field with no history of
85 field pea at the Lenswood Agricultural Centre, approximately 30 km east of Adelaide,
86 South Australia and subjected to DNA analysis by the Root Disease Testing Service
87 (RDTS) at the South Australian Research and Development Institute (Davidson *et al.*,
88 2009a) to confirm it was free from *P. koolunga*. The pH and electrical conductivity (EC)
89 of the field soil were measured using pH and conductivity meters, respectively, and the
90 soil texture was predicted by MIR spectroscopy (Janik *et al.*, 1998). The daily rainfall and
91 average air temperatures for Glen Osmond, 2.8 km from the Waite Campus, were
92 obtained from the Australian Bureau of Meteorology.

93 To initiate formation of pseudosclerotia, twice-autoclaved river sand (3-4 g,
94 Sloan's Sands Pty Ltd) was added to molten potato dextrose agar (PDA) in Petri dishes
95 (9 cm in diameter) and, when solidified, a 2-mm plug of *P. koolunga* (isolate FT07026)
96 was placed in the middle of the plate. The plates were incubated at 22°C in the dark for 4
97 weeks. Pseudosclerotial masses produced in the plates were cut into 2 cm^2 and mixed
98 with 20 g of sterilized sand (Coley-Smith, 1985) in nylon mesh bags (8×8 cm, 20 μm

99 pores; Schweizer Seidengaz-fabrik AG) (Probst, 2011); the edges were sealed and bags
100 were buried in pasteurised or non-pasteurised field soil in 2-L pots in March 2012. The
101 soil was pasteurised at 60°C for one hour.

102 **Recovery of *P. koolunga* and associated mycobiota from stubble in or on soil, or**
103 **from pseudosclerotia**

104 Prior to burying stubble in soil, 15 pieces of randomly selected field pea stubble were
105 tested for presence of *P. koolunga* using the methods described below, which were
106 adapted from Naseri *et al.* (2008). From March 2012 four replicate bags from each
107 treatment, on and in soil, were retrieved monthly for 12 months and a final set was
108 removed in month 15, May 2013. Briefly, the stubble from each bag was rinsed, air-dried
109 and weighed. Representative pieces of stubble were examined for the presence of
110 pycnidia and pseudosclerotial masses. From each of five randomly selected pieces per
111 bag, four 0.5-cm segments were excised, from each end and from the middle. These stem
112 segments then were immersed in 0.5% sodium hypochlorite for 3 min in the first 6 months
113 and thereafter 1 min due to the fragility of the stubble, followed by rinsing in sterile
114 distilled water and drying on sterile filter paper for 2 h. The stem segments were plated
115 onto semi-selective agar medium, developed in preliminary experiments, which
116 comprised PDA amended with 45 mg L^{1} fluorocytocin and 100 mg L^{-1} streptomycin.
117 Fungal growth was recorded during 10 days of incubation at 22°C under 12 h fluorescent
118 and near ultraviolet light and 12 h dark, after which colonies were transferred to PDA in
119 the same incubation conditions.

120 The isolation frequency from each bag was recorded as the number of small stem
121 sections that yielded *P. koolunga* divided by 20 and expressed as percentage. The same
122 formula was applied for other fungi most commonly recovered from stubble from July
123 2012 to May 2013. Fungi were identified to genus on the basis of morphological

124 characteristics (Barron, 1968; Ellis, 1976; Domsch *et al.*, 1980; Barnett & Hunter, 1998;
125 Watanabe, 2010).

126 From April 2012, four replicate bags of pseudosclerotia from non-pasteurised and
127 pasteurised soil were retrieved monthly for 11 months, again in month 14 and a final set
128 was removed in month 18, in September 2013. At each sampling time, four bags from
129 each treatment were randomly removed and the pseudosclerotia were shaken in five
130 changes of sterile water (Dickinson & Sheridan, 1968) amended with streptomycin (100
131 mg L^{-1}), then dried on sterile filter paper. The pseudosclerotia masses were cut to small
132 fragments of approximately 0.3-0.5 mm^2, using a sterile scalpel and then were transferred
133 to PDA, 10 pieces per plate. These plates were incubated in the conditions described
134 above for up to 10 days and viability of pseudosclerotia was assessed as the percentage
135 of pseudosclerotial masses that germinated.

136 **Effect of burial on infectivity of the inoculum of *P. koolunga***

137 The infectivity of inoculum of *P. koolunga* on stubble retrieved over time was tested in a
138 pot bioassay using methods adapted from Davidson and Krysinska-Kaczmarek (2003).
139 Each month, five seeds of field pea cv. Kaspa were sown in each plastic pot (7 × 8.5 ×
140 5.5 cm) containing University of California (UC) potting soil. Four replicate pots were
141 used for each treatment, plants were grown in a growth room at 14-16°C with 12 h light/12
142 h dark and watered as required. Plants at the four-node growth stage were sprinkled with
143 0.5 g milled field pea stubble, maintained in a humidity chamber for 48 h, and then
144 transferred to a growth room and watered as before. For control plants, sterile water was
145 used instead of milled stubble. The number of ascochyta blight lesions on leaves and
146 stems on each plant was counted after 10 days. Representative diseased leaves were
147 examined for presence of *P. koolunga* by isolation on PDA plates.

