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REGULAR ARTICLE

Early events in the life of apple roots: variation in root growth rate is linked to mycorrhizal and nonmycorrhizal fungal colonization

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Abstract Early events of mycorrhizal and nonmycorrhizal fungal colonization in newly-emerging roots of mature apple (*Malus domestica* Borkh) trees were characterized to determine the relationship of these events to fine root growth rate and development. New roots were traced on root windows to measure growth and then collected and stained to quantify microscopically the presence of mycorrhizal and nonmycorrhizal fungal structures. Most new roots were colonized by either mycorrhizal or nonmycorrhizal fungi but none less 25 days old were ever internally colonized by both. Compared to nonmycorrhizal colonization, mycorrhizal colonization was associated with faster growing roots and roots that grew for a longer duration, leading to longer roots. While either type of fungi was observed in roots as soon as 3 days after root emergence, intraradical colonization by mycorrhizal fungi was generally faster (peaking at 7 to 15 days) than that by nonmycorrhizal fungi and often occurred more frequently in younger roots. Only 15 to 35% of the roots had no fungal colonization by 30 days after emergence. This study provides the first detailed examination of the early daily events of mycorrhizal and nonmycorrhizal fungal colonization in newly emerging roots under field conditions. We observed marked discrimination of roots between mycorrhizal and nonmycorrhizal fungi and provide evidence that mycorrhizal fungi may select for faster growing roots and possibly influence the duration of root growth by non-nutritional means.

Keywords Arbuscular mycorrhizal fungi · Fine root production · *Malus domestica* · Root–fungal interactions · Soil pathogens

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Introduction

There has been growing interest in developing a better understanding of perennial root function in the field, both in the context of water and nutrient acquisition and in relation to carbon and nutrient turnover (Eissenstat and Yanai 1997; Pregitzer 2002; Trumbore and Gaudinski 2003). For example, small changes in root diameter in apple fine roots may lead to very different lifespans (Wells and Eissenstat 2001). Similarly, roots of different branching orders may differ markedly in respiration (Huang et al. 2005), anatomy (Eissenstat and Achor 1999), and lifespan

(Eissenstat et al. 2000; Anderson et al. 2003). Besides subtle differences in diameter or branching order, roots change dramatically in physiology and structure as they age (Comas et al. 2000; Bouma et al. 2001). In grape, nitrate uptake and root respiration dropped 50% as roots aged from 0.5 to 1.5 days old and another 50% by the time roots were 4 days old (Volder et al. 2005). During this early period, xylem is maturing, endodermal and exodermal cell walls may be thickening and becoming more lignified, and roots are being colonized by a range of soil microbes (Ma and Peterson 2003). Thus, we are still developing an understanding of factors influencing fine root function and microbial interactions under field conditions.

One important aspect of this dearth of knowledge on function and development of newly emerging roots in the field includes mycorrhizas. In particular, there is a notable lack of information on patterns of mycorrhizal colonization during the first days and weeks of an individual root's life under field conditions. Do mycorrhizal fungi colonize roots more rapidly than competing fungi? Do mycorrhizal fungi colonize fine roots equally or is there some potential for selection of roots that might best benefit the fungi?

Temporal dynamics of fungal colonization of fine lateral roots as they first emerge from the parent root into soil of high microbial diversity is not well understood, especially under field conditions. For mycorrhizas, studies in pot culture in soil media of relatively low microbial diversity suggest that these fungi develop hyphal contact with the root and form appessoria in about 10 days in roots of *Allium porrum* (Amijee et al. 1986) and *Medicago truncatula* (Bonanomi et al. 2001). In a study where roots of transplanted *A. porrum* seedlings developed in soil-less media (Turface®) with an established arbuscular mycorrhizal (AM) symbiosis, mycorrhizal fungi contacted the roots within a day (Brundrett et al. 1985).

Both fungal species and plant species can affect rates of AM colonization. Rates of initial colonization of root systems of four mycotropic host plants (two grasses and two forbs) ranged from <1 week to about 8 weeks among 21 AM fungal isolates (Hart and Reader 2002). Similarly, in a field study of six citrus rootstock genotypes, roots of more mycorrhizal dependent plants tended to be colonized faster than less mycorrhizal dependent plants, ranging from <5% to >30% intraradical colonization of root populations <5 weeks old (Graham et al. 1991).

In this study, we examined daily patterns of AM fungal colonization of individual new roots in a well-established apple orchard in relation to root growth rate and total length extension of individual fine laterals. We also examined if the presence of AM fungi was positively or negatively associated with the occurrence of hyphae of nonmycorrhizal fungi. In the process, we discovered unexpected discrimination occurring among roots and fungi in the first days and weeks of a root's life.

