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Survival, transmission and control of *Phoma koolunga* in field pea seed and reaction of field pea genotypes to the pathogen

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Abstract

Little is known about the epidemiology of *Phoma koolunga,* a component of the ascochyta blight complex of field pea in southern Australia. The aims of this research were to investigate seed infection, efficacy of fungicides as seed dressings and the reaction of current field pea genotypes to this fungus. The frequency of isolation of *P. koolunga* from seed samples from South Australia, Victoria and Western Australia ranged from 0 to 6 %. Disease was transmitted to 98 % of seedlings that emerged from artificially inoculated seeds (AIS) in growth room conditions. Seedling emergence rate from AIS was lower at 8 °C than at 12, 16 and 20°C, and necrotic index was greater at the lower temperature. P-Pickel T® and Jockey Stayer® were the most effective fungicides for reducing disease incidence and severity on seedlings emerged from AIS sown in soil and on germination paper, respectively. The response of 12 field pea genotypes to one isolate of *P. koolunga* was assessed by spraying plants with pycnidiospore suspension in controlled conditions and examining symptoms from 3 to 21 days post-inoculation (dpi). Genotypes Sturt, Morgan and Parafield showed more severe disease on the lowest three leaves than the other genotypes at 21 dpi. In another experiment, four genotypes of short, semi-leafless type field peas were inoculated with three isolates of *P. koolunga* which differed in virulence and assessed as described above. Kaspa showed significantly less disease than Morgan or WAPEA2211 at 21 dpi. Isolates of *P. koolunga* differed in aggressiveness based on % leaf area diseased until 14 dpi, but differences were not significant at 21 dpi.

Keywords Seed-borne, *Pisum sativum*, ascochyta blight, disease severity, seed treatment, resistance

Introduction

Ascochyta blight is one of the most important diseases of field pea (*Pisum sativum* L.) worldwide, including Australia, where it is estimated that crops suffer 15 % annual yield loss and up to 75 % in severe cases (Bretag et al. 2006; Salam et al. 2011a). This disease is caused by a number of closely related fungi, *Didymella pinodes* (*Mycosphaerella pinodes*), *Phoma medicaginis* var. *pinodella*, *Ascochyta pisi* and the recently characterised *Phoma koolunga* (Davidson et al. 2009).

D. pinodes, *P. medicaginis* var. *pinodella* and *A. pisi* are seed-borne (Bretag et al. 1995). Although seed infection by these pathogens rarely results in ascochyta blight epidemics (Moussart et al. 1998; Tivoli & Banniza 2007), the use of infected seed usually leads to poor germination and death of seedlings due to foot rot (Wallen et al. 1967; Xue 2000) or symptoms at or below the soil surface, especially at low temperature (Moussart et al. 1998). Nevertheless, the movement of infected seed not only spreads the fungi over long distance, but also has potential to disseminate compatible mating types of heterothallic fungi which could lead to the formation of teleomorphs in nature and increase the risk of development of new, virulent pathotypes (Kaiser 1997; Tivoli & Banniza 2007). Therefore, it is recommended that seed for sowing be accessed from areas with dry conditions or harvested as early as possible to avoid infection of seed (Hawthorne et al. 2012).

P. koolunga is not known to be seed-borne however isolates of *Macrophomina phaseolina*, which were reported to cause symptoms characteristic of ascochyta blight on field pea (Ali and Dennis 1992), but may have been *P. koolunga* (Davidson et al. 2009), were seed-borne (Ali et al. 1982).

As seedling emergence rate and or yield are negatively correlated with the percentage of seeds infected (Gossen et al. 2010; Xue 2000), treatment of seed with

fungicides is an approach to reduce primary inoculum and seed to seedling transmission of ascochyta blight. Fungicides with the active ingredients thiram and thiabendazole, such as P-Pickel T®, Fairgro® and Reaper TT®, have been recommended to control ascochyta blight seed infection in pulse crops as well as seedling root rot for early sown fields or those in the vicinity of infested stubble (Hawthorne et al. 2010; Hawthorne et al. 2012; Kimber & Ramsey 2001). However, little is known about the efficacy of fungicide seed dressings in control of ascochyta blight caused by *P. koolunga.*

The development of ascochyta blight resistant field peas would be the most economical and long-term approach to control this disease but, despite considerable research, current genotypes are susceptible (Adhikari et al. 2014; Bretag et al. 2006; Davidson & Kimber 2007; Schoeny et al. 2007). Richardson et al. (2009) observed a significant interaction between 31 field pea genotypes and single isolates of ascochyta blight pathogens, including *P. koolunga*, for disease incidence and severity using a detached leaf method, but details were not reported. Evaluation of the response of current and new field pea genotypes to a range of isolates of *P. koolunga* is lacking.

The aims of this study were to investigate (i) aspects of seed infection in relation to *P. koolunga,* such as whether the fungus can be seed-borne and transmitted from seed to seedling, (ii) the most effective fungicides for seed treatment, (iii) the response of a number of current genotypes of field pea to inoculation with isolates of *P. koolunga.*

Materials and methods

Seed infection experiments

Confirmation of seed infection by P. koolunga

Field pea seed samples (cv. Kaspa) were collected in 2010 from the following sources; 18 National Variety Trials (NVT) in South Australia (SA), Victoria (Vic.) and Western

Australia (WA), nine agronomy trials with three times of sowing in SA (Davidson et al. 2013) and four Pulse Breeding Australia (PBA) trial sites in SA. A modified agar plate method (Ali et al. 1982) to isolate seed-borne fungi was used to determine percentage of seed in each sample infected by *P. koolunga*. In brief, three replications of 100 seeds from each sample were surface sterilised in 2 % sodium hypochlorite solution for 2 minutes then dried on sterile filter paper. Ten seeds were placed in each plate contained potato dextrose agar (PDA) amended with 100 μgL^{-1} streptomycin. Plates were incubated at 22 °C under fluorescent and near ultraviolet light; 12 h/12 h dark/light, for up to 21 days. The resulting colonies were identified *P. koolunga* based on morphological and microscopic cultural characteristics (Davidson et al. 2009) or DNA test (Ophel-Keller et al. 2008) when required.

The same procedure was applied to examine infection of seed harvested from severely diseased, experimental potted field pea plants kept outdoors at Waite Campus, South Australia in November 2011. These plants had been artificially inoculated with mixed pycnidiospore suspensions (5×10^5 spores mL^{-1}) of four isolates of *P. koolunga*, 139/03, 142/03, 81/06 and FT07026, until run off at 3 weeks post-sowing. The inoculation process was repeated three times at 6-day intervals.

Examination of seed to seedling transmission of P. koolunga

Artificially inoculated seed (AIS) of field pea (cv. Kaspa) were prepared as described by Kimber et al. (2006). Briefly, surface sterilized seeds were submerged in mixed pycnidiospore suspension (1×10^7 spores mL^{-1}) of *P. koolunga* isolates 139/03, 142/03, 81/06 and FT07026, in a 2-L beaker within a 25-cm Kartell® implosion-proof desiccation bowl. The suction pressure was adjusted to -70 kpa and applied for 3 h. Seeds were dried on sterile germination paper for 24 h in sterile conditions and stored at

4 °C until use. Control non-inoculated seed (NIS) were prepared as above except that water was used instead of spore suspension.