148 The infectivity of pseudosclerotia retrieved from soil was assessed using a method
149 adapted from Yaqub and Shahzad (2005) and Coley-Smith *et al.* (1990) at 1, 4, 7, 10, 14
150 and 18 months post-burial. In brief, plugs of *P. koolunga* containing pseudosclerotia (3
151 cm^2) were dried, crushed and spread on sterilized UC soil in pots (7 × 6 × 6 cm), then
152 covered with a further 4 cm layer of sterilized UC soil. Ten seeds of field pea cv. Kaspa
153 were sown in each pot at a depth of 2.5 cm. The pots were kept in the growth room in
154 conditions described above. After 21 days, roots were washed and assessed for symptoms
155 and isolation of fungus.

156 **Statistical analysis**

157 Analysis of variance (ANOVA) and regression analyses were applied to the data for
158 isolation frequency of *P. koolunga* from stubble, recovery from pseudosclerotia and
159 infectivity tests using GenStat 15th edition SP2. Exponential or Gompertz curves from the
160 regression analyses were used to describe isolation frequencies, recovery and infectivity.
161 To test trends in isolation frequencies of fungi from stubble for sampling month and
162 depth, linear and quadratic analyses were conducted. Tukey's honestly significant test at
163 95% confidence level was applied to compare means in each experiment.

164 **Results**

165 **Stubble burial**

166 The field soil was determined to be sandy-loam with pH 5.38 and EC of 113.7 mg L^{-1}.
167 The monthly average air temperature at Waite Campus, South Australia for the duration
168 of the experiment is shown in Fig. 1. The daily temperature during this research fluctuated
169 between 10.8°C in June 2012 (winter) and 40.5°C in January 2013 (summer). Maximum
170 and minimum monthly rainfalls were recorded in June 2012 (130 mm) and March 2013
171 (11.2 mm), respectively (Fig. 1). Overall, the climatic conditions during this research

172 were similar to the average of the last 10 years. For example, the annual rainfall at Waite
173 Campus for 2012 was 567 mm compared with the average of 626 mm (Bureau of
174 Meteorology of Australia, 2013).

175 After one month, the weight decrease was 4% for both stubble placed on, or buried
176 in, soil but after 2 months the stubble buried in soil lost significantly more weight (9%)
177 than stubble placed on the soil surface (5%) (Table 1). This weight reduction continued
178 over time for stubble both on soil and in soil, however at a greater rate for buried stubble;
179 at the end of the experiment (after 15 months), stubble weight decrease was 28 and 53%
180 for soil surface and buried, respectively. Decomposition of stubble buried in soil was first
181 seen after 4 months and after 8 months some stems were broken in the bags retrieved
182 from soil. The stubble placed on the soil surface did not show signs of decomposition
183 until 12 months, when the pith was decayed. Pycnidia were observed on most pieces of
184 stubble before placement and after retrieval, but pseudosclerotia were not found.

185 ***P. koolunga* and other fungi isolated from stubble**

186 In February 2012, prior to placing or burying field pea stubble on or in soil, *P. koolunga*
187 was isolated from 92% of stubble pieces. After one month of incubation, the same
188 isolation frequency was recorded for stubble placed on the soil surface, but the isolation
189 frequency for buried stubble decreased to 36% (Fig. 2). Isolation decreased over time in
190 an exponential manner for both treatments, except for April, June and September 2012
191 for stubble in soil, and December 2012 and February 2013 for stubble on the soil surface.
192 In addition, Tukey's test ($P < 0.05$) showed that survival of *P. koolunga* on stubble buried
193 in soil for months 9 and 10 was not significantly different, but it was statistically different
194 from month 11 and thereafter, when no isolation was recorded. However, the fungus was
195 isolated from 2.5% and 5% of stubble pieces retrieved from the soil surface in January
196 and February 2013, respectively, but not from the last set retrieved in May 2013.

197 *Fusarium* spp. were the fungi most frequently isolated from stubble buried in soil
198 over time, ranging from 51% in July 2012 to 19% in February 2013 (Table 2). These
199 fungi were recovered at a maximum frequency of 32% of stubble pieces placed on the
200 soil surface after 10 months and a minimum of 5% in July 2012 and May 2013. The
201 second most frequently isolated fungus from buried stubble was *Stachybotrys chartarum*
202 with an average of 25% in 9 months of sampling from July 2012 to May 2013; however,
203 it could not be detected in surface-placed stubble in five of the nine sampling periods.
204 The mean isolation frequency of *Trichoderma* spp. was 12%, fluctuating between 0% in
205 July and 21% in August 2012 for buried stubble and between 0% in December 2012 and
206 February 2013 to 11% in September 2012 for stubble placed on the soil surface.
207 *Gliocladium* spp. were isolated from buried stubble in each of the 9 months of recording,
208 most frequently at 11% in February 2013. This genus was recovered from stubble placed
209 on the soil surface in only four of the nine sampling periods and then only at a frequency
210 of 4% or less.

