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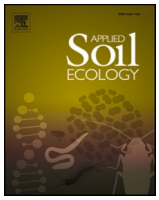

Sequence of introduction determines the success of contrasting root symbionts and their host

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ABSTRACT

Many crop species play host to a diverse range of soil-borne symbionts ranging from parasitic, such as potato cyst nematodes (PCN), to mutualistic, including arbuscular mycorrhizal fungi (AMF). Each of these organisms may establish symbiosis with the host prior to the arrival of another which may impact the fitness of all parties involved. We simulated a range of arrival time scenarios for both AMF and PCN and determined their consequences on potato host plants and subsequent symbionts to reflect the likely complexity of symbioses that occur in the field. Simulations were focussed on the first few weeks of plant growth to identify the importance of symbiont interactions during early plant development. Our data indicate that the order in which symbionts are introduced to crop roots is not only important for their own success, but also for that of the host and its additional symbionts. The presence of AMF increased the PCN population on the host, with earlier introduction of AMF increasing the magnitude of the effect. However, presence of AMF also increased the potato's tolerance to PCN, ameliorating the negative effects of the increased PCN burden. This tolerance was stronger the earlier the AMF were introduced and was sustained even when AMF were introduced after PCN. Overall, we show that the initial few weeks of crop emergence and growth may reflect a window of opportunity where the prosperity of the crop and its tolerance of parasites can potentially be influenced by coordinating application of AMF propagules. Additionally, these timings impact the success of below-ground plant parasites that can persist and impact crops for several years.

1. Introduction

At any given time, plants host a variety of co-colonising symbionts that span the parasitism-mutualism continuum (Johnson et al., 1997) including plant-parasitic nematodes and arbuscular mycorrhizal fungi (AMF). These symbionts can heavily impact outcomes in agricultural systems in terms of crop productivity and health, both when symbionts occur in single and in co-colonisations (Schouteden et al., 2015; Rillig et al., 2016). Plant-parasitic nematodes infect plant roots and extract plant resources for their own growth and proliferation. Of these obligately biotrophic invertebrates, potato cyst nematodes (PCN) cause more than £30 million of economic losses through parasitism-related damage in UK agriculture alone (approximately £400/ha; Price et al., 2021). These losses are a major cause for concern both economically and in terms of crop production systems for global food security. PCN exclusively infect solanaceous species with a widespread incidence across potato growing regions (CABI, 2020) and offer no known benefits to their host plants. The resistance status of the cropped potato variety has a large influence on the infection and fecundity of PCN (Dybal, 2019), however the presence of additional organisms, such as AMF, can also influence PCN fitness (Bell et al., 2022).

AMF are near-ubiquitously occurring root-associated symbionts that are common in agricultural environments (Helgason et al., 1998). These soil-borne fungi are usually mutualistic symbionts of plants, exchanging inorganic nutrients from the soil in return for host photosynthates (Johnson et al., 1997; Kiers et al., 2011). AMF colonise the vast majority of modern land plants, including most economically important crops (Soudzilovskaia et al., 2020), and are increasingly used as soil amendments in agricultural systems (Cely et al., 2016) even though their efficacy remains under debate (Ryan and Graham, 2018; Rillig et al., 2019). AMF propagules are usually present in potato fields (Cesaro et al., 2008) and they readily colonise potato roots, forming mutualistic partnerships (Bell et al., 2022). However, studies into their usage as amendments to increase food production have produced contrasting

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results (e.g. Hijri, 2016; Loján et al., 2017).

