ROOT HETEROGENEITY AND REPLANT DISEASE DEVELOPMENT ON THE FINE ROOT SYSTEM OF APPLE

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ABSTRACT

Root growth and development structure plant interactions with soil-borne pathogens by shaping the spatial and temporal availability of susceptible tissues. To evaluate apple root heterogeneity and susceptibility to apple replant disease (ARD) pathogens, a greenhouse bioassay of two rootstock genotypes, M.26 and CG.210, was conducted. Pathogen abundance was compared across root branching orders and growth trajectories (1st order fine-feeder vs. pioneer) and anatomical development and tissue phenolics were evaluated as mechanisms of tissue resistance. Rootstock growth and defense were then investigated for the two genotypes previously shown to be more (M.26) or less (CG.210) susceptible to ARD.

Quantitative PCR estimates of pathogen DNA concentration were lower in pioneer roots and 3rd order roots compared to 1st and 2nd orders, which corresponded to distinct stages of tissue maturation including the senescence of cortical tissues in 2nd order roots and loss in 3rd order roots. Phenolic profiles indicate distinct compounds are preferentially allocated to different root branching orders. Phloridzin (phloretin 2'-*O*-glucoside) was found in greater concentration in higher branching orders, while defense induction or stress response was only detected in 1st order and pioneer roots. CG.210 roots had lower abundance of *Cylindrocarpon* spp., *Pythium irregulare*, and *P. sylvaticum* DNA in 1st order and pioneer roots compared to M.26, a different phenolic profile, and accumulated two-fold more root biomass than M.26 in both the replant soil and the pasteurized control.

These results suggest that apple root maturation controls tissue resistance and response to pathogen infection, and that root branching order can provide a functional classification of fine-roots meaningful to investigations of plant-pathogen interactions. The ability of CG.210 to

maintain growth in replant soil may be attributable to relative resistance to replant pathogens in distal root branches and tolerance of infection based on increased rates of root growth.

BIOGRAPHICAL SKETCH

Bryan Emmett received his Bachelors of Science with Honors in Natural Resources and the Environment, from the University of Michigan School of Natural Resources and Environment in 2003. Following graduation, he led conservation work crews of high school volunteers for the Student Conservation Association, in National Parks and Monuments of the desert southwest. Following this experience, Bryan moved to Western Massachusetts to work as Stewardship Manager for the Berkshire Natural Resources Council, Inc. from 2003 to 2008. It was during this experience, interacting with landowners as a proponent of conservation of working lands, that a fascination with the ecology of agricultural systems took hold. Perhaps most importantly though, it is there he met his wife Shawnee. After a brief self-study and walkabout in New Zealand, Bryan entered the graduate program in Department of Horticulture at Cornell in 2009. Between classes and research, Bryan can be found in his and Shawnee's garden or out exploring the tributaries of the Finger Lakes and other central New York trout streams.

Dedicated to my wife, friends, family and the many outstanding mentors with whom I have been fortunate enough to work.

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INTRODUCTION

Root growth and development has long been known to exert a high-level control on plant interactions with soil-borne pathogens. Root growth is assumed to initiate the interaction with pathogen propagules, and root development, in conjunction with pathogen life history, will determine the availability of susceptible tissues in time and space (Huisman, 1982; Watt et al., 2006). For the root system of perennial plants, maturation results in highly heterogeneous tissues, which serve as equally heterogeneous habitats for both pathogenic and beneficial microorganisms (Hishi, 2007). Pathogen strategies may limit colonization to cortical tissues and directly disrupt plant resource uptake functions, while other pathogens may colonize vascular tissues, disrupting whole plant resource transport. Yet while histological studies capture cellular and tissue level specification, scaling plant-pathogen investigations to whole root systems poses significant challenges. As a result, sampling efforts and the studies they inform rarely recognize or accommodate this heterogeneity of tissues and pathogen lifestyle.

Hierarchical branching order (Fitter, 1982) provides a classification of fine roots that recognizes a shift in function from absorbance and uptake to transport and anchorage that occurs with increasing root order (Pregitzer et al., 2002). Increasing stele to root ratio, initiation of secondary xylem, periderm formation, and the sloughing of the cortical tissues have been linked with increasing root order in multiple tree species (Guo et al., 2008). Corresponding chemical and physiological shifts include increased carbon to nitrogen ratio, increased lignin content, and decreased respiration (Pregitzer et al., 2002; Hishi, 2007). These anatomical and chemical shifts reflect changes in quality of infection sites for both beneficial and pathogenic microorganisms

(Hishi, 2007). For instance, the presence of a living cortex confines mycorrhizal colonization to the first three root orders in multiple tree species (Guo et al., 2008).

Classification by hierarchal branching order provides a framework for predicting root form and function. However, in a growing root system, all 1st order roots may not be created equal. Zadworny and Eissenstat (2011) drew a distinction between 1st order fine-feeder roots and larger faster growing root tips (pioneer roots) that expand the root system and typically become higher order roots. When comparing these two classes of roots with different growth trajectories at the same stage of development, they found 1st order fine-feeder roots were more likely to be colonized by mycorrhizal and non-mycorrhizal fungi, while the pioneer roots were rarely colonized. They hypothesized that plant defenses are optimally allocated to pioneer roots owing to their greater carbon investment and future role for the plant as a higher order root. Thus, an expanding root system may experience different rates of infection compared to ephemeral roots borne on an established root system.

Hierarchical branching order has been repeatedly shown to provide a good classification of root structure and function, yet surprisingly few studies have attempted to understand pathogen infection based on root order. When attempted, authors have found higher rates of root infection for species such as *Phytophthora* and *Pythium* in root tips compared to higher order roots (English & Mitchell, 1988; Mihail et al., 2002), which may result from localization of infection events at the root tip (English & Mitchell, 1989), constraints of tissue maturation (Guo et al., 2008), or deployment of defenses to higher order roots. To our knowledge this approach has not been used for tree species, where a perennial root system necessitates a functional definition of fine root classes and diseases of the root system are often complex and somewhat cryptic. The goal of this research is to investigate the relationship between tree root order and

pathogen activity, and apply this recognition of root heterogeneity to an investigation of root-pathogen interactions to the apple replant disease (ARD) pathosystem.

Apple replant disease is characterized by the poor growth and yield of replanted apple (*Malus domestica* Borkh.) orchards and is attributed to biotic factors, as evidence by the positive growth response following broad spectrum fumigation (Mai & Abawi, 1981) and the persistence of symptoms following dilution of the soil in a sterilized soil (Jaffee et al., 1982b). The resulting setbacks in orchard re-development are economically significant and failure to adequately control ARD can result in losses up to $100,000 per hectare over a 10-year period (Smith, 1995).

While the etiology of the disease is complex, a core group of common soil-borne pathogens including *Cylindrocarpon* spp., *Rhizoctonia solani*, the oomycete genera *Pythium* and *Phytophthora,* and the root-lesion nematode (*Pratylenchus penetrans*) have been repeatedly implicated in multiple studies in New York State (Jaffee et al., 1981; Jaffee et al., 1982b; Jaffee et al., 1982a), Washington State (Mazzola, 1998; Mazzola, 1999), Australia (Dullahide et al., 1994), Europe (Manici et al., 2003) and South Africa (Tewoldemedhin et al., 2007; Tewoldemedhin et al., 2011b). Complexity and uncertainty surrounding the disease may arise because not all agents are present and active at a given site and some agents may act synergistically (Braun, 1995; Tewoldemedhin et al., 2011b).

Rootstocks, the predominantly clonal genotypes on which desirable apple varieties are grafted, vary in their ability to tolerate replant soils. Select Cornell-Geneva (CG) rootstocks have performed well in both greenhouse screenings (Isutsa & Merwin, 2000) and long-term field experiments (Rumberger et al., 2004; Leinfelder & Merwin, 2006; Fazio, 2009). Various mechanisms have been proposed to explain the performance of CG rootstocks including selection during breeding for resistance to *Phytophthora cactorum*, lower recovery rates of

Pratylenchus penetrans (Isutsa & Merwin, 2000; Mazzola et al., 2009), lower infection rates of *Pythium* species (Mazzola et al., 2009), tolerance through root system vigor or spatial avoidance (Yao et al., 2006), and rhizosphere microorganism mediated disease suppression (Rumberger et al., 2007; St. Laurent et al., 2010).

In the preceding studies, however, the root system is sampled, and effectively conceptualized, as a homogeneous system. Considering the heterogeneity of both root structure and function, the interpretation of counts, isolations, and molecular investigation of microorganisms on the bulk root system may not be straightforward. Histological evidence points primarily to a disease of distal roots in a state of primary development, and symptoms of diseased trees include root systems with few intact roots, extensive sloughing of epidermal and cortical tissues, and limited mycorrhizal colonization (Caruso et al., 1989) In another early study on the pathogenicity of *Pythium* species on apple roots, *P. sylvaticum* only successfully infected the primary roots of apple seedlings just behind the root tip. Roots that grew past the inoculation point were unaffected and escaped infection (Mulder, 1969). These results are tissue specific and may be lost when root systems are evaluated in bulk. However, there is some indication of vascular disruption as well. Authors have also observed fungal hyphae present in the xylem vessels of declining trees at one replant site (Caruso et al., 1989) and orange indistinct lesions apparent on the stele of roots in a replant bioassay (Jaffee et al., 1982b), so the extent to which cortical or vascular colonization contributes to replant disease is not clear.

In this paper we test the hypothesis that root development results in tissue level resistance to replant pathogens. We predict that branching orders vary in resistance to replant pathogens and explore mechanisms of anatomical development and chemical defense to explain observed patterns. We further evaluate the hypothesis that plants invest differentially in roots of different

growth trajectories and predict that pioneer roots are more resistant to pathogen infection, compared to 1st order fine-feeder roots, as a result of greater allocation of chemical defenses. Finally, we evaluate the hypothesis that improved rootstock performance is a result of tissue level resistance to replant pathogens within the root branching system, which may result from the restriction of pathogen spread from distal branches to higher order roots, or limiting infection and spread within the distal branches.

To test these hypotheses we grew Malling (M) 26 and CG.210 rootstocks in a replant bioassay and measured the abundance of commonly implicated replant pathogens on branching orders of the fine root system by using a previously published quantitative polymerase chain reaction (qPCR) assay (Tewoldemedhin et al., 2011b). We evaluated anatomical structure and secondary metabolites across root orders and growth trajectories to investigate mechanisms of tissue resistance. In this investigation we focused on tissue phenolics, a broad class of bioactive compounds recognized for their contribution to structural defenses (Bennett & Wallsgrove, 1994), which have been previously investigated for their role in ARD (Hofmann et al., 2009) and apple interaction with foliar pathogens (Veberic et al., 2005; Petkovsek et al., 2009; Gosch et al., 2010). Finally, we investigated the growth and development of root systems in a replant soil and rootstock resistance to replant pathogens as potential mechanisms of rootstock defense.

MATERIALS AND METHODS

Soil collection, plant material, and plant growth conditions

Soil was collected from the Cornell Orchards in Ithaca, NY, from a known replant site (Leinfelder & Merwin, 2006) in November 2011. Soil was sampled at a depth of 0-30cm under

trees growing on M.26, M.7 and M.9 rootstocks. Soil from this site is a Hudson silty clay loam, glacial-lacustrine, mixed-mesic Udic hapludalf. Soil was mixed with perlite 2:1 (vol/vol) in a cement mixer. Half of the soil mixture was steam pasteurized at 80°C for 2 hours and the remaining field soil (FS) returned to a cooler at 4°C. The pasteurized soil (PS) was allowed to equilibrate at room temperature for one week prior to planting.

Clonal plantlets of M.26 and CG.210 were propagated in tissue culture on Murashige and Skoog media (PhytoTechnology Laboratories, Shawnee Mission, KS) with supplemental vitamins. Plants were rooted in-vitro and planted in a soilless media following root initiation (Cornell Mix, Ithaca, NY). Plants were acclimated for three weeks in a fog tunnel and one week on a greenhouse bench at 25-28°C before final planting in 1.5L plastic pots of bioassay soil. Plantlets were ranked by size and distributed evenly among soil treatments and harvest dates. Plants were grown on a greenhouse bench from December 2011 to February 2012 with a 14hr photoperiod of supplemental light (≈100μM/m^2/s). Day and night temperatures in the greenhouse were 22° and 15°C respectively. Plants were watered daily; three times per week irrigation water included 150ppm nutrient solution (21-5-20 with micronutrients) (Scotts, Marysville, OH).