Materials and methods

Study site

The study was conducted on 'Red Chief' apple trees (*Malus domestica* Borkh) planted on M9 rootstock in 1979 at the Russell E. Larson Agricultural Research Center in Rock Springs, Pennsylvania, USA (40.8°N 77.9°W, elev. 350 m). Trees were spaced 2×5 m apart and trained on a 2-m high trellis system. Grass alleyways were maintained between rows with a 1.5-m wide herbicide-treated strip of bare soil under the trees. Pruning, thinning, and harvesting were done following normal cultural practices used in commercial orchards in the region (Rajotte et al. 1996). The soil at Rock Springs belongs to the Hagerstown series and is characterized by a 0.2-m surface layer of dark-brown silt loam and a 0.9-m layer of reddish-brown silty-clay loam subsoil. Soil at the study site had a bicarbonate-extractable phosphorus concentration of 2.9 μg P g^{-1}, a water pH of 5.8, an organic matter content of 2.7%, a bulk density of 1.25 g cm^{-3} in the top 0.3 m of the soil profile, and was not fertilized for 5 years prior to the experiment. Air temperature and precipitation were monitored daily at the site using a LI-1405 weather station (LI-COR Biosciences, Lincoln, NE, USA).

Root monitoring and collection

Root observation boxes were installed in summer 1996 on alternating sides of a single row of 20 trees. Each box (0.35 m high×0.60 m wide×0.50 m long) was constructed from plywood attached to a wood frame and had an observation window (0.30 m high× 0.50 m wide) made of clear, 0.25-mm thick, acetate sheets built into the front of the box. The window was

angled 15° from vertical, with the base slanted towards the trees to increase the probability of root contact. A lid was attached to the top of the box to exclude rain and sunlight, but was removable to allow access to the root observation window. The top of the boxes were positioned approximately 0.5 m from the base of the trees (within the herbicide strip) in holes dug approximately 0.35-m deep, and soil was filled in around each box. A uniform layer of sifted soil (1-mm sieve), collected during digging for each box, was carefully packed into the gaps (2–3 cm) between the observation windows and the undisturbed soil facing the trees. Removable Styrofoam® insulation was then inserted on the inside of the box against the window to reduce thermal fluctuations and light at the window surface. To allow the soil to settle and new roots to grow against the window, root boxes were in place for 3 months before any observations were made.

After a sufficient number of roots appeared against the observation windows, roots were traced directly on the windows with black permanent marker, which served as a baseline to monitor new root growth. Newly emerging roots were then traced with a color-coded marking system; different colored markers were used each day to track individual root growth over time. At the end of the tracing period, a copy of the tracing of each root was made onto individual 8× 13 cm sheets of acetate, and daily length increases were measured with a ruler. A random sample of individual roots was harvested by making incisions in the observation windows and carefully cutting them from the parent root. Roots were traced over three different marking periods—22 August to 6 September 1996, 9 May to 12 June 1997, and 14 June to 17 July 1997—and collected at the end of each period. Roots collected on 6 September and 17 July were traced daily while those collected on 12 June were traced every 1 to 5 days. This approach gave us a sampling of roots ranging in age from 1 to 15 days old on the first collection date and 1 to 33 days old on the second two collection dates. Once harvested, roots were preserved in 50% ethanol solution (v/v) for later microscopic analyses.

Fungal development

Preserved roots were placed in individual test tubes with 10% KOH (w/w), cleared in a water bath set at 75°C, stained with 0.05% trypan blue in lactic acid: glycerol:water (1:1:1) solution, and finally destained with lactic acid:glycerol:water solution without trypan blue (Brundrett et al. 1996). Heavily pigmented roots were also bleached with 10% ammonia and 3% hydrogen peroxide solution for 5 min before staining. Destained roots were mounted on a microscope slide parallel to the long axis of the slide and then covered with a 40×22 mm cover slip. Both external and internal fungal structures were observed at 400× magnification in 1-mm intersection intervals similar to the method described in McGonigle et al. (1990). To examine each intersection, the plane of focus was moved completely through the root and a note was made of whether the vertical crosshair intersected any external hyphae, penetration points (appressorium), inter- and intracellular hyphae, hyphal coils, arbuscules, vesicles, or other fungal structures. Each individual hypha was carefully examined to determine whether it was mycorrhizal or not; septate hyphae and hyphae attached to nonmycorrhizal fungal structures [*e.g.*, oospores (*Pythium* spp.); haustoria, zoospores, chlamydospores, and oospores (*Phytophthora* spp.); infection cushions and microsclerotia (*Rhizoctonia* spp.)] were categorized as 'nonmycorrhizal' (Rossman et al. 1987). Any roots with nonseptate hyphae but no other distinguishable fungal structures were categorized as 'possibly mycorrhizal'. Roots with missing cortex, which represented less than 3% of the roots sampled, were discarded. Fungal structures at each cross section were related to age of the root segment recorded at harvest. Each root took approximately 0.5 to 4 h to quantify and, when found to be colonized, was scanned a second time to determine the earliest date of appearance of each fungal structure and confirm that each structure was properly documented.

Statistical analysis

All roots were classified as being colonized by mycorrhizal fungi, nonmycorrhizal fungi, both, or none. Differences in growth characteristics (growth rate, days of growth, and final length) of the four classes of roots were analyzed by analysis of variance using the GLM procedure in SAS (SAS Institute 1998). Least-square means of each root class were separated at $P \leq 0.05$ using the PDIFF option in the LSMEANS statement within the GLM procedure.