Seed to seedling transmission of *P. koolunga* was investigated using a method adapted from Kimber et al. (2006). The experiment was conducted as a completely randomised block design with four replications and was repeated once. Forty AIS and ten NIS of cv. Kaspa were sown in University of California (UC) potting mix in Arborcell Easy-grower 50® trays with 50 cells per tray (one replicate). The pots were watered and maintained in a growth room under 12 h light/12 h dark at 16 °C. After 2 weeks, when seedlings were about 3 cm high, the pots were covered with a plastic tent and an ultrasonic humidifier was applied for 4 days to maintain leaf wetness. Seedling emergence and disease incidence were assessed 2 weeks later when plants had approximately eight nodes. Foot rot and foliar disease severity were recorded using a 0 to 5 scale (Moussart et al. 1998): 0, no symptoms; 1, streaks on the hypocotyl or on the epicotyl; 2, streaks on both the hypocotyl and the epicotyl; 3, lesions girdling the hypocotyl and streaks on the epicotyl; 4, lesions girdling both the hypocotyl and the epicotyl; and 5, weak plants with lesions girdling the hypocotyl and the epicotyl.

A necrotic index was calculated for each treatment by multiplying each disease score by the number of seeds in that category, and then dividing by the total number of germinated seeds (Moussart et al. 1998) as follows:

Necrotic index= $\sum_{i=0}^{n} n_i(d_i)/\mathrm{N}$

n_i refers to the number of seedlings in each disease category, d_i is the value of disease category and N is the total number of seedlings assessed.

To study the development of disease on field pea plants infected via seeds, a similar experiment was conducted using the same design and number of plants as above except

131 that the necrotic index was assessed 21 weeks after sowing, at physiological maturity,
132 code 301 (Knott 1987) when lower pods were dry.

133 *Effect of soil temperature on seed to seedling transmission of* P. koolunga

134 The seed to seedling transmission rate of *P. koolunga*, necrotic index and lesion length
135 on basal parts of field pea plants were studied at four soil temperatures (8, 12, 16 and 20
136 °C) as described by Kimber et al. (2006). Briefly, the experiment comprised a
137 randomised complete block design, with five trays, each containing 20 seeds. Each
138 temperature was tested twice. Temperature affected the growth rate of the plants so
139 plants in cooler temperatures were incubated for longer than those at warmer
140 temperatures to enable all plants to reach the eight-node growth stage for assessment.

141 *Comparison of fungicides as seed treatment in soil or on germination paper*

142 The efficacy of six fungicidal seed treatments, registered in Australia for use on pulse
143 crops, was examined. The fungicides are listed here as product name (active ingredient)
144 and dose per kg seed: P- Pickel T® (360 gL^{-1} thiram, 200 gL^{-1} thiabendazole) 2 mL,
145 Thiram® (thiram 600 g/kg) 1.4 g, Jockey® Stayer™ (167 gL^{-1} fluquinconazole) 20 mL,
146 Rovral® (500 g/kg iprodione) 5 mL, Impact® (500 gL^{-1} flutriafol) 3 g and Sumisclex®
147 500 (500 gL^{-1} procymidione) 2 mL (Hawthorne et al. 2010; Sprague & Burgess 2001).
148 NIS and AIS were included as controls. A method reported by Kimber and Ramsey
149 (2001) using germination paper was applied to examine efficacy of these fungicides to
150 control seed-borne *P. koolunga*. Each fungicide was used separately to treat 80 seeds of
151 cv. Kaspa and the seeds were placed on sheets of germination paper (50 cm long and 20
152 cm wide) in two rows 2 cm apart, the top row 5 cm below the upper edge of the paper.
153 There were 20 seeds per sheet. The germination papers were rolled up and fastened by
154 rubber bands, then placed upright in plastic bags with 5 mL water at the bottom which

were sealed and incubated at room temperature (22 °C) in the dark for 2 weeks (Kimber & Ramsey 2001). The experiment was designed as a completely randomised block with eight treatments and four replications, each with 20 seeds, and was conducted twice. Germination rate and necrotic index were assessed as described above.

The six fungicides were also tested in a pot experiment conducted as completely randomised blocks with six replicates in a growth room at 14-16 °C. Every block comprised eight pots (7 × 8.5 × 5.5 cm) containing UC potting mix, six of which were sown with four AIS dressed with a fungicide each, one pot per fungicide. NIS or AIS with no fungicide treatment were sown in the remaining pots as negative and positive controls, respectively. The pots were randomized in each block and watered immediately after sowing and thereafter when needed. Other conditions and disease assessment at eight nodes growth stage were as described for the seed to seedling transmission experiment. This experiment was conducted twice.

Reaction of field pea genotypes to *P. koolunga* isolates

The response of 12 field pea genotypes to one moderately virulent isolate of *P. koolunga*, 139/03 (Davidson et al. 2009), was evaluated in an experiment comprising four replicate blocks. Every block comprised three trays each with 12 pots. Each genotype was planted in a separate pot, four seeds per pot, within each block. The pots were then placed in a growth room at 15 ± 1 °C with 12h/12h light/dark cycle. The plants were watered every 2 days. When all plants had at least four nodes, they were sprayed until run-off with a spore suspension (5×10^5 spores mL^{-1}. Control plants were sprayed with sterile distilled water. All plants were placed in a humidity tent with a humidifier for 2 days after inoculation. Disease severity on the lowest three leaves of each plant was recorded at 2-day intervals starting at 3 days post-inoculation (dpi) to 11

dpi and then, due to fragility of plants, assessment was done at 5-day intervals concluding 21 dpi based on the 0-5 scale of Onfroy et al. (1999) and also as percentage of leaf area diseased (%LAD) (Priestley et al. 1985) at 16 and 21 dpi. Stem lesion length (mm) on internodes 2-4 was also recorded at 21 dpi. The Area Under Disease Progress Curve (AUDPC) for each genotype was calculated using the formula of Shaner and Finney (1977) as follows:

$$\text{AUDPC} = \sum_{i=1}^{n}[(Y_{i+n1} + Y_i)/2][T_{i+1} - T_i]$$

Y_i = disease severity at the i*th* recording, Ti = days at the i*th* recording and n = total number of disease recordings. AUDPC of field pea genotypes inoculated with *P. koolunga* in this experiment was calculated at 21 dpi based on the 0-5 disease scale recorded from 3 to 21 dpi. To standardise, relative area under disease progress curve (RAUDPC) was calculated as AUDPC divided by the number of days from inoculation to recording of disease severity for the desired RAUDPC (Fry 1978).