Plants were destructively harvested at 3, 6 and 9 weeks post planting. Roots were washed free of adhering soil under running tap water. Root systems, or intact branching portions thereof, were scanned on a modified flatbed scanner (Epson Expression 10000XL, Nagon, Japan) and dissected by root branching order (Fitter, 1982). A Strahler-branching order definition was used, with 1st order roots that were larger diameter (approximately ≥0.5mm) and longer than average designated as pioneer roots and assigned their own category. Dissected

roots were frozen at -80°C, lyophilized in a Labconco Freeze Dry System (Kansas City, MO, USA), weighed and then stored at -20°C for downstream analysis.

Root system measurements

Root system scans were skeletonized to black and white, through color thresholding in ImageJ (Abramoff et al., 2004) and analyzed in WinRhizo Pro version 2007d (Regent Instruments, Victoria Canada) for root length. Where subsamples of root systems were scanned, a correction factor (total root biomass/ scanned biomass) was applied to estimate total root length.

DNA extraction and quantitative PCR

Depending on available sample quantity, between 1 and 15mg of lyophilized root tissue was ground in a MiniBeadBeater (BioSpec Products, Bartlesville, OK) for two 45-second cylces in a screw cap microfuge tube with four steel 2mm balls (MoBio, Carlsbad, CA, USA). Following tissue homogenization, DNA was extracted with the Qiagen DNeasy Plant Mini Kit (Valencia, CA, USA) following the manufacturer's protocol. Following extraction, DNA was cleaned and concentrated through an ethanol precipitation (Sambrook et al., 1989) and re-eluted in 50µl of TE buffer (Promega, Madison, WI, USA). DNA concentration was determined with flourometric Quant-iT PicoGreen dsDNA probe (Invitrogen, Grand Island, NY, USA) and diluted to 10ng/µl. Samples with less than 10ng/µl were kept at original concentrations.

Pathogen abundance in root orders was estimated using a qPCR assay developed to target common replant pathogens (Tewoldemedhin et al., 2011b) (Table 1). All reactions were carried out using the SyberGreen based Qiagen Quantifast chemistry on 96-well plates in a BioRad iQ5 real time PCR detection system (Hercules, CA). Each assay consisted of four plates including multiple reactions of negative control root DNA from plants grown in pasteurized soil. Each

plate included duplicate reactions of a standard curve of 10-fold dilutions of pure culture target DNA, non-template controls (water), negative controls (non-target pathogen DNA) and root samples. To generate a standard curve for each target, DNA was extracted from pure cultures of *Pythium sylvaticum*, *Phytophthora cactorum* and *Ilyonectria robusta* (Booth Cylindrocarpon group 3) (Chaverri et al., 2011), *Pythium irregulare*, *Pythium ultimum,* and Rhizoctonia AG-5. DNA extracts were quantified with the Quant-iT PicoGreen kit and serially diluted.

Reactions for all targets except *Pythium sylvaticum* were carried out in 20µl volumes consisting of 10µl of Quantifast 2x, forward and reverse primers at 1µM final concentration and ≤20ng template DNA. *Pythium sylvaticum* reactions contained forward and reverse primers at 0.6µM to balance amplification efficiency and limit primer-dimer formation. Cycling parameters followed the manufacturer's instructions for a two-step protocol: dynamic well factor collection; initial denaturing 95°C for 3 minutes, and 40 cycles of denaturing at 95°C for 10seconds and combined annealing extension for 30seconds.

Temperature gradients were conducted for each primer pair to optimize annealing temperature and maximize separation of target and non-target signals (Table 1). Sensitivity of the assay determined by lowest dilution of standard curve with a cycle threshold below negative controls. Melting curves were generated following all reactions. To test for the presence of PCR inhibitors random samples of root DNA were serially diluted to test for a linear relationship between input DNA and cycle threshold. Amplification data was analyzed with the BioRad iQ5 optical software v2.0. To control for unequal extraction efficiency, pathogen DNA concentrations were normalized to root extracted DNA (fg target DNA /ng root DNA).

Table 1: Target, designation, and sequence of primers used in quantitative PCR estimation of replant pathogen abundance in root samples.

Target - Primer	Annealing Temp	Sensitivity* (fg/reaction)	Target length	Citation
Phytophthora Yph1F/Yph1R CGACCATKGGTGTGGACTTT ACGTTCTCMCAGGCGTATCT	62°	270fg	470bp	(Schena & Cooke, 2006; Schena *et al.*, 2008)
Pythium irregulare PirF1/PiR3 AGTGTGTGTGGCACGTTGTC GATCAACCCGGAGTATACAAAAC	64°	15fg	120bp	(Spies et al., 2011)
Pythium sylvaticum Syl1F/Syl2R GTGTCTCGCTGTGGTTGGTATATTTG CTTCTGCCAATTGCACAAGTGC	65°	5fg	341bp	(Schroeder et al., 2006)
Pythium ultimum PulF2/PulR2 GCA GGA CGAAGGTTGGTCTG GTC CCCACAGTATAAATCAGTATTTAGGT	63°	15fg	102bp	(Spies et al., 2011)
Cylindrocarpon YT2F/ Cyl-R GATGAAGAACGCAGCGAAAT TGTGCTACTACGCAGAGGAA	63°	35fg	233bp	(Dubrovsky & Fabritius, 2007; Tewoldemedhin *et al.*, 2011c)
Rhizoctonia AG-5 RSAG5F / RSAG5R GATATTTGGTTGTAGCTGGCTCATG GCACCAATTGTTCTTAAAAAACAATC	61°	25fg	126bp	(Mazzola & Zhao, 2010)

*Sensitivity of experimental assay (ficograms of target DNA in a 20μl reaction) determined by lowest detection of a serial dilution of standard curve without non-specific amplification.

Root anatomical assessment

Root anatomical development across branching orders and growth trajectory was investigated for each rootstock when growing in either ARD soil or the steam pasteurized control. At six and nine weeks, root segments representative of healthy or symptomatic roots, as assessed by necrosis or abnormal browning, were sampled from each branching order and preserved in FAA (5ml formaldehyde, 5ml acetic acid and 90ml of 70% ethanol). Root segments were dehydrated in a tert-butonal series and embedded in paraplast plus (Leica,

Wetzler, Germany). Sections (14μm) were cut on a Thermo Scientific Microm HM355S rotary microtome (Waltham, MA), stained in safranine-fast green and imaged under a Ziess Axioskop II (Jena, Germany). Cross section images were measured in ImageJ (Abramoff et al., 2004) for diameter, cortex thickness and state of senescence, stele diameter, and presence or absence of a periderm. We characterized the first four root orders, because very few plants had developed a fifth order by the final harvest. While higher root orders appeared later in the assay, their classification is dependent on distal branching, therefore higher order roots do not appear, but are reclassified as such (Fitter, 1982).

Phenolic extraction and quantification

Tissue phenolics were extracted from lyophilized tissue through methanol extraction. Depending on the amount of available tissue, between 1 and 15mg of dry tissue was combined with 500μl of extraction buffer—MeOH with 1% 2.6-di-tert-butyl-4-methylphenol (BHT) (Sigma-Aldrich, USA). The sample was homogenized in a FastPrep-24 instrument (MP Biomedicals, Solon, OH, USA) set on high (6.5M/s) for two 45-second cycles. The lysate was then centrifuged for 15 minutes at 14000rpm at 4°C and the supernatant filtered through a 0.45μm nylon membrane filter (Millipore, Tullagreen, Ireland).

Phenolic compounds were measured on an Agilent 1100 HPLC (Agilent Technologies, Wadbronn, Germany) with a Phenomenex Gemini – NX column (150 x 4.6mm, 3μm) (Torrance, CA, USA) and a diode array detector. Elution solvents were A: acetonitrile and B: .25% phosphoric acid. 15μl of extract was separated using the following protocol: 0 – 4 minutes 5% acetonitrile, 4-24 minutes ramping to 60% acetonitrile, 24-34 minutes ramping to 95% acetonitrile and held at 95% A for 1minute. Phloridzin was quantified at 320nm; phloretin and

other minor peaks were quantified at 280nm. The spectra of compounds were also recorded between 200-400nm.

Peak area was integrated using the HP ChemStation software. Quantifiable peaks that had a signal intensity over 100 and were present in a majority of samples were expressed in terms of peak area/mg root tissue and, when possible, assigned to compound classes based on characteristic UV spectra. Phloridzin concentration in root tissue was calculated as ng/mg by developing a standard curve to relate peak area to mass of phloridzin dihydrate (Simga Aldrich, USA).

Statistical analysis

Pathogen abundance in root branching orders was analyzed in a two-step process. A binomial logistic analysis was conducted to test the main effects of order, rootstock and harvest date on the probability of detecting each pathogen. Analysis was conducted in R (R Development Core Team, 2012) using the geeglm function in the geepack package (Højsgaard et al., 2006). This function fits a generalized estimating equation to solve a logistic model and allows the inclusion of the random (clustering) effect of plant id in the model. Zeros were then removed from the analysis and a full factorial mixed effect analysis of variance (ANOVA) with rootstock, branching order and harvest date as main effects and plant replicate as a random effect was used to analyze natural log transformed abundance.

Differences in anatomical traits among branching orders were tested with a mixed effect ANOVA, with plant replicate included as a random effect. Diameter was natural log transformed and stele to root ratio rank transformed to normality to correct for heteroskadicity.

A multivariate analysis of eleven quantifiable chromatogram peaks was conducted to test for main effects and interactions on phenolic profile of roots. Peak area/mg was z-transformed to

a mean of 0 and standard deviation of 1. The z-transform allows peaks of different wavelengths (scales) and variance to be analyzed together. The effects of root order, soil treatment, harvest date and rootstock were tested in a full factorial model using the permutational multiple analysis of variance (ADONIS) function and a Euclidean distance matrix in the vegan package (Oksanen et al., 2012) for R. A factor analysis was used to group highly correlated chromatogram peaks (Noyer et al., 2011). Factors were extracted using principle component analysis with varimax rotation to construct five synthetic factors, which explained 86% of the variation in the eleven chromatogram peaks. The varimax rotation maximizes high loading values and minimizes low loadings, which improves the interpretability of the resulting factors (McGarigal et al., 2000). Each factor was then used as a dependent variable in a full factorial mixed-effect ANOVA to test the effect of order, rootstock, soil, harvest date and interactions. To evaluate differences in phenolic chemistry between 1st order fine-feeder roots and pioneer roots, higher orders were removed from the mixed-effect ANOVA and the main effect of order and the interaction of order and soil were interpreted at $P < 0.05$.

Differences in plant growth in field soil and pasteurized control were tested with ANOVA independently for each rootstock, with height at planting included as a covariate. Total plant biomass and root biomass were transformed by their natural logarithm to meet assumptions of equal and normally distributed residuals. Pairwise differences between harvest dates were tested using student's t-test with bonferroni correction for multiple comparisons.

Rootstock differences in pathogen abundance, root diameter, cortex thickness, and phenolics were evaluated by root order in a full factorial ANOVA. In order to avoid rates of cortical senescence driving perceived anatomical differences, the condition of the cortex as intact, senescent or absent was included as a covariate for anatomical tests.

Growth inhibition of plants growing in the field soil was calculated as the residual of observed and predicted values (derived from pasteurized soil estimates). To correct for differences in plant size, the residual was expressed relative to the predicted value (FS residual/ predicted value). Biomass and root length relative residuals were analyzed in an analysis of covariance with rootstock and pathogen concentration at each root order to test for correlations between pathogens and growth inhibition. Pearson's product moment correlations were calculated to explore the relationship between phenolic factors and pathogen abundance in 1st order and pioneer roots. Analysis of covariance with rootstock and order as fixed effects and plant included as a random effect was used to test for the significance of the relationship.

Except where noted, all analyses were conducted in JMP pro v9.0 (SAS Institute Inc., Cary, NC). In all proceeding models the order variable contains 1st order pioneer roots and fine-feeder roots as separate levels (e.g. pioneer, 1st order, 2nd order and 3rd order).

RESULTS

Pathogen abundance in heterogeneous tissues

To test the hypothesis that root heterogeneity, as described by branching order or growth trajectory, influences susceptibility to replant pathogens, pathogen abundance was estimated by qPCR analysis. For all assays, the cycle threshold (Ct) for negative control root samples was higher than the detection threshold. For *Phytophthora*, a signal from non-target pathogen DNA was detected at a lower Ct than lowest detectable standard, and the accurate detection threshold was set below the lower Ct value. Amplification efficiency ranged from 94% to 102% and R^2 values for the standard curves ranged from 0.982 to 0.998 for all plates used in the subsequent analysis. Of the pathogens that could be quantified in a reasonable number of samples, *Pythium*

sylvaticum was detected in 90% of root samples and DNA concentrations ranged from 0.33 to 4198fg/ng with a mean of 140fg/ng and median of 25fg/ng. *Cylindrocarpon* spp. DNA was detected in 93% of samples and ranged from 7 to 16,694fg/ng, with a mean of 865fg/ng and median of 290fg/ng. *Pythium irregulare* DNA was present in the lowest quantities and in quantifiable levels in 59% of the samples. Concentrations of *P. irregulare* ranged from 0.5 to 183fg/ng, with an average of 14.4fg/ng and median of 4.6fg/ng DNA. Two of the six pathogens targeted, *Rhizoctonia solani* AG-5 and *Pythium ultimum*, were not recovered in any samples at detectable levels. *Phytophthora* DNA concentration was above the reliable detection limit in only six root samples. These samples had concentrations ranging from 78 – 3714fg/ng. Too few samples were quantifiable to allow further analysis.