Results

Roots examined in this study developed under various weather conditions and at different stages of fruit development (Fig. 1). For example, roots collected in September 1996 were produced under warm, wet conditions and were removed just prior to fruit harvest, while those collected the following spring were produced under cool, wet conditions during flowering and fruit set. The remaining root cohort was produced under warm, dry conditions but in soil sprinkler-irrigated twice prior to collection; fruit were at approximately 30% of their final average weight of 120 g prior to this final collection. Among the three collections, fine root production was generally lowest in August and September, as well as in early-July during drought, and highest in late-May and June (Fig. 1).

A total of 220 fine lateral roots, most of which had no branch roots [i.e., 1st order roots (sensu Fitter 1982)], were examined for fungal colonization over the three collection dates (Table 1). Roots fell into four categories: (1) roots with no evidence of fungi or other hyphal-bearing organisms (e.g., *Phytophthora* and *Pythium*); (2) roots bearing only mycorrhizal structures; (3) roots bearing only non-mycorrhizal structures; and (4) roots with both mycorrhizal and non-mycorrhizal structures. The first three types of roots were generally abundant on each date, each comprising 9 to 49% of the total new roots collected; however, extremely few roots (0–2%) contained both mycorrhizal and nonmycorrhizal fungal structures, and these were only observed in root sections greater than 25 day old.

Figures 2 and 3 show the relative frequency of colonization in root sections of different ages. The accuracy of these estimates depends strongly on the number of roots associated with a particular age category (Fig. 2 insets). In this study, we generally had a least ten or more roots of each age category for assessing relative frequency in each of the three harvests. Nonetheless, in the Jun–July harvest, many more roots were produced, especially in the older categories, leading to higher precision of estimates of percent colonization than what was possible for the other harvests.

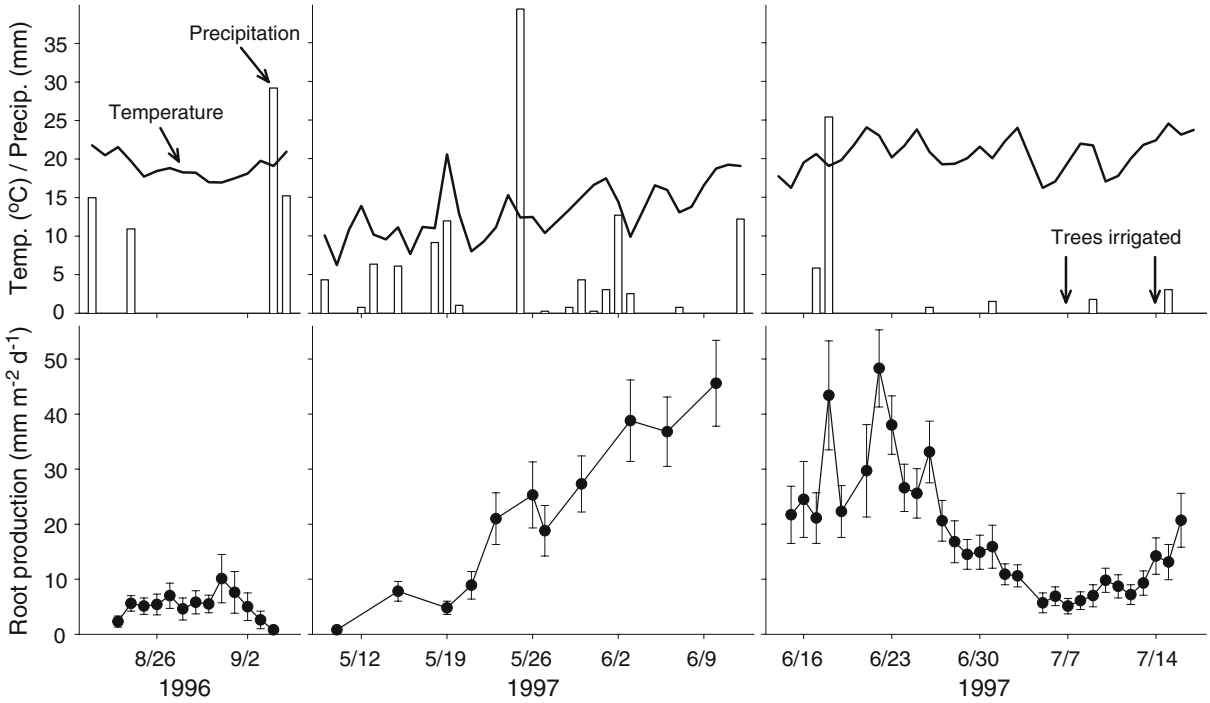

Fig. 1 Weather conditions and fine root production during three collections of new apple roots in 1996 and 1997. Trees were sprinkler-irrigated twice with ≈25 mm of water on 7 July and 14 July 2007. Each symbol used for root production represents the mean of 20 trees and *error bars* represent 1 SE of the mean

Table 1 Proportion of roots either uncolonized by fungi or colonized by mycorrhizal (M) fungi, nonmycorrhizal (NM) fungi, or both M and NM fungi