In the second experiment, four genotypes of short, semi-leafless type field peas that differed in susceptibility to *P. koolunga* isolate 139/03 were inoculated with three individual isolates of *P. koolunga* exhibiting low, medium and high virulence according to Davidson et al. (2009). Methods were as above except that the trial design consisted of three blocks each comprising four trays. Each tray contained three pots of each genotype and the whole tray was inoculated with one isolate. Control trays were inoculated with sterile water. Disease severity was assessed as %LAD at 2-7 days intervals, ending at 21 dpi. Stem lesion length (mm) was assessed at 21 dpi. AUDPC and RAUDPC were calculated for each genotype/isolate combination.

Statistical analysis

Results for repetitions of individual experiments were combined, because t-tests showed no significant difference between repetitions. For the experiment involving the reaction

of plant genotypes to the fungus, as the control plants were not diseased, these data were omitted from the analysis and the experiment analysed as a randomized block. The results for all experiments were subjected to one-way analysis of variance using GenStat 15th edition SP2, except that two-way ANOVA was used to examine the reaction of four field pea genotypes to three isolates of *P. koolunga*. Tukey's honestly significant difference test at 95 % confidence intervals was applied to compare means in each experiment.

Results

Confirmation of seed infection by *P. koolunga*

Most field pea seed samples collected in 2010 from pulse cropping areas in SA, Vic. and WA were infected by ascochyta blight pathogens, mostly *Didymella pinodes*. *P. koolunga* was isolated from most NVT samples from Vic. and SA but not from WA, and from all PBA seed samples from SA (Table 1). Although the identification of *P. koolunga* was based mainly on microscopic and morphological characteristics of cultures, non-sporulating atypical colonies of *P. koolunga* were obtained and identified by DNA testing. The frequency of isolation of *P. koolunga* from NVT seeds ranged from 0 to 6.3 % and from PBA seeds from 0.3 to 5 %. *P. koolunga* was also isolated from seeds harvested from agronomy trials with three times of sowing, most frequently from plants sown early (30th April 2010) and least from plants sown late (11th June 2010). The seeds harvested from severely diseased plants that had been artificially infected and grown at Waite Campus showed the most frequent infection, *P. koolunga* being isolated from 36 % of seeds.

Examination of seed to seedling transmission of *P. koolunga*

Seeds artificially inoculated with conidial suspension of *P. koolunga* and maintained at 16 °C showed a transmission rate to seedlings of about 98 %. The average necrotic index calculated for the seedlings with eight nodes was 1.89, as most seedlings had streaks on both hypocotyl and epicotyl (Fig. 1). This average increased to 3.32 at physiological maturity due to development of lesions girdling the hypocotyl and the epicotyl on more than 53 % of plants. Streaks at physiological maturity ranged from 9 to 51 mm long, with mean of 25.1 mm, and were dark and necrotic. A few infected seedlings were scored 5 as they remained very short, about 7 cm, and weak with lesions girdling the epicotyl and hypocotyl. Many pycnidia were observed on root systems or crowns, and sometimes 20 cm above the cotyledons, on 60 % of plants. Tall plants lodged onto soil or against lower infected parts of plants, allowing the fungus to infect on aerial parts.

Effect of soil temperature on transmission

There was no effect of inoculation on germination (P = 0.05), although at 8 °C germination was reduced from 97 % to 90 %. More than 29 % of seedlings from AIS at 8 °C had lesions which girdled the hypocotyl and or the epicotyl and more than 3 % of seedlings were weak with lesions girdling both the epicotyl and hypocotyl. These plants were usually stunted, 7-9 cm, compared to 23 cm for healthy control plants. Lesions on roots and crowns were longer on seedlings at 8 °C than at other temperatures (Fig. 2), while no statistical difference was observed in lesion length on seedlings at 12, 16 and 20 °C. Necrosis on seedling roots based on the 0-5 necrosis index at 8 °C was also significantly more severe than at other temperatures. Many pycnidia were seen on most decayed seed coats of AIS in soil at 8 °C at the time of recording disease severity while very few pycnidia were found at other temperatures.

Comparison of fungicides as seed treatment in soil or on germination paper

All fungicide treatments in potting soil reduced disease incidence and necrosis compared with the untreated AIS control (Fig. 3a). Disease incidence and necrosis on seedlings that emerged from untreated AIS (positive control) were 97 % and 1.68, respectively, whereas all seedlings from NIS (negative control) were healthy. There were no significant differences among fungicides in terms of disease incidence, whereas P-Pickel T® was more effective than Sumisclex® and Jockey Stayer® (P = 0.05) and as effective as the remaining three fungicides in reducing necrosis.

The efficacy of fungicidal seed treatments examined on germination paper differed from that in potting soil (Fig. 3b). Seedlings from AIS treated with Jockey Stayer® and rolled in germination paper were symptomless. The efficacy of P-Pickel T®, Rovral® and Thiram® was statistically similar to Jockey Stayer® based on disease incidence and necrosis. Impact® was less effective than the other fungicides, except Sumisclex®, for reducing disease incidence. All seedlings from NIS were symptomless and all from AIS without fungicide treatment were diseased. All seedlings germinated from AIS without fungicide treatment showed hypocotyl infection and 54 % also showed symptoms on the first internode, 21 % on second internode, 38 % on scale leaf and 8 % on first leaf.

Reaction of field pea genotypes to *P. koolunga* isolates

In the first experiment, the genotypes differed in reaction to *P. koolunga* isolate 139/03, in that Sturt, Parafield, PBA Percy, Excell and Morgan were more severely diseased on leaves than the others and WAPEA2211, PBA Wharton, PBA Oura, Kaspa and PBA Twilight showed the least disease at 5 and 7 dpi (data not shown). The same trend in reaction to this isolate was seen at 21 dpi in terms of AUDPC and %LAD (Fig. 4), except that WAPEA2211 showed a greater increase in %LAD than the other genotypes

and was one of the more susceptible genotypes at 21 dpi. Field pea genotypes could be grouped into several statistical categories (P = 0.05) on the basis of stem lesion length, such that Morgan was most severely affected, then Sturt and thereafter Excell, Parafield and Moonlight. Although WAPEA2211 had relatively short internode lesions, lesion length was not significantly different from those on PBA Wharton, PBA Twilight, Kaspa, PBA Oura, PBA Percy and PBA Gunyah.

In the second experiment there was a significant interaction effect between isolate and genotype (Fig. 5). Morgan was most severely diseased (P = 0.05) following inoculation with DAR78535 based on RAUDPC, but was statistically similar to WAPEA2211 for the other two isolates. Kaspa was least diseased (P = 0.05) for two isolates but similar to PBA Gunyah for isolate DAR78535. Despite this interaction, ranking of genotypes according to RAUDPC was the same or similar irrespective of the isolate. At 3 and 5 dpi %LAD on Morgan was greater than on Kaspa, but WAPEA2211 and PBA Gunyah were statistically similar to both Morgan and Kaspa. Thereafter, disease progressed more quickly on Morgan than on Kaspa; at 21 dpi 65 % of the leaf area of Morgan was covered with lesions and 42 % on Kaspa. The three isolates of *P. koolunga* differed in pathogenicity on leaves of the three genotypes early in the experiment, for example FT07026 was most and DAR78535 least aggressive in terms of %LAD at 3, 5, 10 and 14 dpi (P = 0.05), but they were statistically similar at 21 dpi. Stem lesions at 21 dpi were longer on Morgan inoculated with 139/03 (P = 0.05) than on the other three genotypes, and FT07026 and DAR78535 produced lesions on Morgan that were longer than those on Kaspa (Fig. 6).