211 **Recovery of *P. koolunga* from pseudosclerotia**

212 Pseudosclerotia in the current study were formed on PDA containing grains of sand and
213 were dark brown, firm, irregular in shape and 105 to 410 µm in diameter. They mostly
214 consisted of dark brown mycelia with thick-walled cells (15.1-20.2 µm in diameter). *P.*
215 *koolunga* was recovered from 100% of pseudosclerotial masses buried in non-pasteurised
216 and pasteurised soil until the fifth and sixth month post-burial, respectively (Fig. 3). Then
217 the percentage recovery decreased in an exponential manner each month for the
218 remainder of the experiment, with a lower isolation frequency ($P < 0.05$) in non-
219 pasteurised soil than pasteurised soil, dropping to 3% and 9%, respectively, in May 2013
220 after 14 months. This delayed response followed by an exponential decrease is best
221 described by the Gompertz equation presented in Figure 3. The fungus did not grow from

222 pseudosclerotial masses onto culture medium after 18 months of burial in either
223 pasteurised or non-pasteurised soil.

224 **Effect of burial on infectivity of *P. koolunga* infested field pea stubble**

225 Most ascochyta blight lesions developed on leaves and stems of plants inoculated with
226 stubble retrieved after one month from the soil surface or buried in soil, *viz*. 26 and 9
227 lesions per plant, respectively (Fig. 4). In general, the infectivity of the inoculum of the
228 fungus on stubble buried in or placed on soil decreased over time in an exponential
229 manner. A sharp decrease in infectivity of the inoculum of the fungus on surface-placed
230 stubble was recorded between June and July 2012, 4 and 5 months after placement, to a
231 level not significantly different from the water control. Disease on plants inoculated with
232 stubble retrieved 6 months and more after burial did not differ from the water control.
233 This was also the case for stubble 3 months after burial. Infectivity of inoculum of the
234 fungus placed on the soil surface at 5 months and later was not significantly different
235 from the water control ($P < 0.05$). Lesions were not observed on control plants inoculated
236 with sterile water.

237 Plants grown from seeds sown in soil inoculated with pseudosclerotia of *P.*
238 *koolunga* showed symptoms as limited (< 4 mm in length) or developed (4-11 mm)
239 necrotic lesions on roots and epicotyls, 3 weeks after sowing. *P. koolunga* was isolated
240 from all representative lesions excised from seedling roots. Root symptoms were most
241 frequently observed on plants grown for 21 days in soil mixed with pseudosclerotia that
242 had been buried for one month, with 82% and 80% disease incidence in non- pasteurised
243 and pasteurised soil, respectively (Fig. 5). Disease incidence on the roots was described
244 with a Gompertz curve, showing an initial delay and then a decrease as duration of burial
245 of pseudoscerotia in soil increased, dropping to 20% and 5% after 10 and 14 months for
246 non-pasteurised and pasteurised soil, respectively. No root lesions were seen on seedlings

247 in soil inoculated with pseudosclerotia retrieved from non-pasteurised or pasteurised soil
248 after 14 and 18 months, respectively.

249 **Discussion**

250 Survival of *P. koolunga* was greater on field pea stubble left on soil than buried in soil;
251 viability of the fungus decreased at different rates over time such that it was not recovered
252 after 11 and 15 months from stubble buried in or placed on soil, respectively. The stubble
253 decomposed more rapidly in soil than on the soil surface. Infectivity of inoculum of *P.*
254 *koolunga* in stubble also decreased over time. Pseudosclerotia remained viable in
255 pasteurised soil longer than in field soil, and infectivity was lost earlier than viability.

256 The infectivity of inoculum of *P. koolunga* on stubble placed on or buried in soil
257 decreased faster than did viability. The mean number of lesions per plant inoculated with
258 stubble after 5 months placement on the soil surface or 6 months of burial during the
259 Australian summer to winter decreased to a level equivalent to the water control. In a
260 similar study, Zhang *et al.* (2005) reported that the ability of *D. pinodes* to produce
261 ascospores on field pea leaf or stem residue placed on or buried 5-10 cm deep in soil
262 decreased to an unmeasurable level after 9 months from autumn to spring in Canada.
263 They stated that disease severity resulting from applying washings from buried stubble to
264 bioassay plants was high for the first 2-4 months. The results of the current study were
265 also in agreement with Davidson *et al.* (1999), in that field pea stubble infested with *D.*
266 *pinodes* and *P. medicaginis* var. *pinodella* remained highly infectious for the first 4
267 months post-burial in soil, but did not initiate severe disease thereafter. In comparison, *P.*
268 *koolunga* survived better in stubble left on the soil surface than in stubble buried in soil,
269 which appeared to be due to lesser decomposition of the stubble on the soil surface.
270 *Leptosphaeria maculans* on stubble remained pathogenic and caused phoma leaf spot on
271 oilseed rape seedlings until 9 months post-burial in soil or sand (Naseri *et al.*, 2008),

272 similar to the duration of time recorded for *P. koolunga* on field pea stubble buried in soil
273 in this research. Environmental conditions such as rainfall and temperature affect the
274 survival and growth of fungi (Baird *et al.*, 2003) and Zhang *et al.* (2005) explained that
275 less decomposition was observed on surface-incubated stubble in Canada as it was drier
276 than buried stubble, especially in winter and spring. The same is likely to be the case in
277 South Australia, although the conditions during winter are much milder than in Canada.