The success of AMF can be influenced by biotic and abiotic interactions such as climatic and soil conditions (Jamiołkowska et al., 2018), species identity (Klironomos, 2003), host responses (Zipfel and Oldroyd, 2017) and the time of arrival at the root surface in relation to other species (Werner and Kiers, 2015; Hoysted et al., 2017). The 'time of arrival' of each symbiont at the root may reflect the sequence of establishment on the host and can have important outcomes in plant-symbiont interactions, particularly those occupying the same ecological niche as competition for space and nutrients may occur (Vos et al., 2014). Colonisation of roots by AMF can be greatly reduced if a different species of AMF arrives and colonises the roots first (Werner and Kiers, 2015). Additionally, the presence of co-occurring symbionts such as aphids (Charters et al., 2020) and PCN (Bell et al., 2022), greatly impact AMF function, disrupting the exchange of carbon for nutrients between AMF and their host by restricting carbon allocation to the AMF whilst the fungus continues to supply nutrients to the host, with a large proportion of these ultimately being ingested by the feeding nematodes (Bell et al., 2022). Simultaneously occurring AMF and plant-parasitic nematodes may compete not only for nutrients within host plant roots, but also for space in which to establish intracellular feeding/exchange structures (Schouteden et al., 2015). As a result of this competition, concurrent infection by PCN and AMF reduces AMF-induced increases in potato yields whilst simultaneously alleviating PCN-induced stresses on photosynthetic efficiency (Bell et al., 2022). It remains unknown whether or how such effects are affected by the order of arrival of each symbiont to the host plant.

Arrival of the symbiont at the root-soil interface and establishment of symbiosis prior to a subsequent organism may determine the success of both (Werner and Kiers, 2015). In nature, a variety of outcomes are likely where each symbiont colonises different plants in a different order, impacting the success of each plant and possibly the mycorrhizal benefits that they receive (Bell et al., 2021a). In agricultural ecosystems, AMF inoculants are increasingly applied as part of sustainable and/or regenerative approaches to improving crop nutrient access (Thirkell et al., 2017; Elliott et al., 2021), however little consideration has been paid to the costs and benefits of such applications to the plant hosts (Verbruggen et al., 2013; Cely et al., 2016), particularly in multi-symbiont scenarios such as those described above. Moreover, it is likely that the timing of such inoculations are important for their success (Verbruggen et al., 2013), possibly due to the order of arrival of the AMF at the root surface relative to other symbionts. Given the ability of AMF to grow across and between multiple host root systems year-round (Isobe et al., 2014), their access to suitable host roots is potentially greater than PCN which typically produce a single generation on a single crop per year following a process known as diapause (Moens et al., 2018). That said, a recently identified Kenyan population of the closely related species *Globodera rostochiensis* can reportedly produce up to three generations per year (Mwangi et al., 2021) and there would be widespread ramifications if this lack of diapause became common in other PCN species. Regardless of their number of generations per year, the host-selective nature of PCN and their prompt hatching after crop planting (Moens et al., 2018), could provide an advantage over AMF in terms of establishing first on the root system.

In order to establish how important order of establishment is on promoting beneficial effects of AMF inoculation over detrimental effects of PCN in potatoes, we investigated whether the timing of inoculation of either AMF or PCN at different stages of host development affects host growth and yield. Secondly, we determined the impact of the timing of inoculation of AMF and PCN on the same host in terms of host yields. Thirdly, we investigated the impact of the arrival order of both symbionts on the colonisation/infection of either symbiont.

2. Methods

2.1. Growth conditions and application of AMF and PCN on potato plants

Potato tubers (*Solanum tuberosum* cv. Désirée) with one chit present were planted in 21 cm pots containing sterilised sand:topsoil (50:50, RHS Silver Sand:Bailey's Norfolk Topsoil, nutritional content in Supplementary Table S1). Treatment pots were inoculated with PCN and/or AMF at different time intervals (Supplementary Table S2). Control pots were inoculated with either AMF-only, PCN-only or AMF + PCN at the selected time points to determine the effects of sequentially introducing the symbionts. All time scales indicate the number of weeks post-planting. To determine the effect of the timing of AMF inoculation, AMF were applied two, three or four weeks post-planting, to pots that were inoculated with PCN from week one (Supplementary Table S2; pot numbers 66-80). Additionally, PCN were inoculated sequentially at weeks two, three or four on hosts that had AMF from week one (Supplementary Table S2; pot numbers 81-95). Plants were randomised for lay-out and grown in a containment glasshouse with a controlled environment (18–20 °C/16 h day length) and watered every other day with no fertiliser applications. Treatments had five biological replicates.