Discussion

P. koolunga was widely distributed but infrequent in seeds collected in SA and Vic. Likewise, Davidson et al. (2009) isolated this pathogen from aerial parts of field pea

300 plants in many cropping regions of SA from 1995 to 2007 and Davidson et al. (2011)
301 detected it in soil samples across SA and western Vic. The absence of *P. koolunga* from
302 seeds from WA is in agreement with Tran et al. (2014), who did not identify *P. koolunga*
303 among 1058 isolates of ascochyta blight pathogens from six locations in WA from 1984
304 to 1996, nor among 150 isolates from field pea leaves and stems in 2010, the year in
305 which WA seed samples examined in our study were harvested. The fact that 14 of the
306 16 SA seed samples yielded *P. koolunga* (0.3 - 6.3 % of seed per sample) supports the
307 report by Ali et al. (1982), in which 72 % of field pea seeds were reported to be infected
308 by *M. phaseolina*, now thought to have been *P. koolunga* (Davidson et al. 2009). The
309 decreasing incidence of infection of seed with later sowing in the agronomy trials in SA
310 reflects disease severity reported by Davidson et al. (2013) which, in turn, was
311 associated with rainfall and timing of ascospore release. More disease on plants is likely
312 to lead to greater pod and seed infection, hence early sown crops are more likely to
313 produce infected seed than later sown crops. The relationship between rainfall and seed
314 infection should be examined through experimentation in field conditions. As a
315 teleomorph has not yet been recorded for *P. koolunga*, infected seed may act as source
316 of primary inoculum to initiate disease in field pea cropping regions in Australia.
317 Plants arising from artificially inoculated seed developed lesions on the epicotyl and
318 hypocotyl that eventually girdled the stem base, a phenomenon often referred to as foot
319 rot, a common feature of the other ascochyta blight pathogens on field pea, particularly
320 *P. medicaginis* var. *pinodella* (Knappe & Hoppe 1995). Girdling increases the risk of
321 lodging and yield reduction. Gossen et al. (2010) reported that lodging was generally
322 more severe among plants that emerged from seed infected with *D. pinodes* when
323 incidence of seed infection was high. They also observed that seed infection with *D.*
324 *pinodes* can lead to reduced yield in some instances. Therefore reduced seedling

emergence due to *P. koolunga* at lower temperatures may impair establishment of plants in the field and should be investigated.

The effects of temperature on transmission of *P. koolunga* from seed to seedlings were generally in agreement with those presented by Moussart et al. (1998), in which seedling emergence from AIS with *D. pinodes* at 8 °C was lower than at 13 and 20 °C. The overall seed to seedling transmission rate at 8-20 °C in this study was 98 %, much more frequent than the 31 % transmission rate from chickpea seed artificially inoculated with *A. rabiei* at 5 to 19 °C reported by Kimber et al. (2006). The necrotic indices for *D. pinodes* reported by Moussart et al. (1998) on field pea seedlings at 8 and 12 °C were higher than at 20 °C. Likewise, the most severe disease caused by *P. koolunga* on epicotyl and hypocotyl of seedlings occurred at 8 °C. Gossen and Morrall (1986) reported that disease incidence on seedlings that emerged from infected lentil seeds was greater in cold than warm soil, which was attributed to the seedling epicotyl growing away from the infected cotyledons in warmer conditions. This may be the case also for transmission of *P. koolunga* from seed to seedlings of field pea as the growth period to eight nodes growth stage at 8 °C was 10 weeks compared to 4 weeks at 20 °C. Furthermore, the observation that more pycnidia of *P. koolunga* developed on epicotyls and hypocotyls at lower temperatures concurs with findings by Moussart et al. (1998) for *D. pinodes*.

P-Pickel T® is the fungicide most commonly recommended for treatment of pea seed to prevent seedling infection due to ascochyta blight in Australia as well as for control of seedling root rot (Hawthorne et al. 2012). P-Pickel T® was generally the most effective of the six fungicides applied to treat seed infected with *P. koolunga* in soil. Thiram® has been shown to increase seedling emergence from seed infected with *D. pinodes* by 35-45 %, increasing yield compared with untreated seeds (Xue 2000), and

Agrosol® (thiram + thiabendazole, similar to P-Pickel T®) increased field pea yield more than Thiram® (Hwang et al. 1991). In the current study, seeds were submerged in spore suspension of the fungus for 3 h, resulting in 100 % infection, much greater than incidence of naturally infected seed. As a consequence, the fungicides may be more effective when applied to naturally infected seeds in the field than to AIS in pots. Although Jockey Stayer® controlled transmission of *P. koolunga* to seedlings on germination paper, producing 100 % healthy seedlings from AIS, it was less effective in soil, with 56 % disease incidence. This difference is not understood.

This study showed that P-Pickel T® and Thiram® can be used as seed dressings for control of *P. koolunga* as well the other ascochyta blight pathogens without further costs to farmers. P-Pickel T® is most effective for crops with a high risk of ascochyta blight, for example where sown early, with a medium to high level of soil-borne inoculum, or close to last year's infested stubble as a source of wind-borne ascospores or pycnidiospores (Hawthorne et al. 2012; Salam et al. 2011b). Although the percentage of seed naturally infected with *P. koolunga* here was less than 10 %, seed harvested from fields with a history of disease, particularly in years conducive for development of disease, should be treated with a fungicide such as P-Pickel T®. Kimber et al. (2007) demonstrated that when a susceptible variety is sown, even 1 % of infected chickpea seedlings originating from seed infected with *D. rabiei* could act as foci for disease dissemination and lead to 60 % yield loss in conducive conditions. In contrast, Bretag et al. (1995), Moussart et al. (1998) and Gossen et al. (2010) found that seed infection was not an important source of inoculum for epidemics, which Gossen et al. (2010) proposed was due to low frequency of transmission of *D. pinodes* from seed to seedling. Nevertheless, Gossen et al. (2010) demonstrated that it is crucial to avoid introducing *D. pinodes*, even at a very low level of seed infection, to regions in Canada that are free of

375 ascochyta blight or that experience this disease infrequently. This would also apply to *P.*
376 *koolunga* as the fungus is not prevalent in states of Australia other than SA and,
377 recently, WA and seed to seedling transmission appears to be frequent. Although *P.*
378 *koolunga* was not detected on field pea plants in WA before 2010, it was detected at
379 several locations in 2012 (Tran et al. 2014), far from the SA border. One explanation for
380 this rapid and broad distribution of *P. koolunga* to new areas in WA could be
381 transmission by infected seeds, as seed harvested in SA, particularly for new genotypes,
382 is sometimes transported to WA (Margetts, K., seednet.com.au, pers. comm., 2014).
383 Alternatively, *P. koolunga* may have been present in WA for some years but not
384 recognised. Therefore, the best measure to control long distance spread of this fungus is
385 to use healthy seed obtained from dry regions for sowing in areas uninfected or with
386 low infection. Furthermore, treatment of seeds with an appropriate fungicide such as P-
387 Pickel T® or Thiram® can provide sufficient protection against not only *P. koolunga*, but
388 also other ascochyta blight pathogens and seedling root rot pathogens.