Analysis of pathogen abundance was conducted in a two-step process to determine whether replant pathogens are less likely to be detected in certain branching orders and, if detected, if they are more or less abundant in different branching orders. Harvest, rootstock and order did not have a significant effect on the likelihood of detecting *Cylindrocarpon* ($P = 0.34$). *Pythium irregulare* was less likely to be detected in pioneer and 3rd order roots compared to 1st and 2nd order ($P < 0.001$) (Figure 1C). *Pythium irregulare* was also less likely to be detected in roots from the final harvest ($P = 0.003$). There were no 3rd order root samples with quantifiable amounts of *P. irregulare* DNA at the final harvest. There were no differences in the likelihood of detecting *P. sylvaticum* across the three branching orders, but this pathogen was less likely to be detected in pioneer roots than 1st order fine-feeder roots ($P = 0.01$).

Root branching order also had a significant effect on target DNA concentration for both *P. sylvaticum* and *Cylindrocarpon* (Figure 1A & 1B). No differences in the abundance of either pathogen were found between 1st and 2nd order roots, while third order roots and pioneer roots

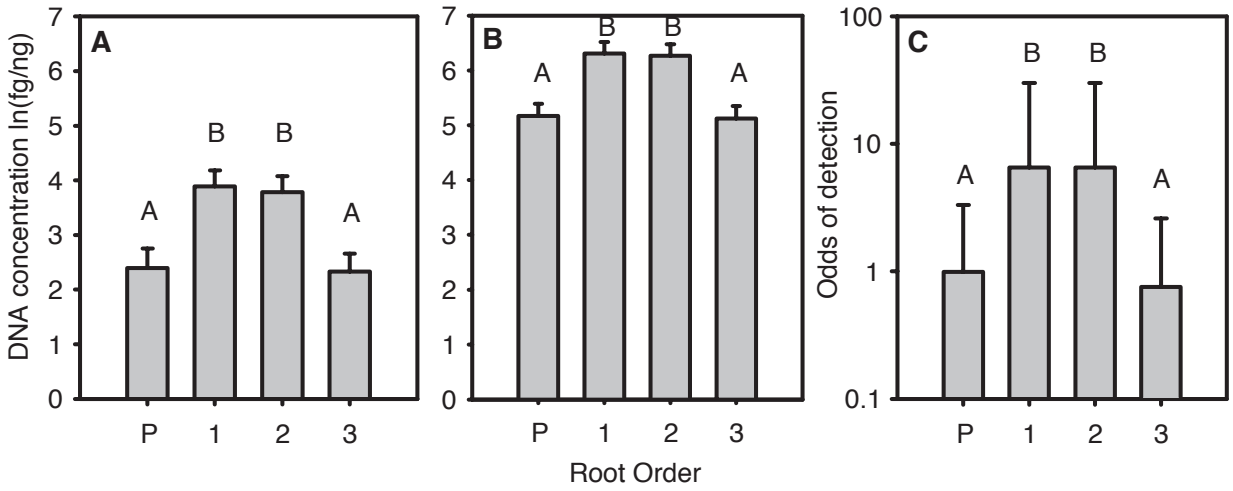

Figure 1: Quantitative PCR estimates of replant pathogen colonization of pioneer (P), 1st, 2nd and 3rd order roots. *Pythium sylvaticum* (A) and *Cylindrocarpon* spp. (B) abundance is expressed as the natural log of target DNA concentration. *P. irregulare* (C) colonization is expressed as odds of detection (p / 1 - p), where p = the probability of detection. Columns are least squares means averaged across rootstocks and harvest dates. Vertical bar is one standard error (A and B) and 95% confidence interval (C). Columns with different letters are significantly different at P = 0.05 Tukey's HSD.

had lower levels of pathogen DNA. Since *P. irregulare* was not detected in any 3rd order roots at the final harvest, they were not included in the factorial analysis for this pathogen. However, the pattern indicated by the logistic analysis of *P. irregulare* and the least squares estimates of *Cylindrocarpon* and *P. sylvaticum* abundance all support decreased populations of replant pathogens in 3rd order roots (Figure 1). For the samples in which *P. irregulare* was detected, there were no differences in the abundance among pioneer, 1st and 2nd order roots (P = 0.97).

Populations of all three pathogens varied with harvest date. *Cylindrocarpon* DNA was found at a higher concentration at the final harvest (P = 0.01). This was attributable to a significant increase in 1st order (281fg/ng to 1096fg/ng; P = 0.03) and 2nd order roots (334fg/ng to 1588fg/ng; P = 0.001) over this time, while pathogen populations in 3rd order roots did not change over time. The interaction between order and harvest was significant for both *Pythium* species (P < 0.05). *Pythium sylvaticum* DNA was recovered at lower concentrations in 3rd order

roots at the final harvest (2.86fg/ng) compared to the first (65fg/ng; $P = 0.0002$), while for *P. irregulare* there were no 3rd order root samples with quantifiable amounts of target DNA at the final harvest. In contrast, both *P. sylvaticum* and *P. irregulare* increased over time in pioneer roots (*P. sylvaticum*: 3.25 to 79fg/ng, $P = 0.001$; *P. irregulare*: 2 to 34fg/ng, $P = 0.007$).

Root development as a constraint on pathogen habitat

Root anatomical development was investigated across branching orders and growth trajectories. This allowed us to evaluate anatomical development as a mechanism to explain observed patterns of pathogen abundance, and to elucidate the structure and function of each branching order. Additionally symptomatic roots were sampled across branching orders to determine the structure of roots susceptible to the replant pathogens and to provide visual signs of tissues colonized by potential pathogens.

Root order separated distinct stages of tissue development with implications for root function and pathogenic interactions. Root diameter increased with root order (Table 2) and concurrently, an increase in stele diameter combined with the senescence and loss of the cortex in 2nd and 3rd order roots (Figure 2) resulted in a higher stele to root ratio. Stele to root ratio has been used to indicate the root area devoted to the alternate functions of resource uptake versus transport (Guo et al., 2008). From the perspective of pathogen interactions, the senescence and loss of the cortex may represent a loss of habitat. Sections from 1st order roots had intact, or in a few cases senescent, cortical tissues. Second order roots varied considerably in the state of the cortical tissues, ranging from intact to absent. Concurrent with this transition, periderm initiation and secondary development of the vascular cylinder was evident in 75% of 2nd order sections. The cortex in 3rd order roots was either senescent or absent entirely and remnants of a cortex were only present in one 4th order root section.

Table 2: Anatomical measurements of root cross sections by branching order and growth trajectory. Mean (± 1 sd) of sampled roots averaged across rootstocks and harvest dates.

	Pioneer	1	2	3	4
Diameter (μm)	545 (141)[b]	270 (67)[d]	431 (163)[c]	588 (230)[b]	1055 (301)[a]
Cortex (μm)	165 (51)[a]	79 (23)[b]	90 (52)[b]	44 (62)[c]	1 (5)[d]
Stele(μm)	157 (41)[c]	72 (22)[d]	198 (102)[c]	437 (217)[b]	990 (301)[a]
Stele: Root	0.3 (0.08)[e]	0.26 (0.05)[d]	0.46 (0.17)[c]	0.75 (0.18)[b]	.93 (0.03)[a]
Cortex[a] (Present/ Senescent/ Missing)	80/7/1	87/3/0	51/42/11	5/32/40	0/1/31
Periderm[b]	10%	2%	74%	95%	100%
n=	88	90	104	77	32

Values followed by different letters are significantly different at $P = 0.05$ Tukey's HSD
a Number of samples
b Per cent of samples with a periderm initiation

By definition, pioneer roots were of greater diameter than 1st order fine-feeder roots. Stele to root ratio was similar between these groups (Table 2), which supports root order as a reliable classification of stage of development. The percent of samples exhibiting periderm initiation was slightly greater than 1st order fine-feeder roots, consistent with the assertion that these roots are destined to become higher branching orders.

To determine the structure of roots visually impacted by the replant pathogens, symptomatic roots were sampled from each root system growing in the field soil. Few 3rd order roots showed symptoms for either rootstock. Across branching orders, almost without exception, a cortex was present (either fully intact or in the process of senescence) in roots that displayed visual symptoms; in contrast, there was a higher frequency of sections without a cortex when sampled from non-symptomatic roots (Figure 2). This localization of symptoms corresponded with fungal and oomycete colonization of the cortex that was observed in many samples.

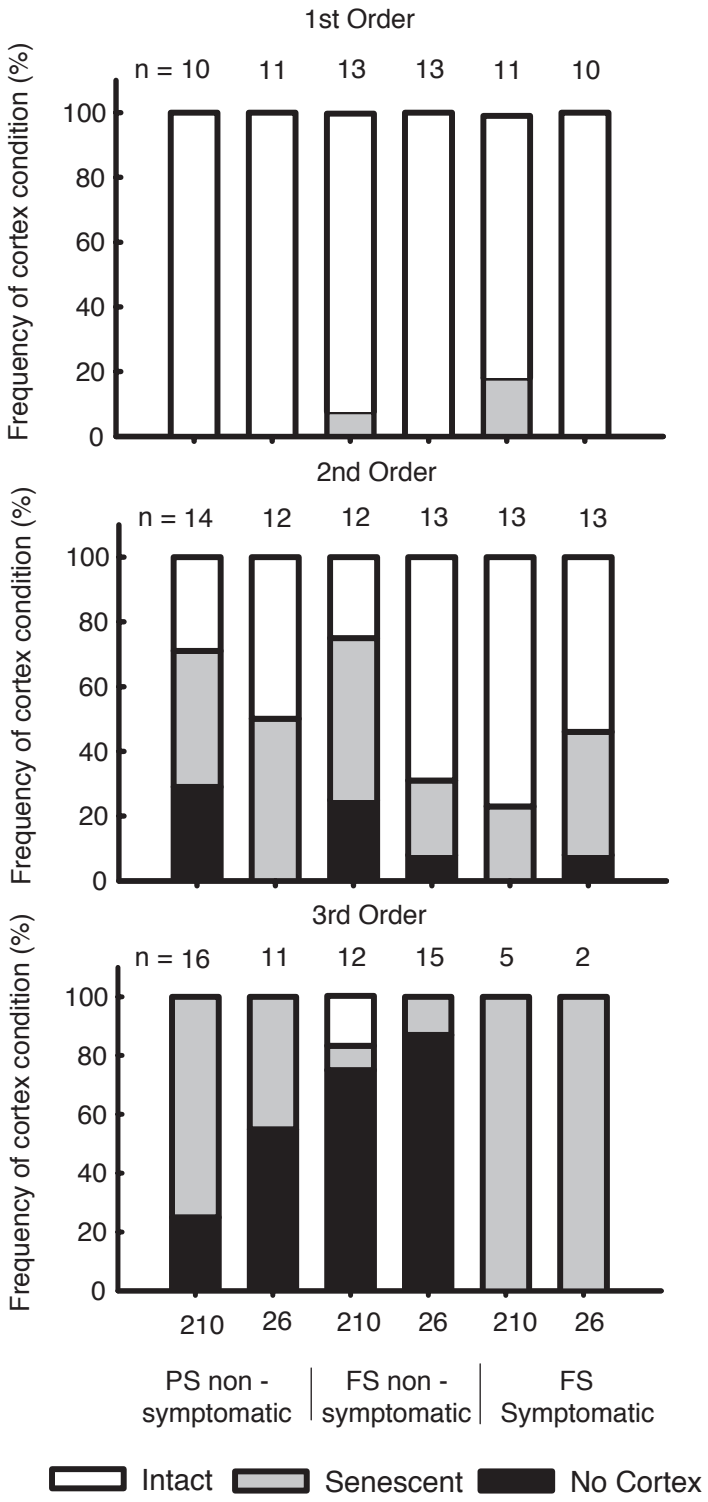

Figure 2: Frequency of 1st, 2nd and 3rd order root cross-sections with intact (white bars), senescent (grey bars), or absent (black bars) cortex. Samples were taken from week 6 and 9 harvests and grouped by soil treatment (PS and FS), appearance of visual symptoms and rootstock ((M.)26 and (CG.)210). Values above each column are number of root samples imaged.