For the AMF inoculum, spores were extracted from a commercially available inoculum of the AM fungus *Rhizophagus irregularis* (PlantWorks Limited, UK) using a sucrose density gradient as described in Brundrett et al. (1994). For the PCN inoculum, cysts of the white potato cyst nematode, *Globodera pallida* (population Lindley), were first extracted from infected sand/loam cultures produced in the glasshouse, using Fenwick's (1940) method. These cysts were then treated with potato root exudate to stimulate hatching of juveniles. Juveniles were collected at 10 days post-treatment with exudates. To apply the liquid inoculums (i.e. AMF or PCN), four P1000 pipette tips were fully inserted into the soil around the potato tuber and the relevant inoculum was introduced through these into the root space to provide in total approximately 5000 spores for the AMF treatments and 3000 second-stage juveniles for the PCN treatments in each pot. An equivalent volume of tap water was introduced via pipette tips to control pots. After approximately 5 min, the majority of the liquid inoculum had entered the soil and 1 ml of water was washed through each pipette tip. To ensure similarly aged inoculum for all treatments, regular spore extractions/nematode hatching protocols were established.

2.2. Plant growth and functional measurements

At weekly intervals, total canopy cover and the maximum potential quantum efficiency of Photosystem II (F_V/F_M) were measured. Total canopy cover was quantified through top-down photographs analysed using FIJI ImageJ (Schindelin et al., 2012) as an indicator of plant growth. The maximum potential quantum efficiency of Photosystem II was characterised by F_V/F_M (Opti-Sciences, OS-30p+ Chlorophyll Fluorometer) as an indicator of plant photosynthetic efficiency under stresses (Cessna et al., 2010). Young leaves of similar sizes were dark-adapted for approximately 20 min before the measurements were taken.

2.3. Quantification of PCN infection and fungal colonisation

Plants were harvested after 12 weeks of growth, where tuber mass was measured and roots were cleaned with tap water. Sub-samples of roots were stored in 50 % ethanol (v/v) at 4 °C for quantification of AMF colonisation. The soil from each pot was mixed and nematode cysts were extracted from a 250 g soil aliquot using Fenwick's (1940) method to get an estimate of nematode cyst density per pot. Approximately ten extracted cysts were then opened and the number of unhatched second-stage juveniles was counted to determine the eggs per cyst and reflect the reproductive capacity of the nematodes under each treatment condition. The preserved roots were stained using the "ink and vinegar" staining method (Vierheilig et al., 1998) to assess AMF root colonisation.

Assessment of percentage root length colonisation was made using the magnified intersection methodology (minimum 150 intersections per pot) (McGonigle et al., 1990).

2.4. Statistical analysis

Tests for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's Test) of the residuals were carried out using OriginPro (OriginLab Corporation, 2021). Data did not require normalising or transforming before the following tests were applied. Data were then analysed by one-way ANOVA. Upon $p < 0.05$, post-hoc Student–Newman–Keuls (SNK) tests were run to identify statistical differences between all treatments for each measurement.

3. Results

3.1. Early AMF colonisation of potato roots results in enhanced potato growth, even under co-colonisation with PCN

When plants were inoculated within the first two weeks of growth with only a single symbiont, total tuber yield decreased with PCN infection and increased with AMF colonisation compared to control plants with no symbionts ($p < 0.05$; One-way ANOVA, SNK; Supplementary Tables S3, S4), whereas inoculation of plants older than two weeks with either symbiont did not affect yield (Fig. 1). Simultaneous co-colonisation with both symbionts did not impact yields compared to controls, however the detrimental effects associated with PCN was negated for plants inoculated concurrently with AMF in weeks 1 and 2 (Fig. 1). AMF inoculation two or three weeks prior to PCN drove enhanced yields, however a one-week period between symbiont inoculations resulted in yields similar to control plants (Fig. 1; $p < 0.05$; One-way ANOVA, SNK). In contrast to this, inoculation with AMF at any point after the introduction of PCN was sufficient to recover yields such that they were similar to those of asymbiotic controls (Fig. 1; $p < 0.05$; One-way ANOVA, SNK).