389 Most of the genotypes assessed in this study have been marketed to Australian
390 farmers as moderately susceptible to ascochyta blight (Hawthorne et al. 2012), however
391 there was some variation in susceptibility to *P. koolunga*. PBA Wharton, a newly
392 released genotype, PBA Oura, Kaspa and PBA Twilight, when inoculated with a
393 moderately virulent isolate of *P. koolunga*, exhibited less disease, based on %LAD, than
394 the other eight genotypes tested. Davidson et al. (2009) indicated that WAPEA2211 and
395 Kaspa at 7 dpi were less susceptible than Parafield to *P. koolunga* in controlled
396 conditions. Although WAPEA2211 has been claimed as the first breeding line
397 moderately resistant to ascochyta blight in WA and was used as a benchmark in a study
398 to compare susceptibility of new breeding lines to *D. pinodes* (Adhikari et al. 2014), the
399 lowest disease severity recorded by those researchers on this line naturally infected in

400 field conditions was 5.1 on a scale of 0-9, while Kaspa was 6. However in our study,
401 AUDPC and stem lesion length on WAPEA2211 at 21 dpi were similar to Kaspa when
402 inoculated with a moderately virulent isolate of *P. koolunga*.

403 The numeric value of %LAD at 21 dpi was very similar to AUDPC (Pearson
404 coefficient R = 0.9075) for the 0-5 disease scale calculated over 3-21 dpi for each of the
405 genotypes assessed (Fig. 4), therefore it was concluded that %LAD provided an
406 accurate assessment of disease severity. In addition this assessment was quicker than
407 using the 0-5 scale, so was adopted in the second experiment. In the second experiment,
408 the genotypes varied in response to isolates of *P. koolunga*. Results for Morgan, the
409 most susceptible genotype of the four tested in this experiment, were similar to the first
410 experiment. Davidson et al. (2009) reported that severity of ascochyta blight on
411 WAPEA2211 inoculated with *M. pinodes* or *P. koolunga* was lower than other lines
412 tested at 7 dpi. In the current study, WAPEA2211 in both experiments showed reduced
413 susceptibility to *P. koolunga* soon after inoculation, but after 10 dpi this effect
414 disappeared. Leaves on WAPEA2211 seedlings expanded more slowly than other
415 genotypes while the fungus progressed at a similar rate on all genotypes, so the percent
416 necrotic area on WAPEA2211 leaves increased at a greater rate than on other genotypes.
417 In this study, Morgan had the longest stem lesions at 21 dpi, regardless of
418 aggressiveness of the isolate, and the largest %LAD. Furthermore, Morgan was the
419 tallest genotype in our second experiment; consequently, this genotype may be at most
420 risk of girdling.

421 The pathogenicity of three isolates of *P. koolunga* on four genotypes differed in the
422 first 14 days after inoculation, in accordance with results presented by Davidson et al.
423 (2009), but by 21 dpi the difference in aggressiveness had disappeared. These isolates

developed at different rates on leaves, but as the rate of leaf area expansion was lower than disease progress, the differences in aggressiveness were not apparent after 14 dpi.

Tivoli et al. (2006) considered assessment of partial resistance in growth room conditions to be more precise than in field conditions as the effect of environmental conditions is minimised. Given that, in this study, the response of field pea genotypes to inoculation with *P. koolunga* in two experiments was generally consistent and overall in agreement with Davidson et al. (2009), it seems that screening in controlled conditions is a valid method to identify genotypes with reduced susceptibility to this pathogen. Further evaluation in field conditions in Australia is necessary to assess the effect of environmental factors on response to *P. koolunga*.

The results of this research suggest that integrated strategies could improve management of ascochyta blight caused by *P. koolunga* on field pea plants. These strategies could include sowing of healthy seed, avoiding movement of infected seed to *P. koolunga*-free cropping areas, dressing seed with fungicides such as P-Pickel T® and, finally, choosing field pea genotypes with reduced susceptibility to this fungus. Evaluation of seed dressing in field conditions at different sowing times, using representative isolates of *P. koolunga* from SA, Vic. and WA on current and newly released field pea genotypes in different rainfall cropping areas, is warranted.

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Survival, transmission and control of *Phoma koolunga* in field pea seed and reaction of field pea genotypes to the pathogen

Australasian Plant Pathology

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Table 1 Percentage of field pea seed infected with *Phoma koolunga* harvested from trials or experiments at several locations in Australia in 2010 or 2011 (means of three replicates)

Seed source[a]	State	Location	Mean	Standard deviation
NVT	Victoria	Beulah	3.33	0.58
		Birchip	3.67	0.58
		Hopetoun	1.67	0.58
		Horsham	2.00	1.00
		Sea Lake	0.00	0.00
		Ultima	1.67	0.58
NVT	South Australia	Bool Lagoon	1.67	0.58
		Lameroo	0.00	0.00
		Laura	2.00	1.00
		Minlaton	3.67	0.58
		Mundulla	0.67	0.58
		Riverton	4.00	1.00
		Rudall	0.00	0.00
		Willamulka	1.67	1.53
		Yeelanna	6.33	1.53
NVT	Western Australia	Dalwallinu	0.00	0.00
		Pingrup	0.00	0.00
		Scadden	0.00	0.00
PBA	South Australia	Balaklava	5.00	2.65
		Kingsford	0.33	0.58
		Snowtown	2.33	0.58
		Willamulka	1.33	1.15
AT	South Australia	Hart,TOS[b] 30/04/2010	2.78	1.48
		Hart,TOS 21/05/2010	2.56	0.88
		Hart,TOS 11/06/2010	0.67	0.71
AIP	South Australia	Waite Campus	35.67	5.51

[a] National Variety Trial 2010 (NVT), Pulse Breeding Australia 2010 (PBA), Agronomy Trials 2010 (AT), Artificially infected plants 2011 (AIP)