Chemical defenses as a mechanism of tissue resistance

Root phenolic chemistry was investigated to determine patterns of chemical defenses on the root branching system in relation to patterns of pathogen abundance. Additionally, we evaluated root orders for an increase in phenolic compounds in the field soil compared to the pasteurized control to determine which branching orders demonstrate an induction of defense compounds or stress response to the presence of replant pathogens.

Root order and trajectory accounted for the greatest portion of the variation in chromatogram peak area ($R^2 = 0.28$; $P < 0.001$) in the full-factorial model (ADONIS; $R^2 = 0.66$). The five synthetic factors extracted from the factor analysis captured 86% of the variance in peak area among samples (Table 3). Branching order influenced the concentration of each factor ($P < 0.05$) except for F5. Two notable patterns emerge from the factor analysis. First, phloridzin, the largest peak in the chromatogram, loaded heavily on F2. This factor was found at highest levels

Table 3: Loadings of z-transformed chromatogram peaks (rows) on synthetic factors (columns) extracted from principle components analysis with varimax rotation. To ease interpretation, only peaks that load above |.40| on a factor are shown. Wavelength of peak absorbance of spectra for each chromatogram peak reported on left.

Peak absorbance λ (nm)	Retention time	F1	F2	F3	F4	F5
285	13.3	0.80				
285	13.85	0.88				
285	14.5	0.92				
285	14.8		0.51	0.63		
315	15.9	0.85				
285	16.9			0.93		
285	17.1	0.47	0.65			
285	18.6 (Phloridzin)		0.90			
285	19.2		0.88			
255	27.6					0.92
325	31.9				0.92	
% of total variance explained		31	22	14	11	10
Eigenvalue		4.20	2.63	1.12	.83	.77

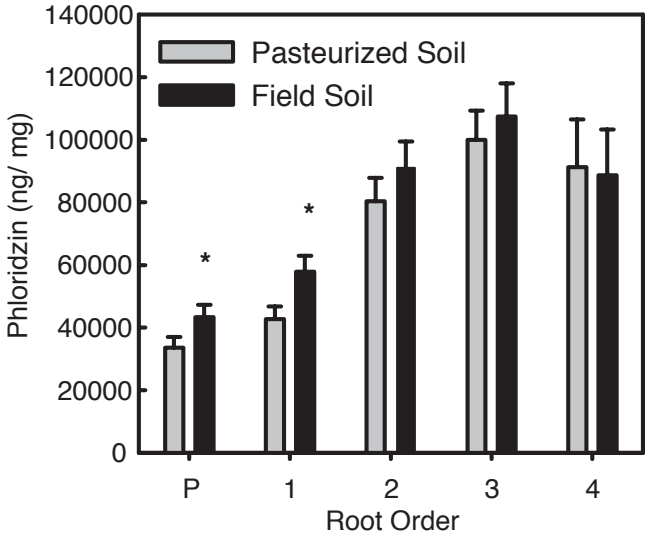

Figure 3: Phloridzin concentration (ng/mg) in pioneer (P) 1st, 2nd, 3rd and 4th order roots growing in field soil and pasteurized soil. Columns are back transformed least square means averaging across rootstock and harvest date. Vertical bar is 95% confidence interval of the mean. * Differences between pasteurized and field soil are significantly different ($P < 0.05$; Student's t-test with Bonferroni correction).

in higher order roots ($P < 0.001$) and phloridzin itself reached concentrations over 10% of root dry weight (Figure 3). Thus, total concentration of phenolics increases with root order to third order roots. Second, in contrast to F2, the other factors were found at higher concentration in 1st order (F1), or 2nd order roots (F3 and F4), which would indicate a shift in composition of phenolic compounds, in addition to the shift in total concentration, among branching orders. Of the compounds loading on F1, four shared similar spectra as phloridzin, with peak absorbance at 285nm, which may indicate precursors or breakdown products, while the spectra of one resembled a hydroxycinnamic acid and had a peak absorbance at 315nm. Factor three also included compounds with spectra similar to phloridzin, while single compounds with peak absorbance at 325nm (putatively caffeic acid) and 255nm loaded on F4 and F5 respectively.

Root order also explained differences among tissues in potential defense induction or stress response when growing in the field soil compared to the pasteurized control (Order x Soil:

$P < 0.05$ for all factors except F3). There was a significant increase for each factor in first order roots growing in field soil ($P < 0.05$). In contrast, in 2nd and 3rd order roots all factors were either lower in the field soil compared to the pasteurized control (2nd order: F1, F3 and F4; 3rd order F4) or there was no significant difference between soil treatments ($P > 0.05$).

First order roots of different growth trajectories also differed in the phenolic chemistry. Pioneer roots had lower concentrations of F2 (phloridzin) and F3 ($P < 0.001$) than smaller diameter fine-feeder roots, but higher concentration and a greater induction of F4 ($P < 0.001$ and $P = 0.02$ respectively). While there was not a significant effect of growth trajectory on F5, this factor was found in higher concentrations in pioneer roots growing in field soil than 1st order fine-feeder roots in the same soil ($P = 0.05$).

Rootstock resistance to replant pathogens

Rootstock growth in bioassay soil

Growth, development, and resistance to replant pathogens were compared between rootstocks to evaluate evidence for resistance or tolerance based mechanisms that support the improved performance of CG rootstocks in ARD soil. Following plant acclimation for four weeks, shoot height at planting ranged from 1-3cm for CG.210 and 1-6cm for M.26. Height at planting had a significant effect on all plant growth metrics ($P < 0.001$). However, interactions between height at planting and treatment effects were not significant ($P > 0.05$).

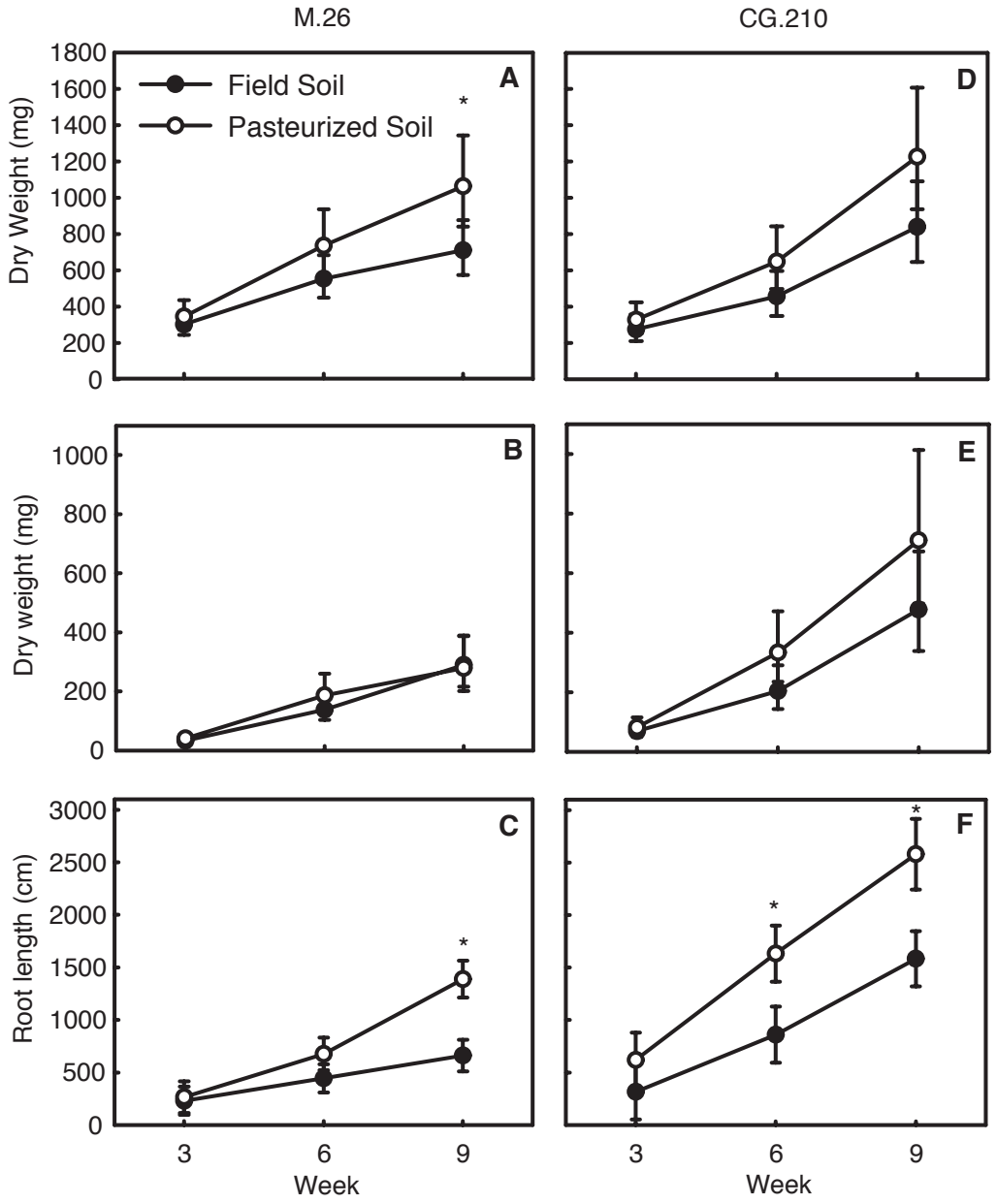

Figure 4: Total biomass (A, D), root biomass (B, E) and root length (C, F) of M.26 (A,B,C) and CG.210 (D,E,F) plants harvested at three, six and nine weeks.
Vertical bar is 95% confidence interval. *Differences between plants grown in field and pasteurized soil that are significantly different ($P < 0.05$; Student's t-test with Bonferroni correction).

Steam pasteurization of soil improved growth of both rootstocks in terms of total biomass and shoot biomass, which points to a biotic inhibition of growth for both rootstocks (Figure 4). Pasteurization improved root biomass accumulation for CG.210 ($P = 0.03$), but not for M.26 (P

= 0.28), though root length was positively impacted by soil pasteurization for both rootstocks ($P < 0.001$). While the effect of soil treatment in the overall model was significant for both rootstocks, pairwise t-tests of biomass accumulation for plants growing in pasteurized vs. field soil at a given harvest were only statistically significant for M.26 at the final harvest (Figure 4).

While growth of both rootstocks was inhibited in the field soil, growth strategy of the two rootstocks differed considerably during this assay. CG.210 accumulated most new biomass below ground (Figure 4), resulting in a higher root to shoot ratio than M.26 ($P < .001$). For both rootstocks, the root to shoot ratio increased from the first to the third harvest (CG.210: 0.38 to 1.38 (R/S); and M.26: 0.13 to 0.53). M.26 initiated above ground growth early in the assay, but this growth halted in the field soil and a terminal bud was set. CG.210 did not initiate measurable shoot extension until the sixth week of the assay, but at week 9, this rootstock was actively growing in both soil treatments.

Rootstock resistance to ARD pathogens

Concentrations of pathogen DNA recovered from roots of the two rootstocks varied with both harvest and root order (Figure 3). Rootstock resistance to ARD pathogens was evaluated by comparing pathogen abundance between the rootstocks by branching order.