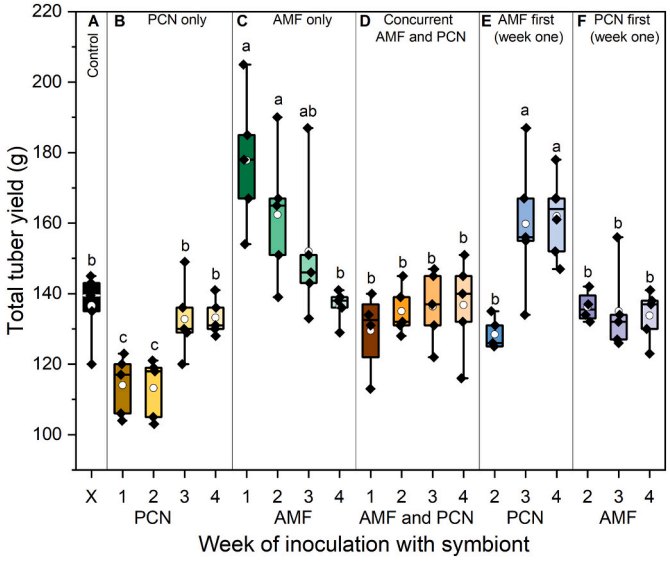

Fig. 1. Earlier inoculation of potato roots by AMF results in enhanced potato yield. Data show total tuber yield per plant that was treated with either PCN only (B), AMF only (C), concurrent AMF and PCN inoculation on sequential weeks (D), AMF at week one and PCN in subsequent weeks (E), and PCN at week one and AMF in subsequent weeks (F). Control pots (A) contained neither symbiont. Boxes represent five biological replicates and extend from the first to the third quartile with the middle bold line representing the median value, white circle representing the mean and whiskers extending to min/max. Different letters denote significant differences between all data ($p < 0.05$; One-way ANOVA, SNK).

In the absence of AMF, PCN inoculation did not affect canopy cover at any time point, compared to controls (Fig. 2; Supplementary Tables S5, S6). For AMF only plants, canopy cover at harvest was only greater when AMF were introduced in week 1 (Fig. 2; $p < 0.05$; One-way ANOVA). For concurrent inoculations, there was an increase in canopy cover compared to non-inoculated controls only when both symbionts arrived at week one (Fig. 2). When AMF were introduced first, there was an increase in canopy cover compared to asymbiotic control plants that was not impacted by PCN inoculation at any of the time points (Fig. 2).

F_V/F_M, the maximum potential quantum efficiency of Photosystem II, was measured each week (Supplementary Fig. 2) and final week means are presented for pairwise comparisons (Fig. 3, Supplementary Tables S7, S8). AMF inoculation did not impact F_V/F_M whereas earlier PCN inoculation ($\leq$2 weeks old) resulted in reduced values (Fig. 3; $p < 0.05$; One-way ANOVA, SNK). AMF alleviated the PCN-induced stress if they were introduced first, concurrently, or up to one week after PCN (Fig. 3).

3.2. PCN inoculation reduces AMF colonisation whereas inoculation with AMF pre- or shortly after PCN leads to greater nematode infection and reproduction

For the AMF only treatment, root colonisation by AMF was greatest when the fungal inoculum was introduced to plants in the first week of plant growth and significantly reduced in plants inoculated in weeks three and four (Fig. 4; $p < 0.05$; One-way ANOVA, SNK, Supplementary Tables S9, S10). Co-inoculation of AMF and PCN in week one as well as inoculation with PCN either pre- or post-AMF resulted in reduced fungal colonisation (Fig. 4C, D, $p < 0.05$; One-way ANOVA, SNK). This reduction in colonisation occurred even when PCN were introduced three weeks post-AMF application.

Increased numbers of PCN cysts per g root and eggs per cyst were recovered from plants that were inoculated at one week compared to four weeks old (Fig. 5; Supplementary Tables S11, S12, S13, S14). Inoculation with AMF first, or concurrent with PCN in weeks one and two, resulted in increased cyst and egg numbers (Fig. 5). Generally, the