278 *Fusarium*, *Gliocladium*, *Trichoderma* and *Stachybotrys* spp. were isolated
279 frequently from field pea stubble. These fungi have been reported to antagonise other
280 fungi and also to decompose plant residue (Domsch *et al.*, 1980). They were isolated
281 more frequently from buried stubble than stubble left on the soil surface, perhaps due to
282 greater moisture content and better physical protection for microbes in soil than on the
283 soil surface (Van Veen & Kuikman, 1990). Burial of stubble may hasten decomposition
284 and promote colonisation of stubble by antagonistic microorganisms, particularly in the
285 relatively dry and temperate conditions typical of the South Australian winter. Carbon
286 released from decomposed residue promotes soil microbial activity and so increases
287 pathogen degradation (Raaijmakers *et al.*, 2009). *Trichoderma* spp., which were isolated
288 from almost all stubble retrieved after burial in soil in this study, are well known for
289 rapidly colonizing plant residue in contact with soil as well as parasitising plant
290 pathogens, such as *Rhizoctonia*, *Pythium*, *Fusarium* spp., in the field and *Mycosphaerella*
291 *phaseolina in vitro* (Harman, 2000; Baird *et al.*, 2003). Baird *et al.* (2003) isolated
292 *Trichoderma* spp. from root segments of soybean in soil and stated that *Trichoderma* spp.
293 with the ability to parasitise pathogens and degrade cellulose may reduce the survival of
294 *M. phaseolina*. The same explanation may apply to *P. koolunga* on field pea stubble
295 buried in soil and this merits further investigation.

296 Pseudosclerotia were formed on PDA that contained grains of sand while on PDA
297 without sand only mycelial growth was observed. In addition, in antifungal sensitivity
298 tests, *P. koolunga* produced large numbers of pseudosclerotia when 100 mg L^{-1}
299 fluorocytocin was included in the medium, although at 45 mg L^{-1} fluorocytocin the fungus
300 produced only mycelium, pycnidia and pycnidiospores (data not shown). Nutritional or
301 mechanical factors or antifungal chemicals appeared to influence the formation of
302 pseudosclerotia by *P. koolunga*, as has been reported for other fungi (Chet & Henis, 1975;
303 Camyon & Gerhardson, 1997). Sclerotia of *Sclerotium rolfsii* formed on culture media in
304 Petri dishes when the mycelium reached the edge and linear growth was restricted (Henis
305 *et al.* (1965) and it is possible that contact with the hard surface of the grains of sand
306 elicited a similar response in *P. koolunga*. Furthermore, antifungal antibiotics such as
307 bacitracin, trichomycin and griseofulvin induced formation of sclerotia by *S. rolfsii* (Chet
308 & Henis, 1975) and a similar effect on formation of pseudosclerotia of *P. koolunga* was
309 seen when fluorocytocin was incorporated into PDA. The pseudosclerotia of *P. koolunga*
310 were morphologically similar to those reported for *Phoma foveata*, the potato gangrene
311 pathogen (Camyon & Gerhardson, 1997) and for *P. chrysanthemicola* (Dorenbosch,
312 1970). Pseudosclerotia of *P. koolunga* were usually initiated at 2 weeks and developed in
313 4 weeks on the above-mentioned media and appeared to be formed as survival structures
314 in adverse conditions. Also they survived in soil and remained pathogenic for longer than
315 other structures of *P. koolunga*, such as pycnidia on stubble.
316 Although 100% of pseudosclerotia buried in soil were viable on PDA plates for up
317 to 5 months, thereafter a reduction was observed; more so for pseudosclerotia in non-
318 pasteurised soil than pasteurised soil. In agreement with these results, the viability of
319 sclerotia of *Colletotrichum coccodes* and *Phoma liguilicola* buried in soil was greater in
320 sterile moist garden soil than non-sterile soil after 6 and 2 months, respectively (Blakeman

321 & Hornby, 1966). The same trend was observed for sclerotia of *C. coccodes* buried in
322 sterile, dry garden soil compared to non-sterile soil for 12 months. It appears that soil
323 microorganisms are responsible for greater decomposition and lysis of buried
324 pseudoscleotia in non-sterile soil than sterile soil (Blakeman & Hornby, 1966).

325 In the current study, 96% of pseudosclerotial masses of *P. koolunga* buried in non-
326 pasteurised soil remained viable after 6 months. Dickinson and Sheridan (1968) also
327 reported that after burial of sclerotia of *D. pinodes* in soil for 4 months, 90% germinated
328 and they attributed this to the persistent nature of sclerotia. Pseudosclerotia of *P. koolunga*
329 buried in field soil in the absence of host plants remained pathogenic for at least 10
330 months, which is sufficient to infect the root systems of the next season's field pea plants
331 and possibly also alternative hosts. In comparison, infectivity of sclerotia of *P. liguilicola*
332 to chrysanthemum cuttings was lost after burial for 2 months in non-sterile compost,
333 whereas 7% of tomato seedlings inoculated with sclerotia of *C. coccodes* retrieved from
334 natural soil after 20 months were infected (Blakeman & Hornby, 1966). It seems that the
335 longevity and infectivity of pseudosclerotia or sclerotia varies and these differences have
336 been attributed to the type of morphological development *viz.* loose, terminal or strand
337 (Blakeman & Hornby, 1966). The type of pseudosclerotia of *P. koolunga* has not yet been
338 determined.