Within 1st order roots CG.210 had lower abundance of both *Pythium* species and a lower abundance of *Cylindrocarpon* at the final harvest compared to M.26 (Figure 3). In pioneer roots, CG.210 also had lower abundance of *P. sylvaticum* and *Cylindrocarpon*, though for the latter, this was attributable to increased levels of target DNA at the final harvest. There was no

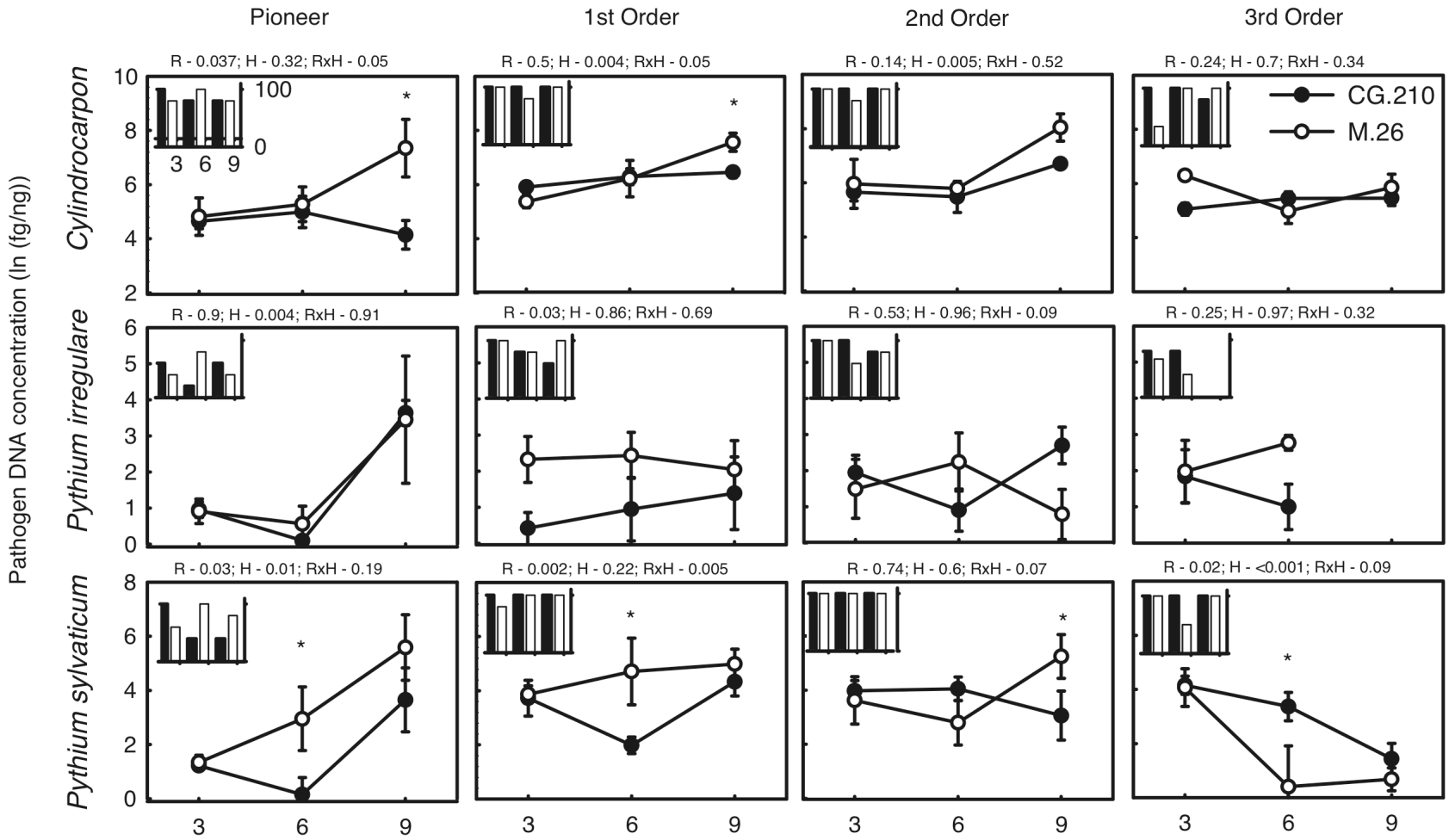

Figure 5 Quantitative PCR estimates of replant pathogen DNA concentration in M.26 and CG.210 root samples with positive detection at three, six and nine weeks. Values are natural log transformed means and vertical bars are one standard error of the mean. Significance value (*P*-value) of effect tests for independent variables rootstock (R), harvest date (H) and interaction (RxH) in ANOVA presented above each plate. *Means are significantly different ($P < 0.05$; Student's t-test). Inset bars: frequency of detecting sample 0-100%

difference between the rootstocks in levels of *P. irregulare* DNA recovered from pioneer roots.

In 2nd order roots there were no overall differences between the rootstocks in populations of *P. sylvaticum*, *P. irregulare or Cylindrocarpon* though populations in both rootstocks were highly variable over time. In 3rd order roots higher concentrations of *P. sylvaticum* DNA were recovered in CG.210 roots compared to M.26 ($P = 0.02$), mainly attributable to differences at the 2nd harvest. There was no difference between the rootstocks in the levels of *Cylindrocarpon* or *P. irregulare* DNA in 3rd order roots.

Growth inhibition was calculated as the relative field soil residual (actual – predicted / predicted) to test if pathogen abundance had an effect on growth suppression. No significant effect ($P > 0.05$) was detected for pathogen concentrations at any root order and the observed growth suppression in terms of biomass or root length. Notably, when analyzed as relative residuals, CG.210 had lower inhibition of growth at the final harvest, but the difference was not statistically significant for either biomass or root length ($P = 0.33$ and $P = 0.33$ respectively).

Anatomical differences between the root branching system of CG.210 and M.26 were evaluated on plants growing in pasteurized soil for the harvests at six and nine weeks. Roots that developed in the assay soil are best reflected by these sampling dates. Overall, CG.210 roots had a finer branching structure with a smaller diameter ($P = 0.002$) and a thinner cortex ($P = 0.005$). When controlling for stage of cortical senescence, CG.210 had smaller diameter among 2nd and 3rd order roots and a thinner cortex among 1st and 2nd order roots than the corresponding roots of M.26 (Table 4).

Phenolic chemistry of the two rootstocks was evaluated as a potential mechanism contributing to pathogen resistance of CG.210, which could result from either differences in constitutive levels of chemical defense compounds or greater induction in response to pathogen

Table 4: Average root diameter and cortex thickness (± se) of pioneer (P) 1st, 2nd and 3rd order roots from CG.210 and M.26 rootstocks growing in pasteurized soil. Data pooled between harvests at six and nine weeks. Differences between rootstocks tested by order in a mixed-effect model with plant as random effect.

	Diameter			Cortex thickness		
Order	CG.210	M.26	*P*	CG.210	M.26	*P*
P	560 (50)	588 (50)	0.69	169(15)	204(15)	0.13
1	229(18)	281 (17)	0.07	67(4)	84(4)	0.008
2	408 (38)	561 (47)	0.04	74(9)	120(11)	0.004
3	571(66)	783(71)	0.06	43(13)	59(14)	0.41

challenge. The two rootstocks had different concentrations of every phenolic factor in first order roots and different concentrations of F1, F2 and F4 in pioneer roots ($P < 0.05$) (Figure 6). Additionally, the two rootstocks responded to the soil treatments by increasing or decreasing phenolic concentration in different ways. First order roots of M.26 growing in field soil had an increase in concentration of F2 (phloridzin) compared to the same roots in pasteurized soil, while CG.210 roots had comparable concentrations of this factor in both soils (Soil x Rootstock; $P = 0.03$). In contrast, the direction of change in the two rootstocks was different for F3 with a much greater induction observed in CG.210 (SxR; $P = 0.002$). Within pioneer roots, the soil by rootstock interaction was significant for F1 and F2 ($P = 0.004$ and $P = 0.05$) (Figure 6).

Phenolic factors were tested for correlations with pathogen abundance to determine if these compounds were linked with a defense or stress response to particular organisms (Table 6). Factor 1, which consisted of putative phloridzin derivatives or precursors and a hydroxycinnamic acid was negatively correlated with *Pythium* DNA of both species (Table 6) and this relationship was robust to the inclusion of rootstock and root classification (fine-feeder vs. pioneer root) in the model. This factor was induced in field soil in both rootstocks, though to a greater extent in CG.210 in pioneer roots (Figure 6).

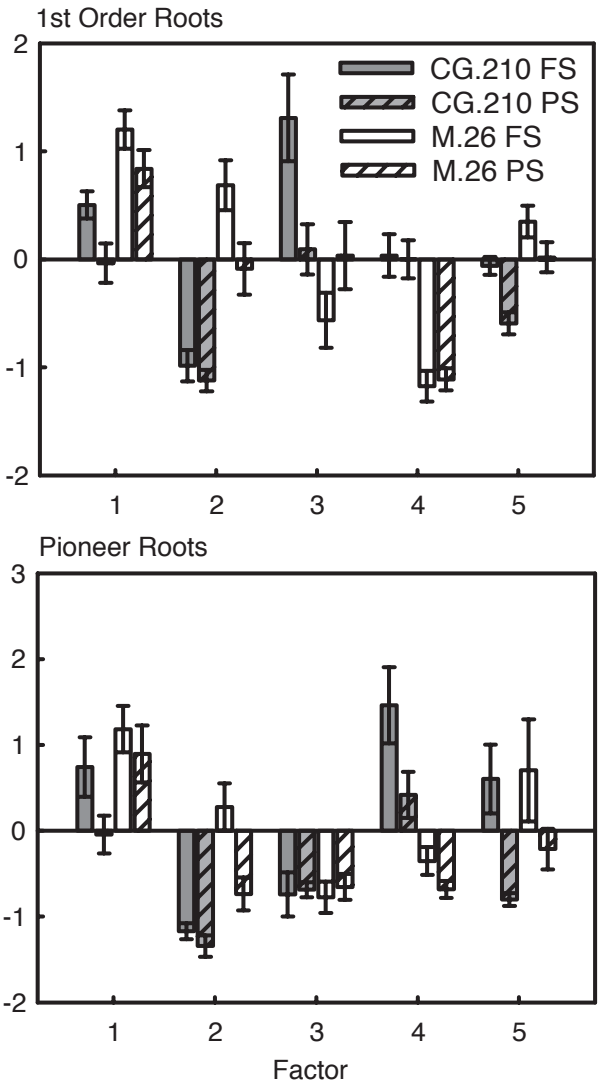

Figure 6: Phenolic factor scores by rootstock and soil treatment. Zero line represents centroid of PCA extracted factors and columns indicate the magnitude and direction of group mean separation from the overall mean. Vertical bar is one standard error of the mean.

F4 (caffeic acid) was also negatively correlated with *Cylindrocarpon* DNA, however this effect was not significant if rootstock and order were included in the model. This factor was found at greater concentrations in CG.210, and particularly high concentrations in CG.210 pioneer roots growing in field soil.

Table 5: Pearson's product moment correlations between pathogen DNA (ln(fg/ng)) and phenolic factors in 1st order and pioneer roots. Values in bold are significant at $P = 0.05$.
* Effect of target DNA concentration on phenolic factor is significant ($P < 0.05$) significant when root order and rootstock effects are included in ANCOVA model.
** $P < 0.01$
*** $P < 0.001$
X following value indicates a significant interaction between rootstock and pathogen concentration.

	Factor1	Factor2	Factor3	Factor4	Factor5
P. irregulare	-0.38***	0.18	-0.27	0.04	0.54***X
Cylindrocarpon	-0.04	0.37	0.10	-0.38	0.17 X**
P. sylvaticum	-0.25**	0.28	0.05	-0.25	0.30

In contrast, F2 was positively correlated with both *Cylindrocarpon* and *P. sylvaticum*. This factor was found at higher levels in M.26 than CG.210, and increased dramatically in the field soil compared to the pasteurized control in M.26. The significance of the correlation between F2 and both pathogens was lost when rootstock is included in the model as factor two was found greater in concentrations in M.26 roots ($P < 0.001$) which also had higher concentrations of target DNA.

Finally, F5 was also positively correlated with both *Pythium* species, though the correlation between F5 and *P. sylvaticum* was not significant when rootstock was added to the model. For M.26 there was a positive correlation with *Cylindrocarpon*, though only a weak negative correlation in CG.210 roots. Factor 5 was found at its highest concentrations in pioneer roots growing in field soil and thus may reflect a defense mechanism of these roots in response to pathogen challenge.

DISCUSSION

Root heterogeneity in structure and function results from both normal growth and development (Hishi, 2007) and initial growth trajectory (Zadworny & Eissenstat, 2011). Our results suggest that this heterogeneity influences plant interactions with soil-borne pathogens by restricting pathogen spread in the branching system, altering the response potential of plant tissues, and altering the relationship of colonization and virulence. Hierarchical branching order, a functional and operational classification of fine roots, captures this heterogeneity in a way meaningful to plant interaction with soil-borne pathogens. The dynamics of susceptibility, resistance, and ultimately disease development at the whole root system level are consequences of tissue level interactions between potential pathogens and fine root branches within the hierarchical branching system. Plant resistance and tolerance to soil-borne pathogens are best understood in this context.

Branching order, root development and root defensese

Quantitative PCR revealed that each of the three taxa detected in our study were less abundant in 3rd order roots compared to 1st and 2nd order roots. This was most dramatic for the *Pythium* species as populations declined sharply by the final harvest. The later harvests likely reflect roots that grew and developed in the assay soil, rather than those present at, and susceptible to the shock of, transplanting. As a mechanism to explain the pattern of abundance across root orders, we found the most support for the restriction of available habitat. Similar to other authors, classification of roots by root order revealed distinct stages of development (Pregitzer et al., 2002; Guo et al., 2008), which have important implications for pathogenic interactions. Most directly, the senescence and loss of cortical tissues occurred in 2nd and 3rd order roots. To the degree that replant pathogens utilize the cortex as a site of resource

acquisition, changes in cortical area mark a loss of available habitat. Within this study there is some evidence of this constraint. Visual symptoms only appeared on roots retaining or in the process of shedding their cortex. Additionally, while our anatomical sectioning was not specifically designed to evaluate infection, we observed no indication of colonization of vascular tissues inside the endodermis or periderm. Meanwhile, fungal and oomycete colonization of the cortex was observed in a many samples. If the organisms targeted in our assay were not infecting the vascular system of higher order roots, the loss of cortex would explain the decrease in target DNA concentration observed in third order roots.