	Proportion of roots (%)		
Type of colonization	22 Aug–6 Sep 1996 (n=47)	9 May–12 Jun 1997 (n=43)	14 Jun–17 Jul 1997 (n=130)
No fungi	38.3	48.8	29.2
M fungi only[a]	21.3	39.5	36.9
NM fungi only	40.4	9.3	32.3
Both[b]	0.0	2.3	1.5

[a] Includes roots categorized as 'possibly mycorrhizal'

[b] Roots colonized by both types of fungi were >25 d old

The greatest percentage of roots with mycorrhizal fungi occurred in root sections developed at 7 to 15 days after emergence where 40 to 58% of the sections examined in these age categories were colonized (Fig. 2). By comparison, nonmycorrhizal fungi were observed more frequently in older roots, comprising 17 to 59% of the roots more than 25 days old. Many of the roots colonized by nonmycorrhizal fungi were damaged (presumably by root herbivores or by cellulolytic and pectolytic activities of necrotrophic fungal pathogens) and heavily penetrated by very fine, septate hyphae. Of the oldest sections of roots in each collection, only 15 to 35% had no fungi (Fig. 2).

Mycorrhizal and nonmycorrhizal fungi were first evident in roots as early as 3 to 5 days after emergence (Fig. 3). Colonization was usually associated with hyphal penetration points (appressoria) and never developed intraradically by way of older, higher-order, and heavily-colonized roots from which the new roots developed. Roots with mycorrhizal fungi often contained hyphal coils within the cortical cells, which appeared as soon as 3 to 4 days after root emergence. Arbuscules also developed but usually more slowly than hyphal coils and often more intensely in older sections of the roots. Vesicles were less common, appearing only in roots produced in late May and June, and never occurring in more than 6% of the total root length. Numerous non-hyphal structures were also produced by nonmycorrhizal fungi, colonizing as much as 13 to 24% of the total root length in each collection (Fig. 3). Interestingly, whether or not roots were colonized by mycorrhizal or nonmycorrhizal fungi, both septate and nonseptate hyphae were frequently observed on the surface of the roots, sometimes as soon as 1 day after emergence (data not shown).

Root growth characteristics often varied among root fungal classes (Table 2). In two out of the three collections, mycorrhizal roots had 20 to 90% faster growth rates than that of nonmycorrhizal roots, even before any internal colonization was evident (i.e., during the first 3 days following emergence). Mycorrhizal roots also exhibited more days of growth and therefore reached 16 to 172% greater total length than nonmycorrhizal roots. Overall, growth of nonmycorrhizal roots was generally similar to roots with no fungi and differed the most from mycorrhizal roots during the August–September collection when root vigor was lowest and differed the least during the May–June collection when root vigor was highest (Table 2).

The entire population of roots sampled over the growing season exhibited growth rates that were reflective of the differences in initial growth rate and the different durations of growth (Fig. 4). Mycorrhizal root populations had faster growth rates for almost the entire 15-day period for the August–September collection. For the June–July collection, growth rate was similar for the first 2 days after emergence, but for the next 3 days, mycorrhizal roots on average grew faster than the other root types. After 7 days, few roots of all root types were still growing. Type of colonization was not associated with different root growth rates for the May–June collection.

Discussion

When new roots emerge into soil, they encounter a myriad of soil organisms—some beneficial, others not. Few studies have examined the daily process of mycorrhizal colonization at the individual root level