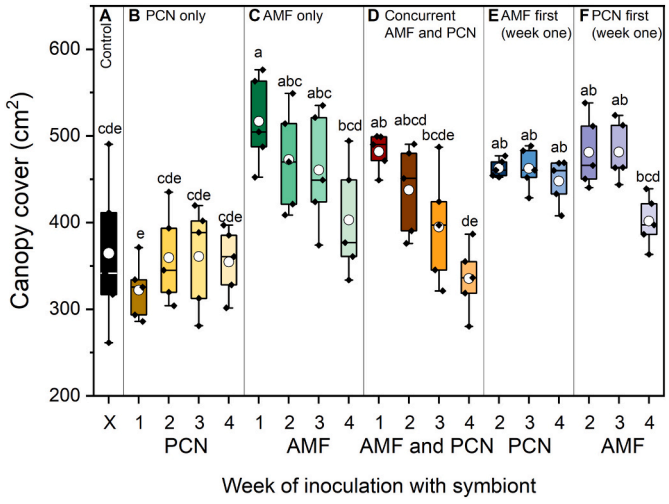

Fig. 2. Earlier inoculation of potato roots by AMF enhances leaf area, even under co-inoculation with PCN. Data show total leaf area per plant prior to harvest when treated with PCN only (B), AMF only (C), concurrently with AMF and PCN (E), AMF first (D) or PCN first (F), sequentially over four weeks. Control pots (A) contained neither symbiont. Boxes represent five biological replicates and extend from the first to the third quartile with the middle bold line representing the median value, white circle representing the mean and whiskers extending to min/max. Different letters denote significant differences between all data ($p < 0.05$; One-way ANOVA, SNK).

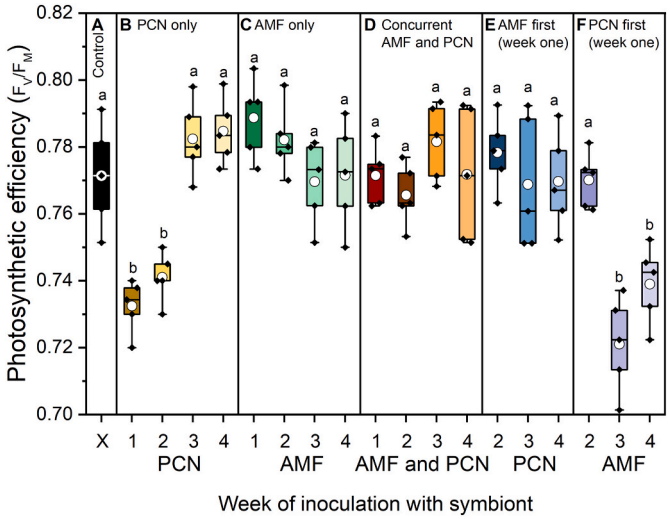

Fig. 3. AMF inoculation ameliorates PCN induced reduction in F_vF_M. Data show F_V/F_M values per plant prior to harvest when treated with PCN only (B), AMF only (C), concurrently with AMF and PCN (E), AMF first (D) or PCN first (F), sequentially over four weeks. Control pots (A) contained neither symbiont. Boxes represent five biological replicates and extend from the first to the third quartile with the middle bold line representing the median value, white circle representing the mean and whiskers extending to min/max. Different letters denote significant differences between all data ($p < 0.05$; One-way ANOVA, SNK).

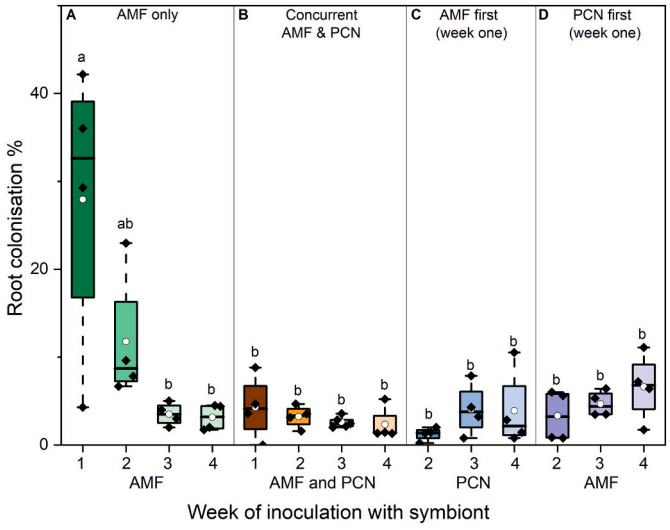

Fig. 4. Inoculation of younger, PCN-free roots results in the greatest AMF colonisation. Data show root colonisation of potato (cv. Désirée) with AMF when: AMF were inoculated on weeks 1–4 (A), AMF and PCN were concurrently inoculated over sequential weeks (B), AMF were inoculated at week one and PCN in subsequent weeks (C), and PCN inoculated at week one and AMF in subsequent weeks (D). Boxes represent four biological replicates and extend from the first to the third quartile with the middle bold line representing the median value, white circle representing the mean and whiskers extending to min/max. Different letters denote significance between all data ($p < 0.05$; One-way ANOVA).