339 Davidson *et al.* (2011) reported that DNA of *P. koolunga* could be detected in field
340 soil 4 years after a field pea crop was grown and predicted that the level of soil borne
341 inoculum could initiate an ascochyta blight epidemic, but they did not assess infectivity
342 nor indicate the means by which the fungus might survive. The current study suggests
343 that *P. koolunga* may not have been viable 4 years after the last field pea crop.

344 Although this research showed that infested field pea stubble left on the soil surface
345 did not initiate severe disease after 5 months, this window may not be sufficient in all

346 conditions and also could allow early sown or volunteer field pea or alternative hosts to
347 be infected by *P. koolunga*. Burying stubble would shorten survival of the pathogen and
348 limit release and dissemination of conidia (Davidson *et al.*, 2013). On the other hand,
349 burying plant debris is not compatible with zero tillage or residue retention farming
350 systems which have been adopted in recent decades. Davidson *et al.* (2011) found that *P.*
351 *koolunga* was coincidental with *D. pinodes* in field pea cropping soils. As a consequence,
352 the general recommendation of delayed sowing or distance from field pea stubble infested
353 with *D. pinodes* (Davidson *et al.*, 2013) as well as a 4-year interval between crops of field
354 pea should be applied to minimise the risk of ascochyta blight epidemics . In addition,
355 other legume crops may be alternative hosts of *P. koolunga* (Bretag *et al.*, 2006;
356 Davidson *et al.*, 2009b). Possible alternative hosts might be infected not only by
357 pycnidiospores and mycelia, but also by pseudosclerotia via root systems if sown in the
358 same field in the next season. Therefore, research on host range of *P. koolunga* is required.
359 If any other crop plants become infected by survival structures of this fungus, their use in
360 rotation with field pea or even in the vicinity of infested stubble from the previous year
361 would need to be reviewed.

362 So far, no sexual stage has been reported for *P. koolunga* and this study showed
363 that pycnidia or mycelia on plant residue are not likely to survive for long periods of time.
364 Thus, pseudosclerotia may be responsible for longer survival than other structures and
365 may pose a greater risk to the next season's field pea crops than infested stubble buried
366 in soil. The question remains as to whether or not *P. koolunga* can produce
367 pseudosclerotia in natural conditions. Investigation of the formation of these structures
368 on plant material or in soil and understanding their importance in survival of the fungus
369 and possible role in the epidemiology of the pathogen in field pea crops is recommended.

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474 survival of *Mycosphaerella pinodes* in Manitoba. *Canadian Journal of Plant*
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477 **Table 1** Mean percentage decrease in weight of field pea stubble buried in or placed on soil in February 2012 over 15 months

	Sampling month												
	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	May
On soil surface[a]	4.53	5.63	7.87	9.44	11.54	10.79	13.53	16.16	19.22	21.35	24.17	24.86	27.86
In soil	4.64	9.27	13.19	16.11	18.37	19.45	20.75	22.87	26.88	30.11	32.32	36.59	52.76

478 [a]Mean percentage decrease of stubble weight in four replicate bags; LSD = 1.55 (C data set) , $P < 0.01$.

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489 **Table 2** Mean isolation frequency (%) of common fungi from field pea stubble placed on or buried in soil from July 2012 to May 2013

Fungus genus	Stubble retrieved from	Sampling month									L[a]	Q	LSD[b]
		Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	May			
Alternaria	Soil surface	2.5	2.5	0	1.25	0	5	0	0	3.75	NS	NS	4.90
	In soil	11.25	5	3.75	6.25	5	5	2.5	10	7.5	NS	NS	8.47
Aspergillus	Soil surface	0	1.25	0	7.5	0	0	0	0	0	NS	NS	4.89
	In soil	0	0	0	6.25	0	0	0	0	0	NS	S	2.32
Fusarium	Soil surface	5	15	13.75	7.5	7.5	32.5	18.75	3.75	5	NS	NS	12.02
	In soil	51.25	48.75	33.75	41.25	21.25	43.8	30	18.75	22.5	S	NS	21.54
Gliocladium	Soil surface	0	2.5	1.25	0	0	0	1.25	0	3.75	NS	NS	3.92
	In soil	1.25	1.25	6.25	7.5	6.25	7.5	2.5	11.25	10	S	NS	7.66
Penicillium	Soil surface	0	0	0	0	1.25	0	0	5	0	NS	NS	4.86
	In soil	0	2.5	1.25	3.75	1.25	0	0	0	0	NS	NS	3.72
Rhizoctonia	Soil surface	0	2.5	0	1.25	0	0	0	0	3.75	NS	NS	3.76
	In soil	10	11.25	18.75	20	13.75	3.75	10	16.25	16.25	NS	NS	10.18
Rhizopus	Soil surface	0	0	0	0	1.25	0	0	0	0	NS	NS	2.76
	In soil	1.25	7.5	1.25	10	6.25	7.5	2.5	7.5	2.5	NS	S	6.05
Stachybotrys	Soil surface	1.25	0	0	1.25	1.25	0	0	0	2.5	NS	NS	3.00
	In soil	13.75	26.25	27.5	31.25	32.5	38.8	18.75	11.25	25	NS	S	10.44
Trichoderma	Soil surface	2.5	7.5	11.25	7.5	2.5	0	1.25	0	1.25	S	NS	5.42
	In soil	0	21.25	11.25	12.5	16.25	15	6.25	17.5	8.75	NS	NS	11.40

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491 [a]L refers to a linear response and Q refer to quadratic response of the mean isolation frequency (%) for each fungus genus; NS non-significant; S significant ($P < 0.05$).