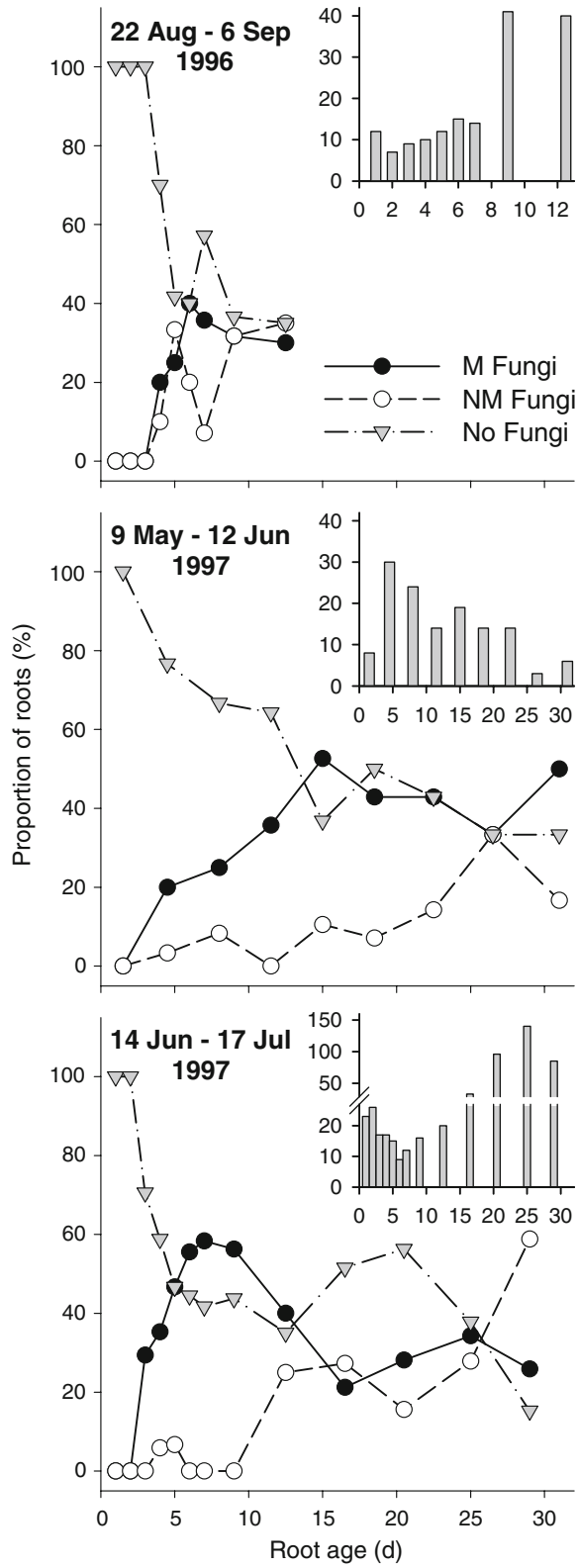

Fig. 2 Percentage of root sections of a particular age that either had no evidence of fungi or contained mycorrhizal (M) or nonmycorrhizal (NM) fungal structures. Pooled root section lengths averaged 6.2±0.3 mm with no fungi (n=398), 6.9± 0.3 mm with M fungi (n=232), and 5.6±0.3 mm with NM fungi (n=166); errors represent 1 SE of the mean. *Insets*: total number of root sections examined in each age category

in established inoculum under controlled conditions (e.g., Brundrett et al. 1985), and none that we are aware of examined this in the field where a complex of soil microbes interacts. Consequently, the events that occur in the first days of a root's life under field conditions are poorly understood. While this study was not designed to examine mechanisms of root colonization by mycorrhizal and nonmycorrhizal fungi, our observations provide evidence that is consistent with several hypotheses: (1) roots are colonized infrequently by both mycorrhizal and non-mycorrhizal fungi in the first weeks of life, perhaps due to a combination of causes, including direct interference by the mycorrhizal fungi and vigorous roots being less susceptible to non-beneficial or pathogenic nonmycorrhizal fungi; (2) mycorrhizal fungi selectively colonize faster growing roots, thus, providing greater opportunity for occupancy of a longer-living host that receives a greater fraction of carbohydrates from the shoot; (3) mycorrhizal fungi may increase the length of time and rate of growth of colonized roots, which would increase the benefits the fungus may gain from the host; and (4) soil pathogens may affect root growth and colonization directly, whereby roots that encounter such pathogens grow slower and become less likely to be colonized by mycorrhizal fungi.

Methodological considerations

Roots were traced during three periods in the study and collected once at the end of each tracing period to examine fungal colonization. From this we determined if roots were colonized by mycorrhizal or nonmycorrhizal fungi and whether the type of colonization was related to root growth. We also determined the youngest age at each collection date in which various fungal structures appeared in the roots. For example, we found that mycorrhizal hyphae and hyphal coils appeared in roots or portions of roots as young as 3 days old, arbuscules appeared as early as 4 days, and vesicles appeared no earlier than 8 days.