[b] Time of sowing

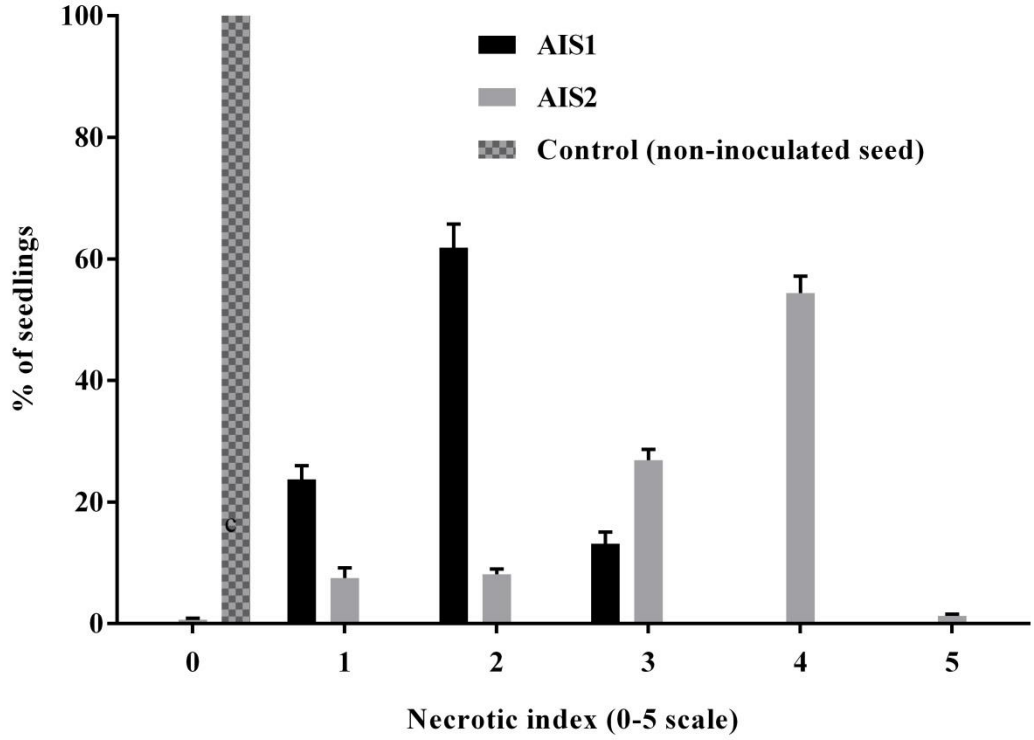
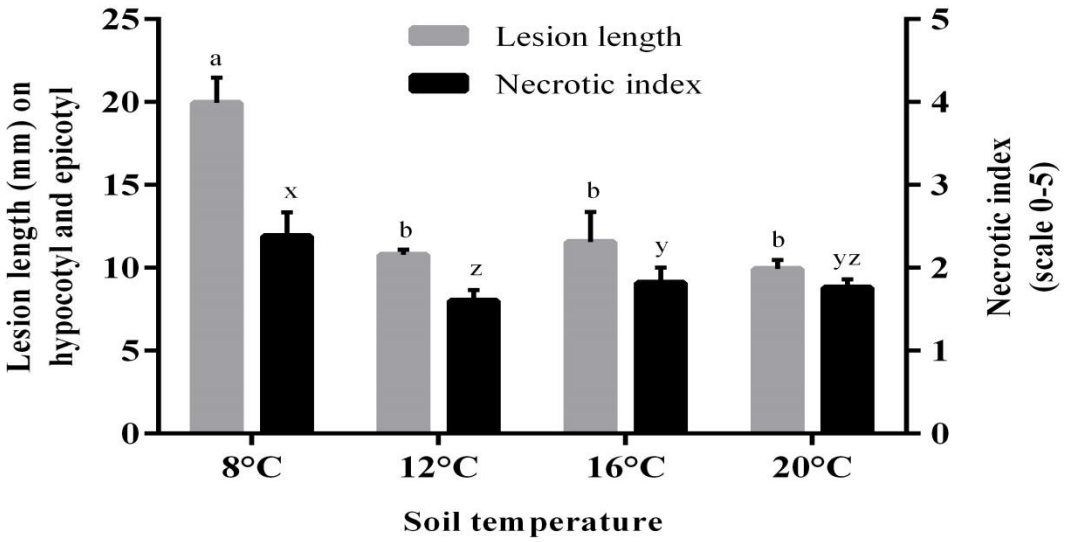


Fig. 1 Necrotic index on field pea plants infected via seed artificially inoculated (AIS) with *Phoma koolunga* and kept in a growth room at 16 °C, assessed at the eight-node growth stage (AIS 1) in the first experiment and at physiological maturity (AIS 2) in the second experiment, error bars represent SE.



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34 **Fig. 2** Necrotic index and lesion length on field pea seedling roots infected via seed
35 artificially inoculated with *Phoma koolunga,* which were incubated in potting soil at
36 four temperatures, transferred to a growth room and assessed at eight node growth
37 stage. Bars with the same letters (a-b and x-z) are not significantly different by Tukey's
38 test ($P = 0.05$), bars represent SE.

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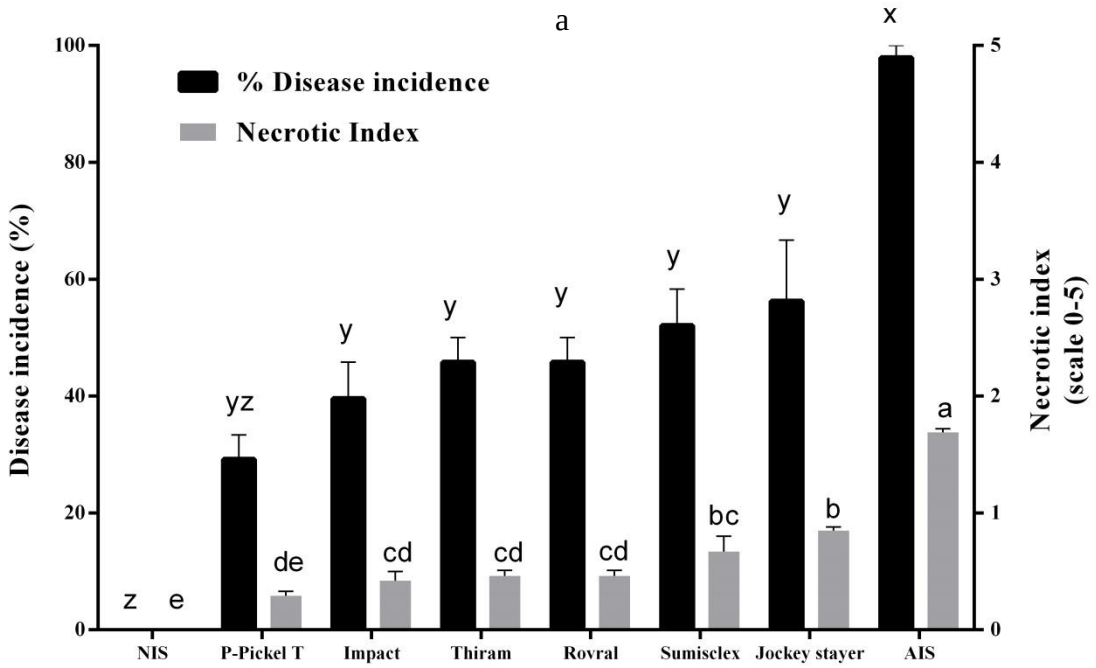