The structural changes observed over the first four root orders, including increasing diameter, increasing stele to root ratio, and senescence of the cortex, mirror those found on multiple tree species and are indicative of a shift in function from resource uptake to resource transport (Guo et al., 2008). Even though many 2nd order roots had an intact cortex, the initiation of the periderm, which was observed in a majority of samples, will isolate the cortex and seal its fate as senescent tissue. Thus, in this assay, it is likely the contribution of 2nd order roots to resource uptake is significantly decreased compared to that of 1st order roots. If the function of 2nd and higher order roots is primarily resource transport, then the functional significance of pathogen colonization of the remaining cortical tissues in 2nd and 3rd order roots is questionable and may not constitute a disruption of plant function or contribute to disease. It is possible that replant pathogens have other mechanisms of virulence when colonizing higher order roots. Inhibition of branch root initiation and disruption of the vascular system could both contribute to declines in root system performance. However, we did not observe any vascular disruption by presence of mycelium, occlusion of vessels or the presence of tyloses.

The secondary development and initiation of the periderm in 2nd order roots in our study may contrast with roots of field grown and established trees. Eissenstat and Anchor (1999) found that the fibrous 1st and 2nd order roots of citrus did not undergo secondary development, while seedling 2nd order roots did develop secondary xylem and periderm. Thus, the specific link between branching order and development may shift with age, soil, and establishment of the root system (Wells & Eissenstat, 2002), as will conclusions of the functional importance of specific root orders to the plant-microbe interaction of interest.

Phenolic chemistry also varied by root order and may contribute to the patterns of pathogen abundance observed in this study. Third order roots had the highest concentrations of phloridzin and it's associated factor; however, 2nd order roots also had higher concentrations of this compound and yet we observed no reduction in target DNA concentration, so it is unlikely that this defense compound alone limits the spread of these organisms. Phenolics are generally recognized for their role in plant defense as precursors to lignin, suberin, and callose formation (Bennett & Wallsgrove, 1994). The build up of phloridzin in higher order roots may act directly to inhibit certain organisms or contribute to structural defenses that isolate the vascular tissues from infection. In particular, *Pythium* species are generally unable to able to penetrate secondary wall thickenings (Hendrix & Campbell, 1973). Viewed as a component of structural defense, the pattern of increasing phloridzin allocation with root order is consistent with the replacement of primary development with the suberin deposits and heavily lignified tissues of secondary development.

Classification by root order highlighted groups of compounds preferentially allocated to different tissues within a root system. While phloridzin increased in concentration between 1st and 3rd order roots, other chemical factors were found at their highest concentration in 1st order

and pioneer roots. Additionally, the response of all compounds to the field soil treatment was order specific. Increased concentration in field soil, possible evidence of induction or a stress response, was significant only in 1st order fine-feeder and pioneer roots. These are the roots that have the highest proportion of metabolically active cortical tissue and are reasoned to be of primary importance for the pathogenic interaction of interest.

Overall there is considerable evidence that tissue heterogeneity is important in terms of tissue resistance to replant pathogens and that root branching order provides a classification of heterogeneous tissues with functional relevance to plant interactions with soil-borne pathogens.

Root growth trajectory and pathogen susceptibility

There is a growing body of evidence that tissue development is predetermined in early stages of root growth (Resendes et al., 2008; Zadworny & Eissenstat, 2011). Our findings provide qualified support for the assertion that pioneer roots and smaller diameter 1st order roots differ in pathogen resistance. Overall, pioneer roots had lower amounts of target DNA than 1st order fine-feeder roots (Figure 1). However, this pattern was highly dependent on harvest date, limiting this strength of this conclusion. Furthermore, in this study, we cannot differentiate root age, so it is possible that age distribution, and not resistance, led to the observed patterns of organism abundance.

Zadworny and Eissenstat (2011) hypothesized that optimal defense allocation could account for differences in infection rates between these classes of roots and found evidence of increased structural defenses—fewer passage cells and increased number of layers in the hypodermis—in pioneer roots. We evaluated phenolic chemistry between 1st order roots of different diameters and found distinct chemical profiles. Interestingly, phloridzin (F2) was found at lower concentrations in pioneer roots, while caffeic acid (F4) and F5 were found at higher

concentrations in these roots, particularly when growing in field soil. The functional significance of these differences is unclear, but evidence of distinct phenolic chemistry is consistent with altered allocation of defenses to pioneer roots.

The discussion of pioneer roots and 1st order fibrous roots highlights a challenge for the implementation of a branching order classification of root interaction with soil-borne pathogens. If on an expanding root system, characteristic of a replant scenario, some roots are predestined to become higher order roots and others destined to remain in primary development as fine-feeder roots, then the same branching order may capture subclasses of roots of different structure and function (Zadworny & Eissenstat, 2011).

Rootstock Resistance

Given the differences in pathogens abundance, anatomical development, and tissue phenolics among root orders, there is a compelling reason to investigate rootstock resistance to replant pathogens with explicit recognition of the tissues being sampled. We used the data collected by hierarchical branching order to evaluate the hypothesis that tissue level resistance to replant pathogens contributes to previously observed performance of CG.210.

Support for the hypothesis of resistance to replant pathogens comes primarily from 1st order and pioneer roots. CG.210 roots had decreased populations of all three target organisms in these roots, which are reasoned, to be of primary importance for the interaction of interest. Mazzola et al. (2009) also found decreased populations of *Pythium* species on roots of CG rootstocks and, in a previous study at the Ithaca orchard, oomycete communities in the rhizosphere of CG.210 were distinct from those on Malling rootstocks (Rumberger et al., 2007). To our knowledge, this is the first report of lower abundance of *Cylindrocarpon spp*. on a CG rootstock. In contrast, CG.210 did not have significantly lower concentrations of target DNA in

higher order roots, suggesting that resistance mechanisms limiting initial infection and colonization in distal branches, rather than mechanisms that restrict systemic spread, may contribute to the improved performance of this rootstock in replant soil.

The dynamic populations of *P. sylvaticum* observed in our study suggests other factors contribute to the population abundance at a given time point. One explanation may relate to the timing and rate of root growth. Since root extension is assumed to initiate interactions with soil-borne pathogens (Huisman, 1982), differences in timing and progression of infection may result from different rates of growth between the two rootstocks. M.26 displayed its greatest rate of root growth between the harvests at six and nine weeks, while CG.210 expanded its root system early in the assay (Figure 4C & F). If interactions with propagules were initiated at an earlier time, then these organisms would have more time to increase in abundance in these root tissues.

Overall, we did not find a correlation between plant growth suppression and target DNA from any root order, so the link between observed resistance and plant performance remains unclear. This is consistent with the absence of a correlation between pathogen DNA in seedling roots and growth response in replant soil from multiple orchards in South Africa (Tewoldemedhin et al., 2011b), which may result from multiple causal organisms with synergistic or antagonistic effects (Braun, 1995; Tewoldemedhin et al., 2011b). For *Cylindrocarpon* this may result from primers that pick up non-virulent strains, or a weak relationship between seedling growth suppression and pathogen DNA recovered from plant roots (Tewoldemedhin et al., 2011c). Importantly, quantitative PCR is a measure of organism abundance and fitness, which need not have a direct relationship with virulence or plant defense as measured in growth outcomes.

A stronger link has been demonstrated between target DNA and virulence for *P. irregulare and P. sylvaticum*, as DNA recovered from inoculated plants has been negatively correlated with plant weight (*P. sylvaticum*) and positively correlated with visual root rot ratings (*P. irregulare*) (Tewoldemedhin et al., 2011a). Additionally, Bent et al. (2009) found that *Pythium ultimum* DNA in root tips was inversely related to shoot biomass in a study of peach growth in replant soil.

On a physiological level, we observed differences between these rootstocks in each of the five phenolic factors in 1st order and pioneer roots in terms of absolute quantity and direction or magnitude of response to the replant soil. A number of these factors were directly correlated with target DNA, while for others the correlation was closely linked with the rootstock effect. However, this could be evidence of either differences in defense induction or a modulated stress response.

The role of phenolics in apple resistance to pathogens has been a subject of considerable debate. In general, phenolics are recognized for their role in structural defenses, as a building block of lignin in secondary wall thickenings and component of papillae wound plugs that isolate pathogen infections (Bennett & Wallsgrove, 1994). Phloridzin has demonstrated toxicity to *Phytophthora cactorum in vitro* (Alt & Schmidle, 1980; Gosch et al., 2010) and foliar concentrations of this compound and the hydroxycinnamic acids, caffeic and *p*-coumaric acid, have been found at higher levels in varieties resistant to apple scab (*Venturia inequalis*) (Petkovsek et al., 2009). In our study, CG.210 had lower concentrations of phloridzin than the more susceptible M.26, however, greater concentrations or induction was observed for factors putatively associated with hydroxycinnamic acid and caffeic acid in CG.210. Alternately, authors have suggested phloridzin can be stimulatory to fungal growth, while precursors and

breakdown products can be either stimulatory or fungitoxic (Barnes & Williams, 1961). Hoffman et al. (2009) found that phloridzin content of root exudates peaked at the onset of ARD symptoms, but then decreased with time. We did not observe a decrease for either rootstock in tissue concentration of this compound and its correlation with pathogen abundance was mainly a result of higher concentrations found in the more susceptible M.26.

An alternate hypothesis of rootstock performance relates to root system vigor and tolerance based mechanisms of root system defense. Root biomass and root length accumulation were two-fold greater for CG.210 than M.26. If pathogen populations are restricted to distal branches, the production of a more extensive and highly branched root system may compensate for proportional loss. There is some evidence for such vigor-based tolerance of root herbivory (Bauerle et al., 2007). However, plant vigor was not significantly related with replant soil performance in Isutsa and Merwin's (2000) screening for replant resistance. Additionally, root proliferation in infested soil has been linked with *Phytophthora* resistance in citrus (Graham, 1995), but plant vigor was not a predictor. It is not vigor though, measured in terms of total biomass accumulation over the period of the assay, that differed between the two rootstocks, rather it is the relative allocation of biomass above and below ground. The extra biomass allocation below ground and associated proliferation of root length may contribute to the ability of CG.210 to maintain shoot growth in the replant soil, even when loosing some uptake capacity. In this vein, CG.210 roots were of smaller diameter in the lower branching orders. A finer and more highly branched root system would also be cheaper to construct in terms of investment in root length (Eissenstat et al., 2000), which could also contribute to tolerance of root loss.

Overall, CG.210 had a different growth habit, lower pathogen abundance in roots of primary development, and an altered phenolic profile compared to M.26. Given the previous

reports of this and other CG rootstock performance in replant soil, direct and functional investigations with single replant pathogens would help to elucidate the mechanisms of plant defense against replant pathogens.

Methodological Considerations

Our assay was timed to capture early stages of disease development on the expanding root system. Sampling at three, six and nine weeks post planting spans the range of assay lengths typical of seedling based investigations of replant severity and etiology which have ranged from five (Mazzola, 1998) to twelve weeks (Tewoldemedhin et al., 2011b). While differences between rootstock growth in pots and the field must be recognized as a limitation of this study, this type of assay does reflect the pot studies that provide the basis of our understanding of the etiology of replant disease (Jaffee et al., 1982a; Jaffee et al., 1982b; Mazzola, 1998; Mazzola, 1999; Tewoldemedhin et al., 2011b). Such studies have been less likely to confuse saprobic organisms inhabiting older roots with pathogens colonizing young roots (Tewoldemedhin et al., 2011a). Furthermore, propagation of our plant material in tissue culture ensured clean starting material and a growth stage comparable to other seedling based assays. While overall plant growth was modest during the assay, sampling captured the period when the root systems were expanding to fill the soil volume.

Our analysis is conducted on roots pooled by branching order and, as a result, the qPCR analysis cannot distinguish between a difference in infection events and rate or extent of colonization of roots. Likewise metabolite chemistry represents average values for a root branching order and not the individual roots where this interaction is occurring. Therefore local induction of defenses following infection events will be diluted among other roots of that order.

Neither *Pythium ultimum* nor *Rhizoctonia* AG-5 were detected in any root samples. In earlier work on this site Rhizoctonia AG-G was the most frequent isolate recovered from apple roots (Appendix A). While many strains of this anastomosis group have not demonstrated pathogenicity to apple (Mazzola, 1997; Tewoldemedhin et al., 2011a), some have (Mazzola, 1997), and we cannot rule out that *Rhizoctonia* strains not targeted by the AG-5 primer contributed to the growth reduction observed in the replant soil. Additionally, while previous work on this site found parasitic nematodes to be far below the damage threshold (Leinfelder & Merwin, 2006), their contribution, or synergistic effects with other species, is not addressed in our study and conclusions about the disease dynamics we observed must leave space for the contribution of these organisms.