earlier the AMF was inoculated the larger the impact on PCN cyst and egg counts. Additionally, even if the AMF was introduced shortly after PCN there was still an increase in PCN on the root system compared to treatments absent of AMF (Fig. 5). PCN that were applied in week four on a host that had AMF present since week one showed similar numbers of eggs/cyst as nematodes that were added at week one with no AMF

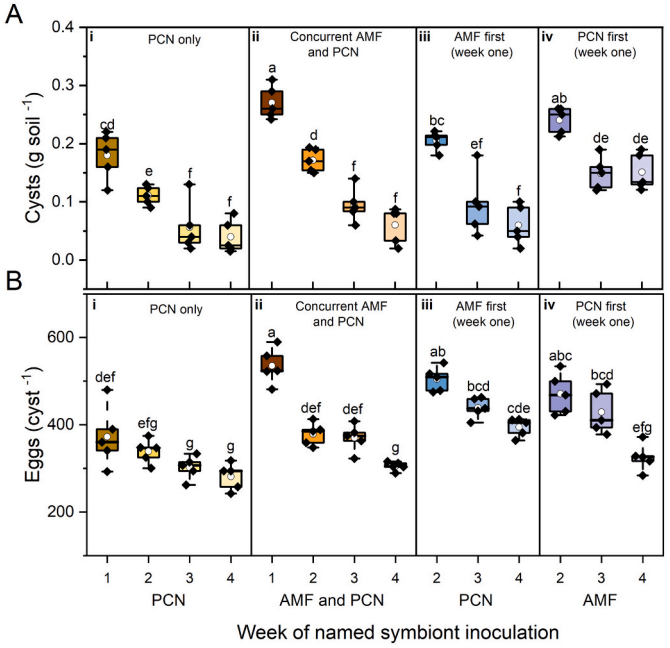

Fig. 5. AMF colonisation pre- or shortly after PCN inoculation results in greater nematode infection and reproduction. Cysts/g soil (A) and eggs/cyst (B) of PCN on plants treated with PCN only (i), AMF and PCN concurrently in sequential weeks (ii), AMF at week one and PCN in subsequent weeks (iii), and PCN at week one and AMF in subsequent weeks (iv). Boxes represent four biological replicates and extend from the first to the third quartile with the middle bold line representing the median value, white circle representing the mean and whiskers extending to min/max. Different letters denote significance between data within A or B ($p < 0.05$; One-way ANOVA, SNK).

(Fig. 5).

4. Discussion

In this study, we investigated the impact of the timing of introduction of two plant symbionts that are important components of agricultural systems, AMF and PCN. We found that concurrent PCN infection reduced AMF colonisation, however host plant growth was still increased when compared to PCN only pots. This was apparent when symbionts were added during the first two weeks, possibly due to the reduced impact of PCN inoculation in weeks three and four. Furthermore, AMF colonisation of PCN-infected hosts also enhanced the density and fitness of the nematode compared to nematodes infected for similar time points in the absence of AMF. AMF-induced tolerance appeared to be stronger the earlier the AMF were introduced, even if this was after inoculation with PCN. Our data indicate that the order of symbiont introduction is not only important for their own success, but also for that of the host and its additional symbionts, and may have wider implications on the function of agroecosystems.