492 [b]Least significant difference ($P < 0.05$) of the mean isolation frequency (%) for each burial depth.

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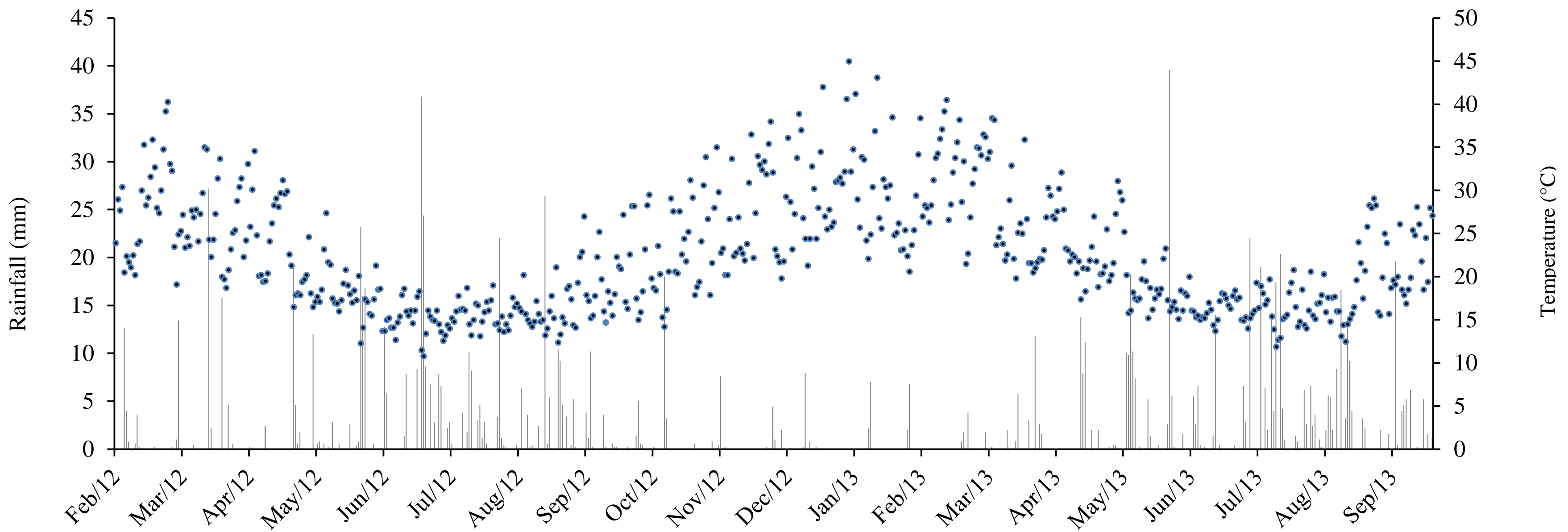

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495 **Figure 1** Mean daily air temperature (dark circle) and daily rainfall (grey column) at Glen Osmond, South Australia for 20 months from

496 February 2012 (data obtained from the Australian Bureau of Meteorology).

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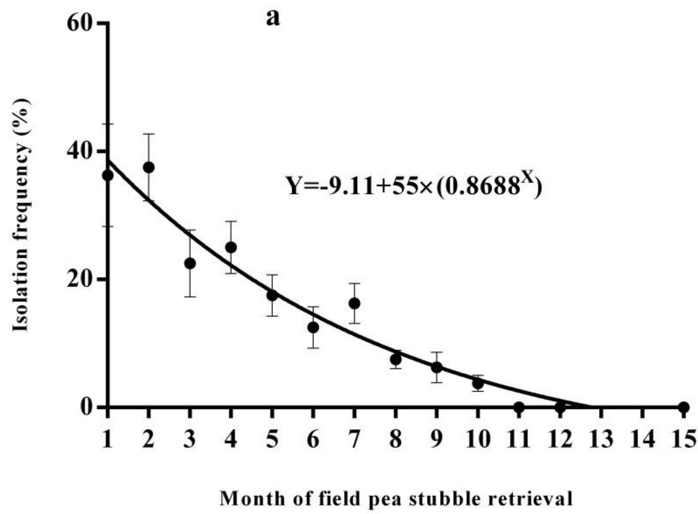

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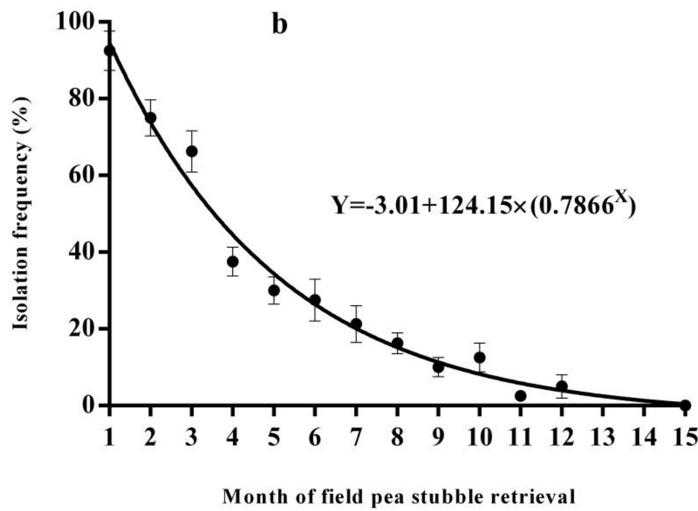

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500 **Figure 2** Mean isolation frequency of *Phoma koolunga* from infested field pea stubble
501 buried in pots of field soil (a) or left on the soil surface (b) in February 2012 over 15
502 months (mean of four stubble samples per treatment per month, bars represent standard
503 error).