CONCLUSION

An investigation of the link between root order and plant interaction with soil-borne pathogens revealed differences in tissue resistance and distinct stages of development among roots of different orders. Additionally, the functional significance of pathogen colonization of host tissues is brought into focus by explicitly addressing the type of tissue and its contribution to root function. In this case, we make the argument that colonization of cortical tissues by common replant pathogens is most significant for 1st order roots and of lower significance in higher order roots. Concurrent with changes in anatomy, tissue secondary metabolites were differentially allocated to different orders and induction of metabolites is only observed in 1st order and pioneer roots. Finally, when rootstocks (M.26 and CG.210) with contrasting performance in a replant soil are compared, tissue level resistance is apparent in 1st order and

pioneer roots of CG.210, which corresponds to shifts in phenolic chemistry in these same root tissues.

APPENDIX A

In the summer of 2010 a study was undertaken to investigate root physiological development of two apple rootstocks in a replant soil.

MATERIALS AND METHODS

Soil, plant material, and growth conditions

Soil was collected from the Cornell Orchards in Ithaca, NY. The soil was a Hudson silty clay loam, glacial-lacustrine, mixed-mesic Udic hapludalf. Soil was mixed with perlite 1:1 (vol/vol) in a cement mixer. Half of the soil mixture was steam pasteurized at 65°C for 45 minutes and the remaining field soil (FS) was returned to a cooler at 4°C. The pasteurized soil (PS) was allowed to equilibrate at room temperature for one week prior to planting.

Rootstock liners of M.26 and CG.6210 (CG.210) were planted in FS and PS soil in 7.65L tree pots (Steuwe and Sons Inc., Tangent, Oregon, USA) with two 3" x 12" windows cut out and replaced with mylar plastic. Prior to planting, liners were rated for quality by the number and size of roots present. Pots were wrapped in black plastic to exclude light and held in double pots in an outdoor nursery at the Cornell Orchards. Pine mulch was bermed around pots to keep plants upright, and exposed black plastic was wrapped in aluminum foil insulation to moderate temperature. Liners were potted on May 28th, 2010 and moved to randomized locations in outdoor nursery June 1st, 2010. Plants were irrigated as needed and fertilized bi-weekly with 200ml of complete nutrient solution at a concentration of 3g/L (24N-8P-16K) (Miracle Grow, Scotts Company, Marysville, OH, USA). From each rootstock and soil treatment combination,

plants were randomly assigned to three subsets for sequential destructive harvests on July 15th (16th), August 25th (26th), and October 7th (8th).

Rootstock growth and morphology

Rootstock stem caliper at 10cm above the soil line and shoot extension were measured bi-weekly. At harvests, material was washed free of adhering soil, dried for two days at 60°C and weighed for biomass (Tables A2-A4). During the July and August harvests, whole root branches of plants (n=7) from each rootstock and soil combination were randomly sampled and scanned using an EPSON scanner. Following root scanning, the scanned and un-scanned portions of the root system were dried separately so root measurements could be scaled accordingly.

Root tracking

The subset assigned to the final harvest was also used to track root dynamics over the growing season. Root windows were photographed weekly from shortly after planting to the final harvest. A jig was used to maintain a consistent distance from the camera to the window.

Two weeks prior to the July and August harvests, root tracing commenced on the subset of plants to be harvested. All roots visible in mylar windows were traced with black marker. Following the initial tracing, roots were traced as they appeared, color-coded by day (Table A1). Prior to the October harvest, there were very few new roots appearing in the windows, so root tracing ceased.

Table A-1: Date and color-coding of root tracing in root box windows.

July Tracing Color	Date of Tracing	August Tracing Color	Date of Tracing
Black	June 30th	Black	August 10th
Gold	July 5th	Silver	August 15th
White	July 9th	Blue	August 18th
Blue	July 11th	Green	August 19th
Silver	July 13th	Yellow	August 20th
Red	July 14th	Red	August 21st
Green/Clear	July 15th	White	August 23rd
Clear	July 16th	Green circles	August 24th
		Clear/yellow circles	August 25th
		Clear	August 26th

Respiration

Roots of known age were excised through the mylar windows and pooled with roots of the same age within replicate plant. Respiration was measured using an Oxygraph electrode chamber (Hansetech LTD, Norfolk, England). Samples were kept on ice until measurement and allowed to equilibrate in the respiration buffer until reading stabilized, then run for 10 minutes. Roots were then dried at 60°C for two days and measured for dry weight. Respiration was calculated as O_2 consumption over time (nmol O_2/mg dry weight/sec).

Carbon and nitrogen

Root samples gathered for respiration analysis were analyzed for total carbon, nitrogen and carbon: nitrogen ratio on the Goodale Lab elemental analyzer. Roots were pooled into three age groups (0- 4 days, 5-11 days, and 12+ days) to generate enough mass for analysis and, when necessary to generate adequate mass, samples from multiple plants were combined into one.

Root anatomy

At the July and August harvests, roots of known age were preserved in FAA (5ml formaldehyde, 5ml acetic acid and 90ml of 70% ethanol).

Root infection by fungi and oomycetes

During destructive harvests in August, 1st and 2nd order root segments were plated in water agar (Bacto Agar, BD, Franklin Lakes, NJ) amended with 15μg/ml each of rifampicin and penicillin (Sigma Aldrich, St. Louis, MO, USA) (WARP). Hyphae emanating from the root segments were transferred to 1/5 strength potato dextrose agar (Himedia Laboratories Ltd., Mumbai, India). Cultures were purified through consecutive hyphal tipping and agar plugs were placed under WARP to force hyphae to grow through media and remove bacterial contamination.

Mycelium from pure cultures was harvested by growing the culture on sterile cellophane over 1/5 strength PDA. After five days mycelia was harvested and lyophilized overnight in a Labconco Freeze Dry System (Kansas City, MO, USA). DNA was extracted from the lyophilized mycelium using either the FastDNA® kit (MP Biomedicals, Santa Ana, CA, USA) following manufacturers instructions, except for the lysing stage, which was conducted using the bead matrix on a vortex for 45 seconds. DNA was alternately extracted using a CTAB protocol with 0.8% betamercaptoethanol (Baker & Mullin, 1994; Martin & Rygiewicz, 2005).

A portion of the internal transcribed spacer (ITS) region was amplified using the ITS1 and ITS4 primer pair (White *et al.*, 1990). Reactions were carried out in a volume of 35μl with 1.25μl of REDTaq® DNA polymeraze (Sigma Aldrich, St. Louis, MO, USA), 1x PCR buffer (Sigma), 2.5mM $MgCl_2$, 0.2μM forward and reverse primers, 0.2mM DNTPs (Sigma) and 1μl DNA template. Amplification was conducted in a Techne 3000 thermocylcer (Bibby Scientific Ltd., Staffordshire, UK) under the following cycling conditions: 3 minutes at 95°C; 30 cycles of 95°C for 30seconds, 50°C 30seconds, and 72°C for 2 minutes; followed by 72°C for 10 minutes. Resulting PCR products were run on a 1% agarose gel and visualized under UV illumination. All sets of reactions included a positive and negative (water) control. Amplified products were

cleaned for 15 minutes at 37°C with 2µl of ExoSAP-IT® (Affymetrix, Santa Clara, CA, USA). Sequencing was carried out at the Cornell University Core Facility. ABI trace files were viewed using FinchTV v1.4.0 (GeoSpiza Inc., Seattle, WA, USA). Sequences were assigned the putative taxonomic identity of the nearest matching non-environmental entry, with coverage greater than 95% and max identity greater than 97%, resulting from a BLAST search of the NCBI genebank database.

Table A-2: Above and below ground growth of M.26 and CG.6210 rootstocks from July harvest growing in field (FS) and pasteurized soil (PS).

			Caliper (mm)		Shoot extension (cm)		Biomass (g)				
Sample plant	Liner rating (1-10)	Shank depth	June 8th	July 15th	June 8th	July 15th	Above ground	Below ground shank	Roots not scanned	Roots scanned	Below ground total
FS 26-11	7	22.0	8.3	9.2	19.7	32.9	15.58	5.92	1.35	1.15	8.42
FS 26-14	6	25.2	8.0	8.7	2.9	22.0	8.72	5.56	0.00	0.64	6.20
FS 26-17	8	26.4	8.5	9.0	17.8	57.6	15.49	7.95	2.50	1.20	11.65
FS 26-20	7	22.2	8.8	9.4	10.5	35.3	12.99	5.04	0.50	0.65	6.19
FS 26-29	7	24.0	9.5	10.2	15.2	43.2	17.27	9.89	0.88	0.80	11.57
FS 26-35	8	25.0	9.1	10.0	12.7	51.8	16.73	6.65	1.81	0.00	8.46
FS 26-4	7	25.4	10.9	11.7	17.5	68.3	22.96	12.51	3.00	0.00	15.51
FS 26-8	4	25.5	6.9	7.8	10.8	42.7	9.61	4.21	1.30	0.00	5.51
FS 26-9	4	23.0	7.4	7.9	8.6	26.8	8.07	5.09	1.03	0.00	6.12
FS 6210-11	5	21.2	5.9	6.5	13.0	76.5	7.53	3.81	3.23	0.00	7.04
FS 6210-14	5	24.3	7.4	7.9	17.5	73.6	15.85	6.21	3.09	1.87	11.17
FS 6210-17	4	20.0	5.8	6.5	12.1	50.5	10.61	2.90	1.30	1.02	5.22
FS 6210-18	4	22.7	5.6	5.7	12.1	44.7	8.34		6.54	0.00	
FS 6210-24	7	24.5	7.9	8.5	14.6	57.1	15.21	6.16	4.70	1.91	12.77
FS 6210-25	3	21.0	5.1	5.8	10.2	64.1	9.10	3.22	0.96	0.83	5.01
FS 6210-3	3	25.7	4.9	5.8	15.2	60.7	11.68	4.45	7.29	0.00	11.74
FS 6210-33	3	21.8	5.4	6.5	12.7	60.4	12.53	3.50	4.18	0.00	7.68
FS 6210-34	4	21.4	5.7	6.6	15.2	70.3	14.17	7.14	3.15	0.98	11.27
PS 26-10	5	25.6	8.9	9.5	18.1	25.2	14.08	8.06	2.00	0.00	10.06
PS 26-17	6	26.9	8.1	8.6	12.7	46.2	10.57	6.40	2.06	0.00	8.46
PS 26-19	5	23.4	7.6	8.5	6.4	28.0	11.00	5.75	0.50	0.73	6.98
PS 26-21	9	32.6	8.2	9.3	14.9	53.8	15.37	7.76	1.42	1.06	10.24
PS 26-22	10	26.7	9.0	10.0	21.0	77.9	20.96	10.29	2.39	1.69	14.37
PS 26-3	4	15.0	6.7	7.5	5.1	22.0	8.27	3.53	1.35	0.00	4.88
PS 26-32	2	21.6	10.4	11.0	6.4	46.7	17.80	10.60	1.09	0.70	12.39
PS 26-40	9	23.5	9.0	9.7	8.3	26.8	12.40	6.87	1.01	0.93	8.81
PS 26-6	4	25.9	9.0	10.3	13.0	85.3	22.48	8.28	3.16	0.00	11.44
PS 6210-1	4	30.0	7.4	8.4		88.5	17.32	8.59	7.28	0.00	15.87
PS 6210-10	2	20.5	6.0	6.7	9.5	54.9	13.30	4.65	3.58	1.49	9.72
PS 6210-19	2	19.9	3.4	4.7	4.5	61.0	8.32	4.00	1.87	1.20	7.07
PS 6210-2	2	23.8	5.6	6.7	16.2	57.6	13.96	4.10	4.91	0.00	9.01
PS 6210-20	2	20.0	5.7	6.2	7.9	73.6	11.88	5.37	1.52	0.92	7.81
PS 6210-21		22.2	4.9	5.6	1.1	38.7	8.41	3.10	0.53	0.57	4.20
PS 6210-24	3	24.0	5.9	6.6	8.3	51.3	12.08	5.26	0.68	0.68	6.62
PS 6210-29	2	20.5	4.6	5.7	6.0	56.1	10.10	2.86	2.95	0.00	5.81
PS 6210-37	2	22.0	6.6	7.6	17.1	65.9	20.43	6.29	4.18	0.00	10.47
PS 6210-9	1	21.5	5.8	6.3	6.7	46.9	9.55	2.67	0.75	0.00	3.42

Table A-3: Above and below ground growth of M.26 and CG.6210 rootstocks from August harvest growing in field (FS) and pasteurized soil (PS).