4.1. Earlier inoculation with AMF leads to reduced plant stress and enhanced host yields, even under co-colonisation with PCN

The earlier the AMF were applied, the greater the degree of colonisation of host roots and the greater their impact on tuber yields. There could potentially be a correlation between the extent of AMF colonisation and plant performance, however this remains equivocal (Thirkell et al., 2017). Furthermore, when PCN were present there was reduced colonisation by AMF yet fungal interactions still provided benefits, such as increased yields, compared to plants infected only with PCN at the same time points. This indicates that even low colonisation rates by AMF may be sufficient for the host plant to derive benefits. These data also

indicate that the developmental stage of the host may impact the propensity for AMF colonisation, with younger roots being more readily colonised. However, root staining is not indicative of the function or activity of the intracellular fungal structures. It is thus possible that in younger plant roots, fungal structures are produced more rapidly but are not as active as those in older roots, which are potentially longer established. Due to the relatively short life span of arbuscules (approx. 8 days) (Luginbuehl and Oldroyd, 2017), it is likely that root staining highlights the remains of previously functional arbuscules as well as currently active structures which may further distance this measurement from being an accurate representation of mycorrhizal functionality and significance. Additionally, the increased enzymatic cleavage of sucrose into fructose and glucose in inoculated roots after 35 days (Schubert et al., 2004), potentially indicates that as the symbiosis becomes more established, the plant host may return greater benefits to the fungus.

Similar to AMF, there was also a greater abundance of PCN on younger plant roots, suggesting that the yield reduction in plants inoculated with PCN at an earlier time point may be directly induced by the greater nematode burden on these hosts. Greater reductions in crop yields have previously been suggested to be linked with earlier plant-parasitic nematode infection as the pests can establish and impact the host whilst it is still young and likely less tolerant and/or resistant, thereby increasing the probability of crop death (Kayani et al., 2017). This may be directly related to the vast proportion of host resources PCN acquire from host roots, therefore resulting in fewer resources available for plant growth and tuber development (Bell et al., 2022).

To explore symbiotic scenarios that are likely to occur in the field, we applied AMF and PCN sequentially on host roots. We found that reduced potato yields in plants infected with PCN during the first two weeks of growth can be alleviated if AMF are introduced to the same host pre-PCN inoculation, concurrently or even up to three weeks post-PCN inoculation (Fig. 1). This is likely due to the enhanced nutrient status of AMF-colonised host potatoes, where AMF increase P and N assimilation in the host whilst inducing no additional C burden to the PCN-infected hosts (Bell et al., 2022). If AMF were introduced two weeks prior to PCN, then AMF-derived benefits on host yields prevailed, suggesting that although the colonisation level was the same, their functionality may be enhanced. This may also be due to the nutrients that AMF have exchanged with the host that may promote the tolerance response (Bell et al., 2022). Additionally, we have shown previously that AMF supplied more phosphorus to potato plants when in the absence of PCN (Bell et al., 2022), indicating that this enhanced phosphorus in the few weeks before PCN arrival may be important for establishing tolerance towards future pests.

Alongside yields, AMF can also ameliorate the negative impact PCN feeding has on the hosts' photosynthetic efficiency. Suppression of photosynthesis has been previously reported for plant-parasitic nematode infected hosts (Blouin et al., 2005) and may be a symptom of nematode feeding, which reduces root function and induces greater stress from lack of resources. AMF are known to alleviate photosynthetic stress induced by other common factors such as salt, heavy metal and drought stresses by improving the utilisation of photons and alleviating the inhibition of electron transport (Borkowska, 2002; Yang et al., 2015; Wang et al., 2019). It appears that AMF had a similar effect in our experimental systems, resulting in the alleviation of the stress PCN induce when inoculated in weeks one and two.

4.2. Pre-colonisation of plants by AMF enhances parasitism by PCN

We found that when AMF were introduced first, or shortly after the PCN, the PCN reproduction rate was enhanced. Increased numbers of PCN are likely due to the enhanced nutrition of nematode feeding sites, achieved by enhanced nutrient uptake via AMF partners. Once established within the roots, the nutritional quality of each feeding site will determine the number of eggs produced by the female nematode (Goheen et al., 2013). Enhanced nutrition of the host through mycorrhizal interactions increases plant tissue nitrogen and phosphorus, and this is then available for acquisition by co-colonising PCN (Schouteden et al., 2015; Bell et al., 2021a, 2021b). We found that inoculation with AMF before the introduction of PCN appeared to further increase PCN egg production, with these plants supporting greater PCN populations than PCN-only or PCN-first inoculated plants. These hosts also produced higher yields indicating that prior inoculation with AMF may further increase host nutrient availability, which can be beneficial to the host as well as to other symbionts.