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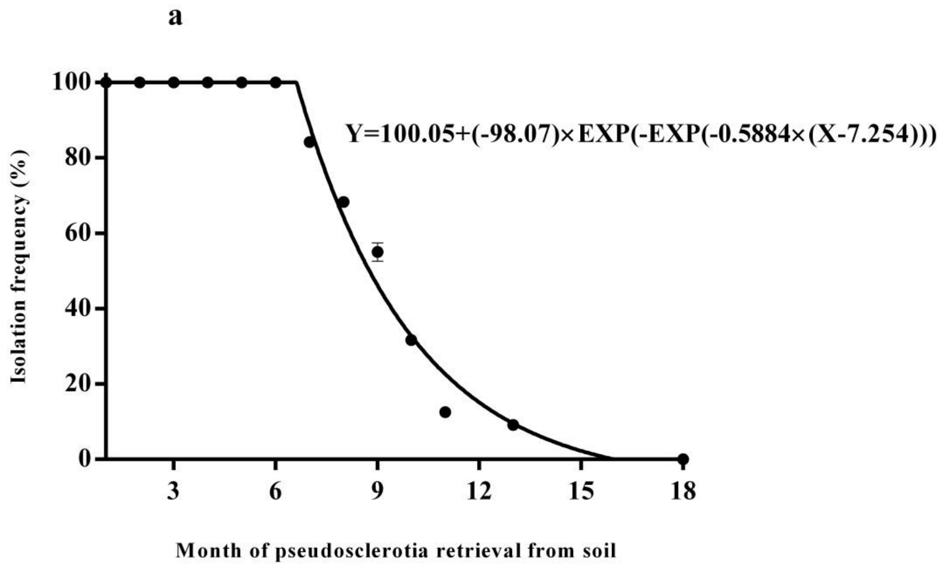

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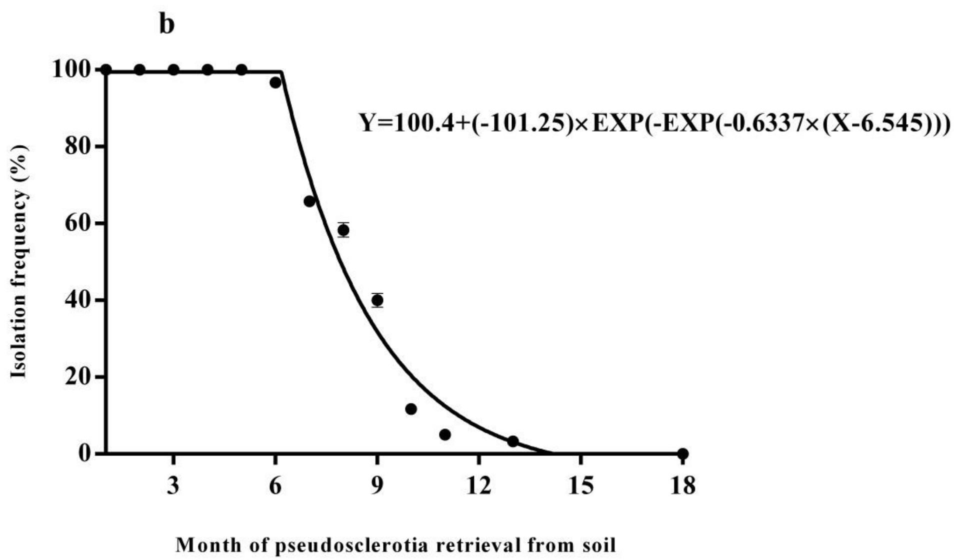

509

510 **Figure 3** Percentage isolation frequency of *Phoma koolunga* from pseudosclerotial
511 masses buried in non-pasteurised (a) and pasteurised soil (b) in pots in March 2012 over
512 18 months (bars represent standard error).