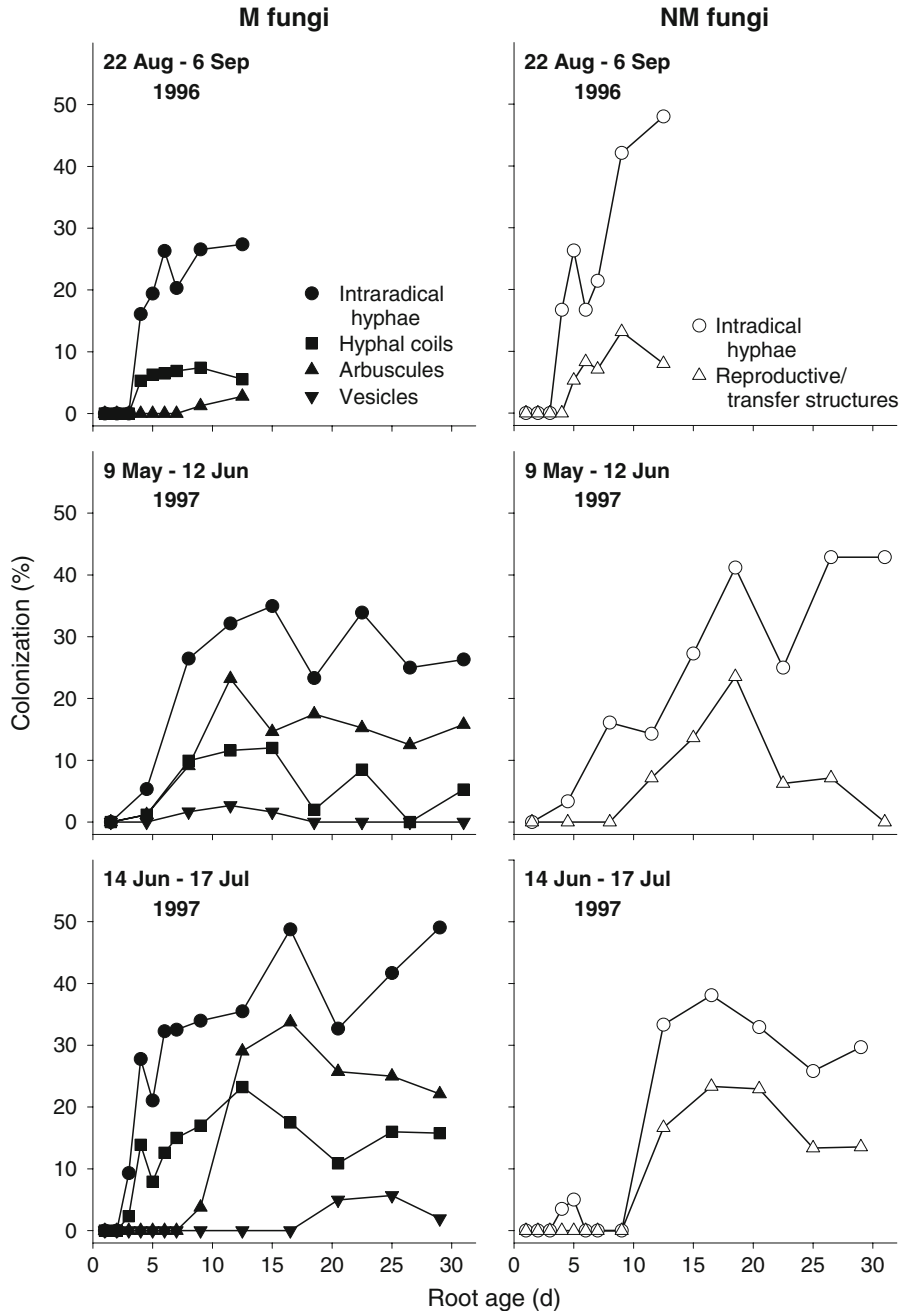

Fig. 3 Development of mycorrhizal (M) and non-mycorrhizal (NM) fungi in new roots during the first few weeks after emergence. Each symbol represents the percent root length colonized by intraradical hyphae, hyphal coils, arbuscules, and vesicles in M roots and by intraradical hyphae and other fungal structures in NM roots

If we could follow fungal colonization rates non-destructively, this of course would yield higher temporal resolution of exactly when the roots were first colonized. Because detection of fungi requires sampling roots and clearing and staining, our approach depends on obtaining sufficient samples of every root age class to estimate colonization. Such an approach requires continuous root production during the observation period as well as sufficient replication of the root boxes to assure adequate sample size. In apple roots in the summer and using 20 root boxes, we were reasonably successful in getting good representation of all the age classes (Fig. 2 inset).

We estimated fungal colonization by staining with trypan blue. While some nonmycorrhizal fungi or fungal-like organisms, such as *Phytophthora* and

Table 2 Growth characteristics of roots either uncolonized by fungi or colonized by mycorrhizal (M) or nonmycorrhizal (NM) fungi

Collection	Type of colonization	Initial growth rate (mm d^{-1})	Days of growth	Final length (mm)
22 Aug–6 Sep 1996	No fungi	2.9 b	2.6 b	12.4 b
	M fungi only	5.9 a	6.6 a	37.8 a
	NM fungi only	3.1 b	3.2 b	13.9 b
9 May–12 Jun 1997	No fungi	5.1 p	10.3 p	41.0 pq
	M fungi only	4.8 p	11.2 p	43.9 p
	NM fungi only	5.3 p	9.5 p	38.0 q
14 Jun–17 Jul 1997	No fungi	3.7 ab	4.0 b	16.1 b
	M fungi only	4.2 a	6.0 a	22.6 a
	NM fungi only	3.5 b	5.5 a	17.5 b

Initial growth rate was measured during first 3 days after root emergence. Different letters within each collection indicate significant differences ($P \leq 0.05$)

Pythium, might be missed because of lack of chitin, this likely was not a serious problem in our study. For the rare instances where a root section exhibited unstained hyphae, it was readily observable and recorded because of the high magnification and careful inspection used. Nonetheless, coupling microscopic approaches with molecular techniques targeting fungal DNA (as well as other microbes) would provide greater insight into patterns of microbial succession both in the root and on the root surface.

Lack of colonization by both mycorrhizal and nonmycorrhizal fungi

In over 200 roots collected at various times during the growing season, we never observed any roots less than 25 days old that were colonized by both mycorrhizal and nonmycorrhizal fungi (Table 1). Causes may include both fungal–fungal and plant–fungal interactions.