In our experiments, we observed greatest colonisation from AMF that were inoculated in the first week of plant growth. However, introduction of PCN at any time point, even after AMF, reduced colonisation. Previous studies have also shown AMF colonisation to be reduced by migratory as well as sedentary plant-parasitic nematodes, including PCN (Borowicz, 2001; Deliopoulos et al., 2008; dos Anjos et al., 2010; del Mar Alguacil et al., 2011; Alban et al., 2013). Given that PCN and AMF colonise similar regions within the host root, this may be a result of competition for space and resources (Bell et al., 2021a) in a similar manner to that hypothesised in AMF-AMF competitive interactions (Werner and Kiers, 2014). The lower colonisation in plant roots may be due to the AMF expanding throughout the soil in search of another host to obtain carbon (Werner and Kiers, 2015), which could be initiated by the perception of a “poorer” (e.g. PCN infected) host.

It is possible that certain species of AMF may exhibit increased fitness on PCN-infected hosts compared to others and have evolved as such from extensive and consistent monoculturing of crops. Different species of AMF are known to preferentially colonise roots infected with *Meloidogyne incognita*, another highly-damaging plant-parasitic nematode, compared to uninfected roots of the same host species (del Mar Alguacil et al., 2011), raising the possibility that the same may be true for PCN-infected roots whereby AMF species/populations might have evolved to preferentially colonise PCN-infected tissues. This may be due to spatial competition (Vos et al., 2014), defence responses, or differential exudation between infected and uninfected root tissues.

The timing of the introduction of AMF within the first four weeks of plant growth has far-reaching consequences on PCN populations as well as host quality and yields obtained eight weeks later, as seen in this study. This is consistent with other biotic stresses, such as sudden death syndrome of soybean where the severity of the pathogen decreases heavily with plant age (Gongora-Canul and Leandro, 2011). As such, it appears that the initial period of plant emergence and growth may present a window of opportunity, where application of AMF inocula may have a greater impact than at a later growth stage.

The application and use of mycorrhizal fungi in agricultural settings is gaining traction (Rillig et al., 2016; Thirkell et al., 2017) and the debate as to whether farmers should specifically manage for AMF is ongoing (Ryan and Graham, 2018; Rillig et al., 2019). Different crops alter the soil AMF community extensively (Emery et al., 2017) and the selection of inter- as well as over-winter crops can have profound effects on mycorrhizal colonisation and yields of subsequent crops by maintaining the AMF content of the soil (Isobe et al., 2014). At the end of one generation, the encysted PCN eggs generally require diapause over winter before the second generation can hatch and infect a new host (Turner and Rowe, 2006) upon detection of cues from host roots, such as monosaccharides (Bell et al., 2021b). Therefore, enhancing over-winter levels of AMF in the soil by cover crops may assist tolerance in young potato plants the following year by allowing colonisation of hosts prior to PCN. That said, farming practices such as intensive tilling may potentially disrupt the hyphal network and reduce field populations of AMF (Bowles et al., 2017). Farming methods may also have wider implications on other organisms, including PCN, if they impact the AMF content of the soil. Due to the persistence of PCN in the soil (Koehler et al., 2021) there may be long-term effects of increasing PCN populations by maximising the AMF content of the soil, especially if AMF-induced tolerance fails. It is possible that abiotic conditions as well as the presence and density of other symbionts may impact the level of

tolerance that AMF provide the host. Furthermore, the emerging ability of certain cyst nematodes to produce multiple generations per year may indicate that the impact of AMF on cyst nematodes could potentially be exacerbated and result in the faster development of larger plant-parasitic nematode burdens on crops.

The first few weeks of plant development appear to be a crucial period for establishment of beneficial AMF-crop interactions, particularly in promoting AMF-induced tolerance to PCN. As such, coordinating the application of AMF propagules at an early stage of crop development is likely to be critical for farmers seeking to gain maximum benefit and performance from AMF inoculants in the field. Further research is now needed into the mechanisms underpinning the strength of AMF-induced tolerance and how they are impacted by symbiont arrival times. Understanding these mechanisms will assist the development of new measures to mitigate the impact of PCN and reduce agricultural chemical inputs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2022.104733.

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