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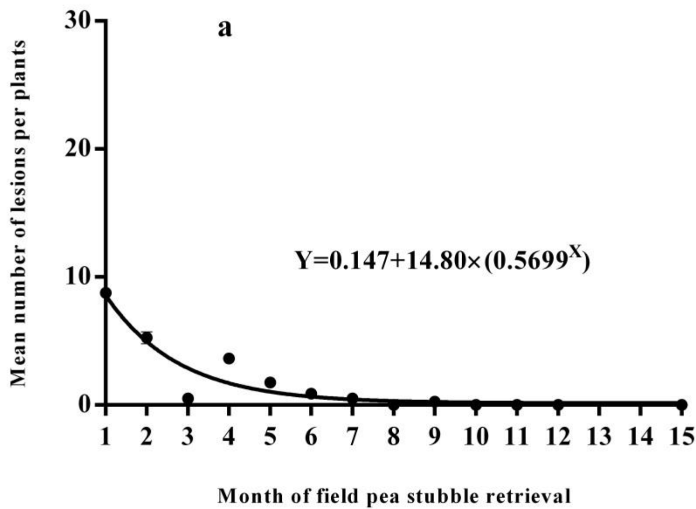

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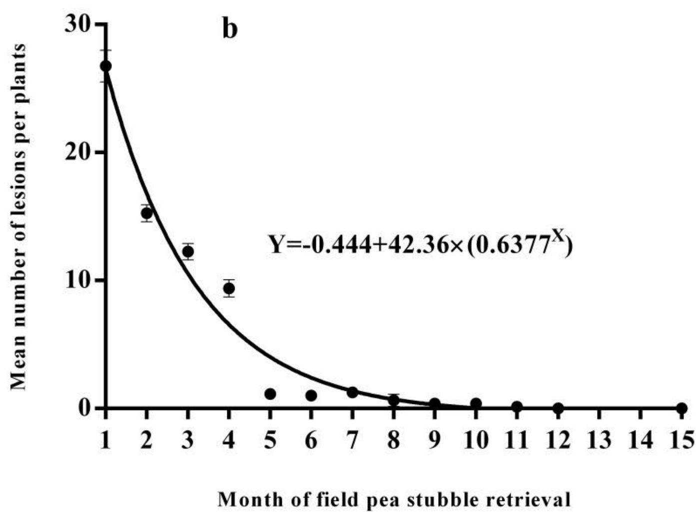

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520 **Figure 4** Mean number of ascochyta blight lesions on field pea plants sprinkled with
521 milled *Phoma koolunga*-infested stubble that had been buried in pots of soil (a) or left on
522 the soil surface (b) and retrieved from one to 15 months after placement (bars represent
523 standard error). There were no lesions on non-inoculated control plants.

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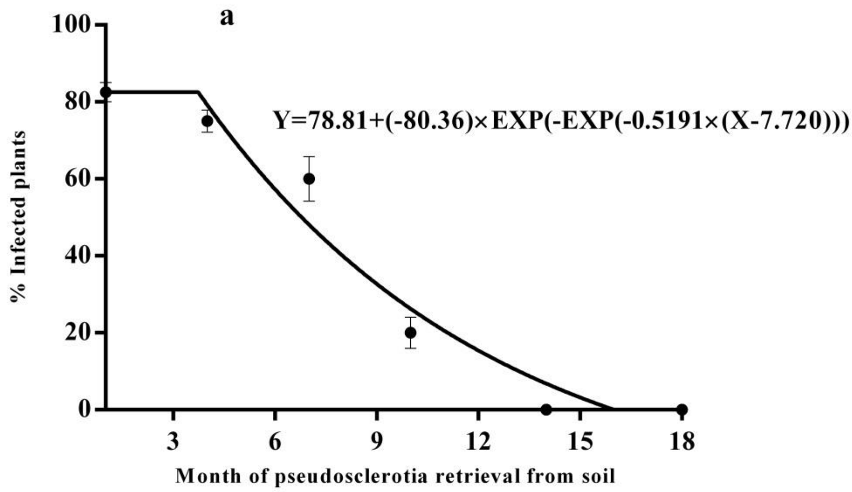

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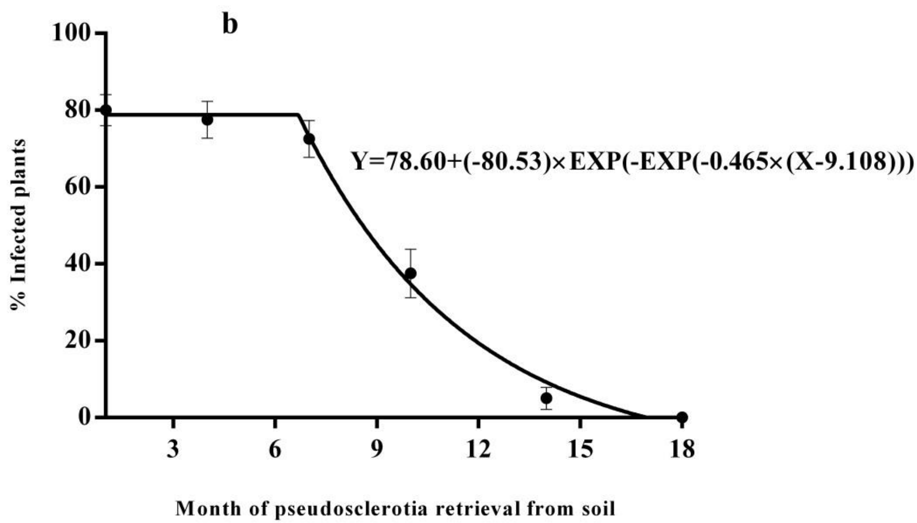

530

531 **Figure 5** Percentage of field pea plants, in pots of soil, infected by pseudosclerotia
532 retrieved from non-pasteurised (a) and pasteurised soil (b) over 18 months (data are means
533 of 40 plants, bars represent standard error).

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