One explanation is that only mycorrhizal fungi would (or could) colonize healthy, vigorous roots, while only weak (e.g., due to low carbohydrate availability) or compromised (e.g., due to herbivory) roots were susceptible to colonization by nonmycorrhizal fungi. No interference between fungi, direct or indirect via modification of plant defenses, needs to be invoked to explain differences in colonization. We did see evidence that if a root was injured, the root was more susceptible to nonmycorrhizal fungal or fungal-like colonization, which is consistent with numerous studies that indicate secondary fungal infection following injury by nematodes (Taheri et al. 1994; van der Stoel et al. 2002) and insects (Graham et al. 2003). Uninjured new roots may also be more susceptible to fungal pathogens if, due to limited energy reserves, their cell membranes are leaky and attractive to pathogens (Katan 2002; Graham et al. 2003; Bais et al. 2006). Our data indicated wide variation in growth rates of roots less than 3 days old (especially between 2 and 3 days; Fig. 4), which may reflect wide variation in the amount of carbohydrates received from the shoot. Reduced carbohydrate supply may be caused by smaller meristematic regions in the tips of new roots (Kosola and Eissenstat 1994), leading to reduced sink activity, or because of inadequate vascular connections and higher resistance to phloem transport. In addition, roots of low vigor may produce less defense compounds, which also may cause them to be more susceptible to nonmycorrhizal fungi (Duffy et al. 2003; van der Putten 2003).

A second explanation is that mycorrhizal fungi actively defend the root against colonization by other microbes (Benhamou et al. 1994; Trotta et al. 1996; Filion et al. 1999; Vigo et al. 2000; Kasiamdari et al. 2002). The mechanisms may include direct fungal–fungal interactions (Filion et al. 1999; Norman and Hooker 2000), AM-fungal modification of plant defenses towards other microbes (Benhamou et al. 1994), or simply competition for limited resources or infection sites (Borowicz 2001). For example, Vigo et al. (2000) found that AM colonization reduced the number of infection loci but not internal spread of the root pathogen, *Phytophthora parasitica*, in tomato (*Lycopersicon esculentum*). In one of the few field

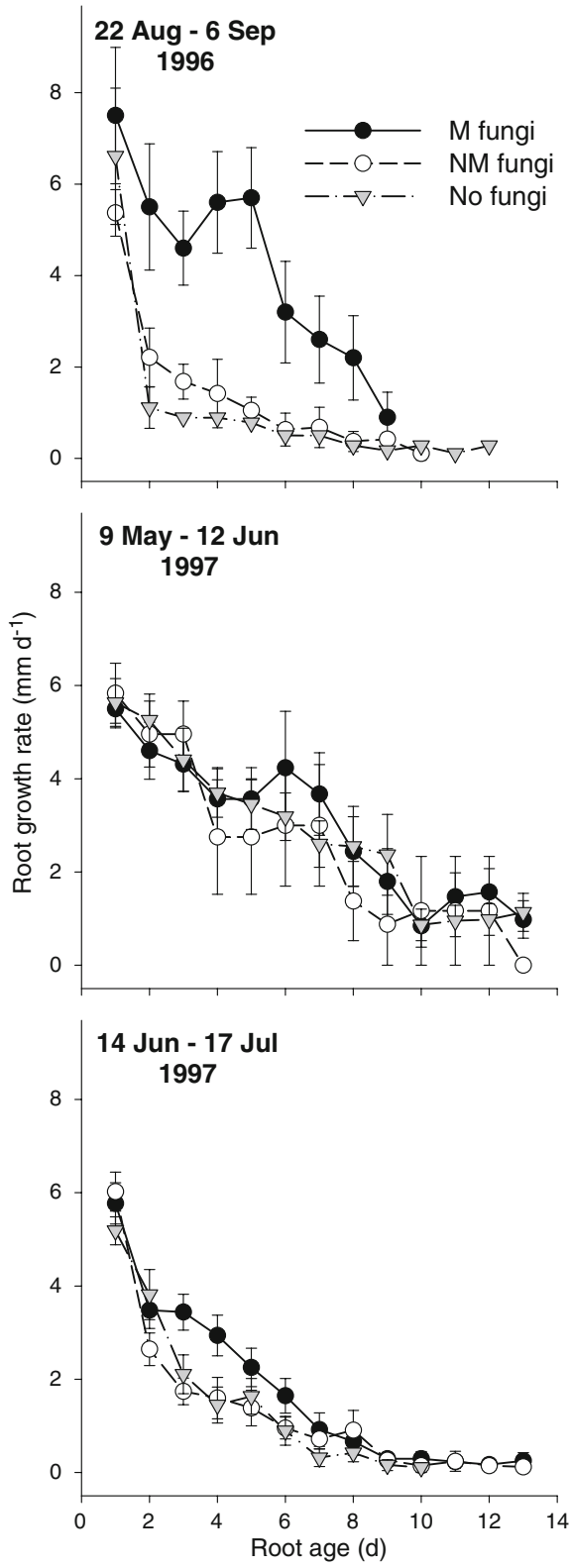

Fig. 4 Average growth rate of roots either uncolonized by fungi or colonized by mycorrhizal (M) or nonmycorrhizal (NM) fungi. Each symbol represents the mean of four to 48 roots (varying depending on type of colonization and collection date) and *error bars* represent 1 SE of the mean

studies of this question, AM colonized roots of the annual grass, *Vulpia ciliata*, limited infection by the pathogenic fungi, *Fusarium oxysporum* and *Embellisia chlamydospora,* two species of fungi associated with reduced fecundity in this plant (Newsham et al. 1995).