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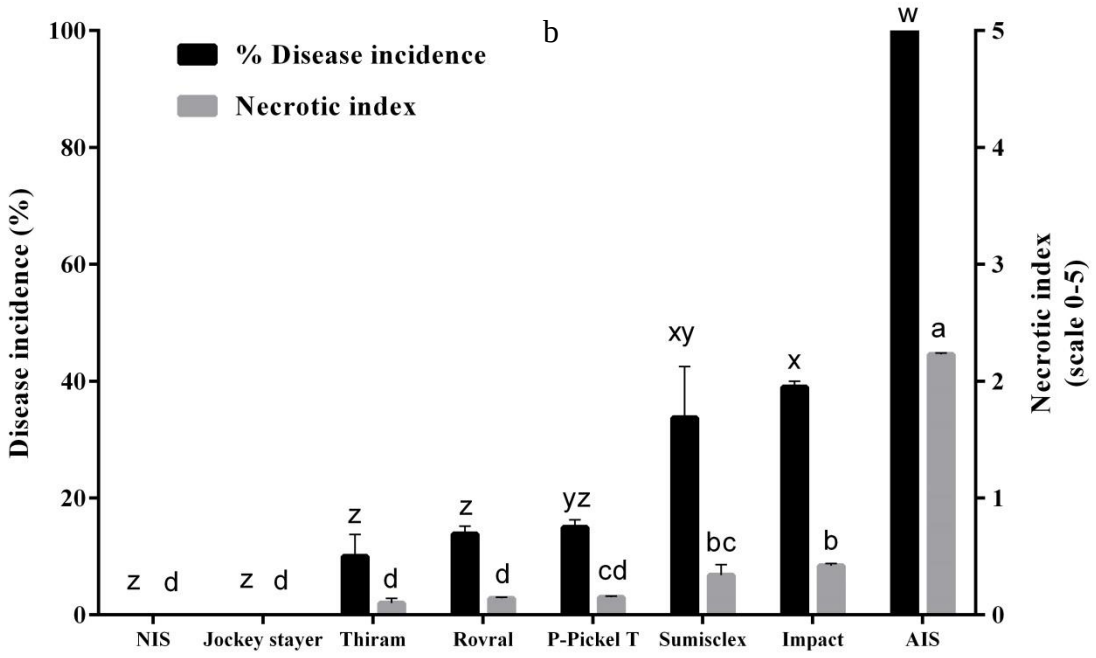

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49 **Fig. 3** Efficacy of fungicidal seed treatments on field pea seed artificially inoculated
50 with *Phoma koolunga* and incubated in (a) potting soil and (b) on germination paper.
51 Disease was assessed based on disease incidence (DI) and necrotic index on seedlings
52 roots. Treatments with the same letters (a-e and w-z) are not significantly different by
53 Tukey's test ($P = 0.05$), bars represent SE.

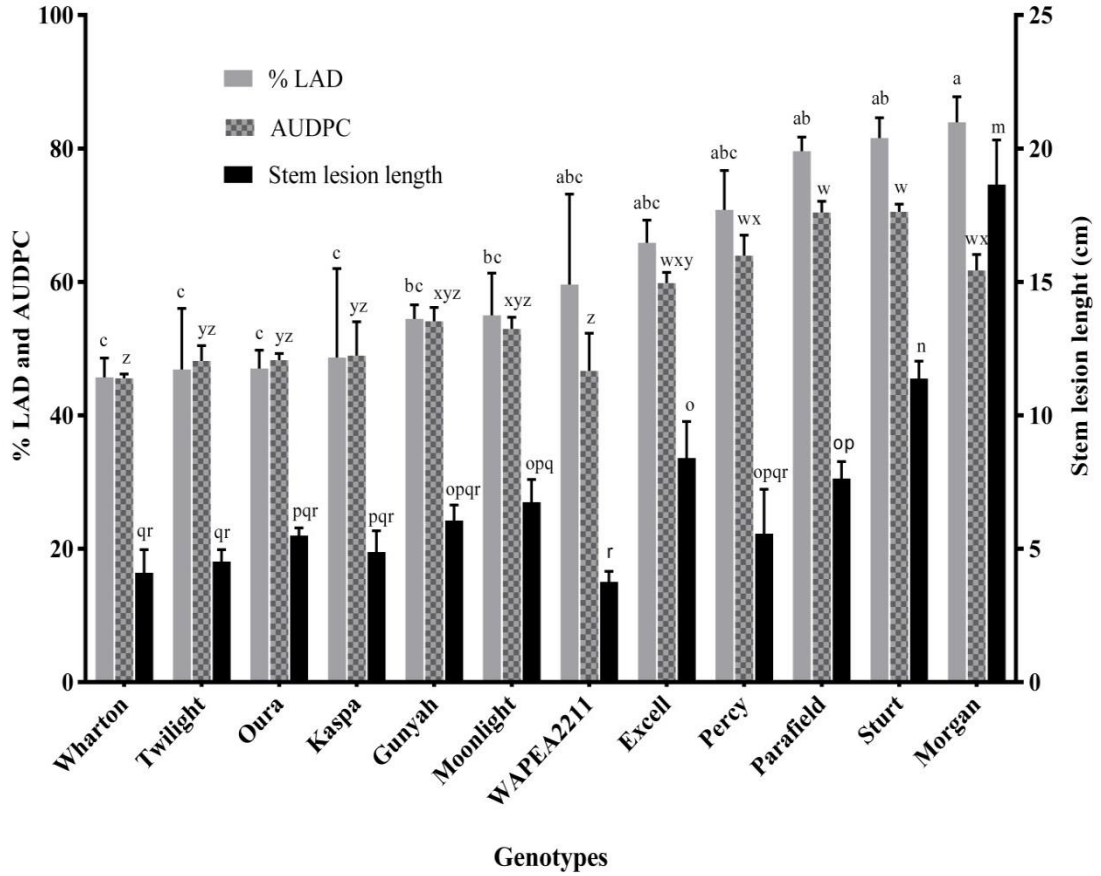


Fig. 4 Reaction of 12 field pea genotypes incubated in a growth room at 16 °C after inoculation by spraying foliage with a spore suspension of *Phoma koolunga* isolate 139/03. Disease was assessed as % Leaf Area Diseased (%LAD), Area Under the Disease Progress Curve (AUDPC) and stem lesion length at 21 dpi. Means with the same letters (a-c, w-z, and m-r) are not significantly different by Tukey's test ($P = 0.05$), bars represent SE.