Both plant–fungal and fungal–fungal interactions likely occurred in our study. Many of the roots colonized by nonmycorrhizal fungi were 20 or more days old and therefore likely had lower metabolic activity (Bouma et al. 2001) and fewer resources available for inducible-defense production than younger roots. Conversely, young roots colonized by nonmycorrhizal fungi may have been either compromised in metabolic activity or damaged by soil fauna. Nonetheless, the presence of both mycorrhizal and nonmycorrhizal fungi in roots less than 25 days old was highly unlikely, presumably because mycorrhizal fungi either directly or indirectly interfered with the nonmycorrhizal fungi.

Did mycorrhizal fungi selectively colonize fast-growing roots?

In two of the three collection periods, mycorrhizal roots were faster growing or growing for a longer period of time than nonmycorrhizal roots or uncolonized roots (Table 2). One explanation for this observation was that mycorrhizal fungi could "sense" the healthiest roots and were only stimulated to colonize those that were most actively growing.

Mycorrhizal fungi may be attracted to fast-growing roots, avoid slow-growing roots, or do a combination of both. Roots varied widely in growth rates at only 2 days of age (Fig. 4), which likely influenced chemical signaling compounds that could be sensed by external organisms (Giovanetti et al. 1993; Pinior et al. 1999; Buée et al. 2000). In the first 15 days of life, 30 to 40% of apple roots remained uncolonized in undisturbed field soil where mycorrhizal inoculum potential was not likely limiting, given the limited soil disturbance (Fig. 2). If the fungi were not being

selective, more roots probably would have been colonized. A fungal propagule that utilizes its limited reserves to colonize a root with little photosynthate, especially if it soon dies, may be a bad investment for future fungal reproduction. This kind of selection among roots that likely have different payback potentials should be explored further.

Did mycorrhizal fungi stimulate root growth?

In August–September, roots colonized by mycorrhizal fungi were faster growing, even before any internal mycorrhizal structures developed; mycorrhizal root growth was also faster in June–July, even when internal hyphae were evident for only a day (Figs. 2 and 4). For either collection, it is unlikely that improved nutrient acquisition by the mycorrhizal fungi was an explanation for faster root growth, especially given the lack of arbuscules and hyphal coils and the limited amount of internal hyphae (<10%) in roots less than 3 days old (Fig. 3). Mycorrhizal fungi may be capable of altering root physiology in non-nutritional ways. For example, Fusconi et al. (1999) observed that AM fungi increased the size of the root apex in tomato, which was associated in this case with more sustained root growth. This may have also occurred in our study, where sustained growth rate was an important feature of mycorrhizal apple roots (Fig. 4).

Seasonal patterns

Both mycorrhizal and nonmycorrhizal colonization peaked later during the May–June collection than during the other two collection periods (Fig. 2). Colonization may have been slower at this time of year due to a reduction in the number of fungal propagules over winter (Kabir and Koide 2002) and to reduced hyphal growth rates associated with lower soil temperatures (Smith and Roncadori 1986; Gavito et al. 2005). Perhaps not surprisingly, however, more roots were colonized by mycorrhizal fungi than by nonmycorrhizal fungi in the spring (Table 1) when allocation of carbohydrates to roots is often highest (Pregitzer 2003).

Soil moisture became limited during the June–July collection and air temperatures were the highest of the study (Fig. 1). Initial root growth rates were thus lower on average during this period than those observed in the cooler and wetter May–June period (Table 2). Limited soil moisture might also have contributed to the more variable colonization rates in older roots, as shifts in soil water availability can have complex effects on mycorrhizal– and nonmycorrhizal–fungal interactions with the plant (Garmendia et al. 2005).

The August–September collection was the warmest and wettest period among the collections (Fig. 1). Such conditions are very conducive to fungal pathogens (Agrios 1997) and, coupled with a build up of inoculum over the season and reduced energy for root defenses due to high fruit C demand, probably account for the higher incidence of nonmycorrhizal colonized roots observed during the fall collection (Table 1).

Implications for root function

We observed subpopulations of roots—differing in growth and fungal associations from the first days of birth—that likely vary widely in their benefit to the plant. We are not aware of any study that has previously identified such differences in 1st-order root subpopulations. Faster growing subpopulations were more likely colonized by mycorrhizal fungi, which may have in turn enhanced their growth, restricted infection by pathogenic organisms, and presumably enhanced root lifespan (see also Espeleta et al. 1999). A typical cohort of apple roots during the spring and summer in this orchard may experience about 25% mortality in 15 days and 50% mortality in 30 days (Eissenstat et al. 2000). Observations in this study suggest that if the roots are colonized by mycorrhizal fungi, they likely would contribute less to the fraction of the root population that exhibited shortened lifespans. In addition, for an important fraction of the root's life, mycorrhizal fungi may *not* be contributing substantially to nutrient acquisition. Hyphal coils and arbuscules were only observed, on average, when roots were about 10 days old, which might be a third of the lifespan of an average root during the summer (Eissenstat et al. 2000). To the extent roots benefit from nutrient exchange with internal hyphae (van Aarle et al. 2005), longer root lifespan would extend the period of benefit mycorrhizal fungi provide for nutrient acquisition. These observations of patterns of fungal colonization of newly emerging roots provide a more dynamic perspective on the role of mycorrhizal fungi on root function.

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