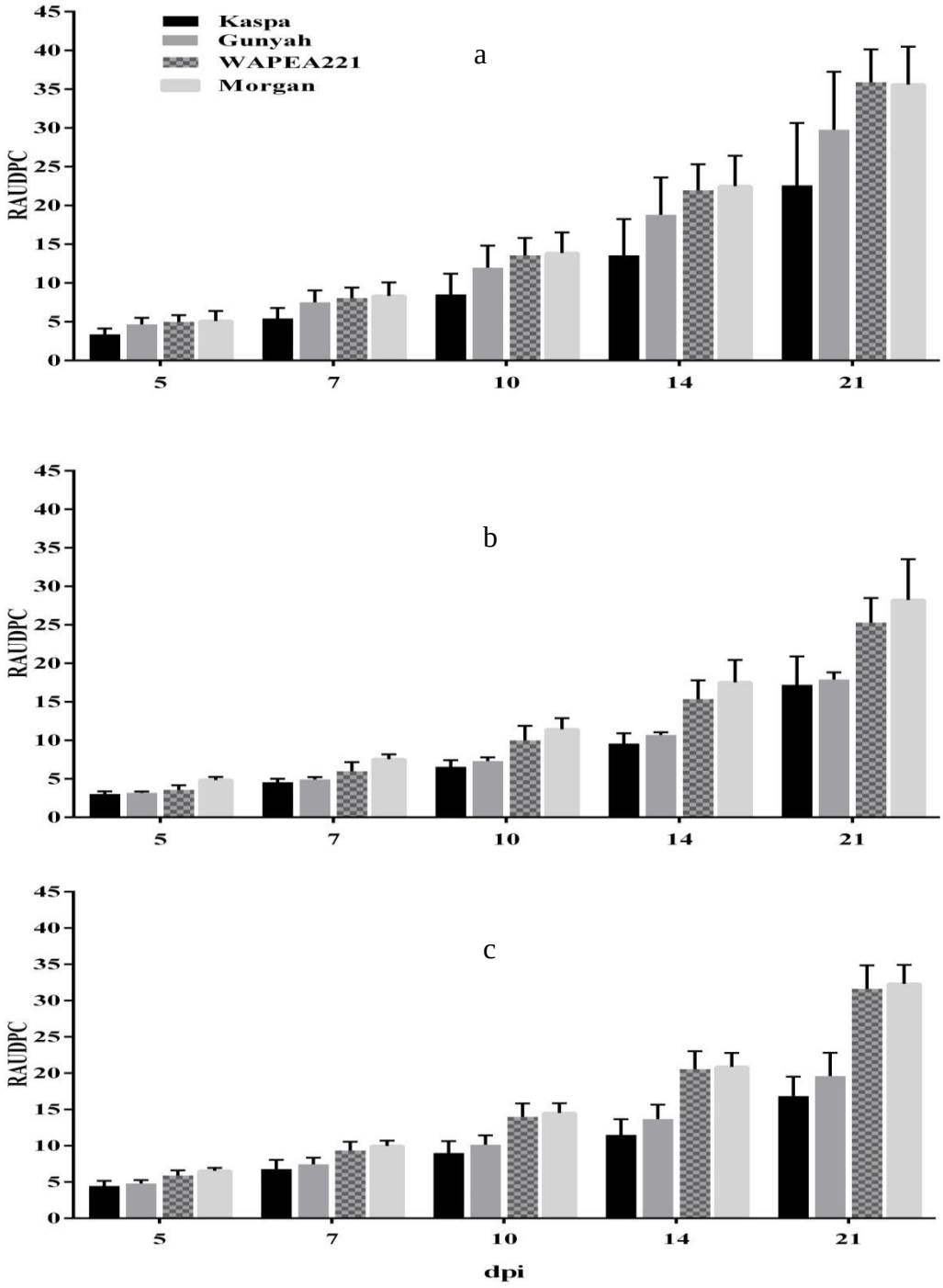
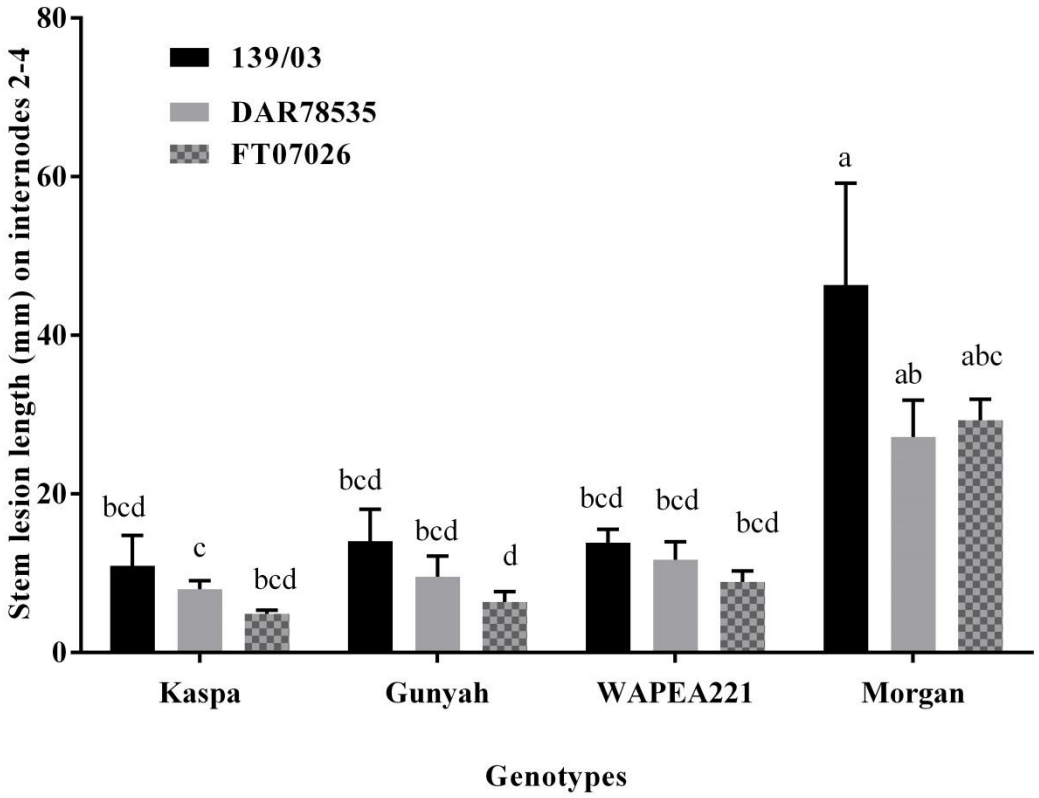


Fig. 5 Relative Area Under the Disease Progress Curve (RAUDPC) based on %Leaf Area Diseased on four field pea genotypes inoculated with spore suspensions of three *Phoma koolunga* isolates (a) 139/03, (b) DAR78535, (c) FT07026 and assessed to 21 dpi, bars represent SE. Least significant difference ($P < 0.05$) = 8.70 (a), 13.67 (b) and 5.38 (c) at 21 dpi.



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77 **Fig. 6** Stem lesion length (mm) on four pea genotypes inoculated with spore
78 suspensions of three individual *Phoma koolunga* isolates, 21 dpi in a growth room at
79 16°C. Means with the same letters are not significantly different by Tukey's test
80 (P = 0.05), bars represent SE.

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