			Caliper (mm)		Shoot extension (cm)		Biomass (g)				
Sample plant	Liner rating (1-10)	Shank depth	June 8th	Aug 25th	June 8th	Aug 25th	Above ground	Below ground shank	Roots not scanned	Roots scanned	Below ground total
FS 26-15	4	29.8	8.6	10.1	21.3	39.0	20.55	10.76	3.17	1.72	15.64
FS 26-19	7	25.3	9.3	9.8	8.9	72.0	20.55	9.01	1.12	0.66	10.79
FS 26-23	6	25.9	7.4	8.4	6.4	54.1	15.13	5.25	1.42	1.46	8.13
FS 26-25	7	22.8	8.6	10.2	19.4	37.5	25.09	6.99	5.71	0.00	12.70
FS 26-3	2	26.8	7.8	9.5	9.2	49.8	19.54	8.76	1.50	1.56	11.81
FS 26-30	7	24.0	9.4	10.9	18.4	45.5	22.72	7.87	1.50	1.40	10.77
FS 26-6	9	26.5	8.8	10.2	19.1	62.0	17.90	8.46	4.84	0.00	13.30
FS 26-7	6	24.0	7.8	9.2	14.6	63.3	17.95	6.44	2.63	0.00	9.07
FS 6210-16	3	21.0	6.3	7.0	14.6	49.7	15.55	4.87	5.47	3.12	13.47
FS 6210-20	5	20.0	5.4	6.4	17.5	54.2	13.12	5.23	10.58	0.00	15.81
FS 6210-26	3	21.0	5.9	7.3	13.3	66.1	20.43	5.38	6.33	2.34	14.05
FS 6210-28	3	20.3	5.0	6.3	21.9	44.5	14.45	4.75	5.27	2.37	12.40
FS 6210-30	2	22.7	5.3	6.6	3.8	36.5	12.78	4.18	8.32	0.00	12.50
FS 6210-36	4	23.0	7.4	8.1	9.2	36.9	17.76	11.34	7.42	3.17	21.94
FS 6210-6	3	26.5	6.8	8.4	21.6	43.4	20.30	6.43	7.35	2.47	16.25
PS 26-1	7	21.8	9.1	9.3	6.4	40.0	21.03	5.55	1.17	0.58	7.30
PS 26-14	4	23.0	8.1	9.2	8.6	70.4	19.07	5.24	1.81	0.00	7.05
PS 26-2	8	28.4	9.0	10.1	1.9	49.2	17.81	10.37	1.23	0.97	12.56
PS 26-33	7	25.0	8.2	9.5	12.4	45.2	22.77	7.86	1.88	1.37	11.11
PS 26-37	7	26.5	9.3	10.7	6.7	49.9	22.22	8.80	1.18	1.36	11.34
PS 26-39	8	27.5	8.3	8.7	11.1	64.0	18.21	6.13	1.62	1.01	8.76
PS 26-8	9	27.0	7.8	9.7	8.3	59.5	20.46	8.27	4.26	0.00	12.53
PS 6210-13	8	23.1	7.8	9.6	23.5	50.1	26.69	19.74	13.20	4.24	37.18
PS 6210-23	2	17.0	4.8	6.5	5.7	22.3	10.65	3.39	6.09	0.00	9.48
PS 6210-3	3	27.5	5.2	6.4	17.5	28.5	13.62	6.25	7.34	0.00	13.59
PS 6210-32	4	26.5	4.9	6.4	8.3	38.7	13.14	5.67	6.44	2.29	14.40
PS 6210-35	2	23.4	6.1	7.3	15.9	40.9	15.26	5.46	4.36	3.01	12.83
PS 6210-36	3	23.0	4.9	6.2	12.1	40.9	15.39	6.63	3.84	2.46	12.93
PS 6210-8	3	24.5	4.8	7.4	9.8	56.0	18.82	7.19	6.25	2.75	16.19

Table A-4: Above and below ground growth of M.26 and CG.6210 rootstocks from October harvest growing in field (FS) and pasteurized soil (PS).

			Caliper (mm)		Shoot extension (cm)		Biomass (g)				
Sample plant	Liner rating (1-10)	Shank depth	June 8th	Oct 7th	June 8th	Oct 7th	Above ground	Below ground shank	Roots not scanned	Roots scanned	Below ground total
FS 26-10	4	24.0	9.5	10.7	5.7	40.8	23.68	9.04	5.84	0.00	14.88
FS 26-16	7	25.0	9.4	10.4	5.1	47.1	25.93	10.83	5.02	0.00	15.86
FS 26-2	6	24.0	7.0	8.1	8.9	62.9	17.42	5.94	3.22	0.00	9.16
FS 26-22	7	26.7	8.8	9.8	6.4	57.5	22.16	9.62	5.71	0.00	15.33
FS 26-24	6	27.8	9.3	10.5	13.7	42.3	26.12	10.81	10.46	0.00	21.26
FS 26-26	5	21.7	9.6	10.9	15.6	55.6	30.15	9.06	9.18	0.00	18.24
FS 26-36	9	30.5	8.4	9.8	8.6	52.0	21.55	10.54	10.24	0.00	20.78
FS 26-40	7	28.1	8.3	9.3	5.1	49.1	21.92	9.82	4.92	0.00	14.74
FS 6210-1	2	26.5	4.5	4.9	6.0	20.1	6.87	3.99	8.93	0.00	12.92
FS 6210-10	2	22.3	9.6	10.3	8.3	86.7	22.51	13.00	14.19	0.00	27.18
FS 6210-13	4	21.8	7.9	13.0	18.4	52.7	24.11	11.48	14.34	0.00	25.82
FS 6210-22	5	23.5	6.4	6.9	11.1	25.5	13.03	7.29	14.70	0.00	21.99
FS 6210-29	4	21.5	8.1	9.0	14.6	45.0	16.54	7.89	17.94	0.00	25.83
FS 6210-35	5	25.4	5.4	7.4	17.8	53.8	18.91	10.72	14.67	0.00	25.40
FS 6210-4	3	22.0	6.4	7.6	17.8	44.6	18.41	9.94	20.49	0.00	30.43
FS 6210-9	3	21.5	4.9	7.3	7.0	67.6	18.13	7.83	12.97	0.00	20.81
PS 26-11	7	26.6	8.2	9.5	12.1	45.5	21.27	8.67	5.80	0.00	14.46
PS 26-15	7	22.0	9.5	10.9	8.6	32.9	21.32	7.78	4.48	0.00	12.25
PS 26-23	4	26.7	8.6	9.8	10.2	46.7	19.59	8.84	6.23	0.00	15.07
PS 26-25	8	26.5	9.2	11.3	10.2	52.8	27.46	13.60	10.57	0.00	24.17
PS 26-26	6	26.0	8.1	8.5	6.7	36.1	18.87	5.88	4.68	0.00	10.57
PS 26-35	7	26.3	7.0	8.8	7.6	73.4	17.92	8.68	6.74	0.00	15.42
PS 26-4	5	24.5	8.3	9.7	6.0	54.0	21.22	7.60	3.99	0.00	11.59
PS 26-7	5	23.1	7.3	8.8	11.7	40.9	16.60	9.51	7.32	0.00	16.83
PS 6210-15	1	20.9	6.4	7.7	11.4	50.2	20.89	7.67	16.69	0.00	24.36
PS 6210-16	2	20.0	6.1	8.0	7.6	66.7	20.75	9.07	31.29	0.00	40.36
PS 6210-18	1	20.0	5.0	5.8	13.0	30.8	10.52	3.79	8.59	0.00	12.37
PS 6210-22	2	24.5	5.8	6.9	9.5	53.3	12.89	6.69	10.37	0.00	17.06
PS 6210-25	5	24.3	8.1	9.1	15.9	71.5	24.63	11.12	35.07	0.00	46.19
PS 6210-27	2	21.0	4.6	5.6	6.7	22.4	9.24	3.48	7.21	0.00	10.69
PS 6210-31	4	12.5	8.4	10.1	7.6	75.3	26.31	10.02	25.75	0.00	35.77
PS 6210-7	3	22.6	5.8	7.7	16.2	55.6	19.43	7.22	23.89	0.00	31.11

Table A-5: Respiration of CG.6210 and M.26 roots by age, July 2010.

Sample plant	Color	Channel	Sample day	Age (days)	Dry weight (mg)	Manual O2 in	O2 out	Time	Respiration rate (nmol/ mg/sec)
FS 6210-11	Clear	1	7/15/10	0.5	0.704	283.30	254.53	600	0.170
FS 6210-11	Red	2	7/15/10	1.5	0.060	220.80	210.67	612	0.690
FS 6210-11	White	1	7/15/10	8	1.471	279.50	263.50	607	0.045
FS 6210-11	Gold	2	7/15/10	12	0.190	213.04	208.84	600	0.092
FS 6210-11	Black	1	7/15/10	15	1.558	267.26	262.27	600	0.013
FS 6210-11	Blue	2	7/15/10	5	0.305	213.90	206.79	600	0.097
FS 6210-11	Silver	1	7/15/10	3	0.128	266.73	261.75	600	0.162
PS 6210-1	Gold	2	7/15/10	12	0.418	209.48	205.87	600	0.036
PS 6210-1	Black	1	7/15/10	15	10.350	260.17	247.05	600	0.005
PS 6210-1	White	2	7/15/10	8	2.132	209.16	198.28	600	0.021
PS 26-22	Gold	1	7/15/10	12	1.376	257.94	250.99	600	0.021
PS 26-22	Black	2	7/15/10	15	10.475	207.98	189.76	600	0.007
FS 26-9	Black	1	7/15/10	15	6.520	261.75	252.87	600	0.006
PS 6210-29	White	2	7/15/10	8	0.681	206.68	196.45	600	0.063
FS 26-9	White	1	7/15/10	8	3.426	259.38	248.76	600	0.013
PS 6210-29	Gold	2	7/15/10	12	2.889	204.31	194.83	600	0.014
FS 29-9	Gold	1	7/15/10	12	1.371	257.42	251.25	600	0.019
PS 6210-29	Black	2	7/15/10	15	7.023	203.23	181.14	600	0.013
FS 26-11	White	1	7/15/10	8	1.471	259.70	239.18	600	0.058
FS 26-11	Clear	2	7/15/10	0.5	0.831	203.13	192.46	600	0.053
FS 26-11	Silver	1	7/15/10	3	1.785	271.06	235.84	600	0.082
FS 26-11	Red	1	7/15/10	1.5	2.289	257.29	226.72	600	0.056
FS 26-11	Gold	2	7/15/10	12	1.861	201.62	190.41	600	0.025
PS 6210-10	Silver	1	7/15/10	3	0.839	255.97	245.35	600	0.053
FS 26-11	Blue	2	7/15/10	5	8.968	200.32	178.34	600	0.010
PS 6210-10	Red	1	7/15/10	1.5	0.167	252.04	250.59	600	0.036
PS 6210-10	Clear	2	7/15/10	0.5	0.143	197.95	195.48	600	0.072
PS 6210-10	White	1	7/15/10	8	1.625	255.71	236.95	600	0.048
PS 6210-10	Black	2	7/15/10	15	3.064	201.51	194.72	600	0.009
PS 6210-37	Blue	1	7/15/10	5	1.555	255.05	241.41	600	0.037
PS 6210-37	Clear	2	7/15/10	0.5	0.165	200.65	194.94	600	0.144
PS 6210-37	Silver	1	7/15/10	3	0.553	271.20	261.51	600	0.073
PS 6210-37	Black	2	7/15/10	15	2.637	287.20	272.92	600	0.023
PS 6210-37	Red	1	7/15/10	1.5	0.475	216.77	247.24	600	-0.267
PS 6210-37	White	2	7/15/10	8	0.731	290.19	274.28	600	0.091
PS 26-17	Gold	1	7/15/10	12	5.387	268.58	244.10	600	0.019
PS 26-17	Black	2	7/15/10	15	6.080	286.79	274.28	600	0.009
PS 26-17	Silver	1	7/15/10	3	0.630	257.71	245.19	600	0.083
PS 26-17	Red	2	7/15/10	1.5	0.454	286.38	277.00	600	0.086
PS 26-17	Clear	1	7/15/10	0.5	0.080	261.51	253.26	600	0.430
FS 6210-34	Blue	2	7/15/10	5	1.107	287.33	259.73	600	0.104
FS 6210-33	White	1	7/15/10	8	1.481	263.35	251.09	600	0.034

Table A-5 (cont.)

Sample plant	Color	Channel	Sample day	Age (days)	Dry weight (mg)	Manual O2 in	O2 out	Time	Respiration rate (nmol/ mg/sec)
FS 6210-33	Black	2	7/15/10	15	6.089	281.35	238.92	578	0.030
FS 26-20	White	1	7/15/10	8	1.548	261.51	237.29	600	0.065
FS 26-20	Red	2	7/15/10	1.5	0.359	296.58	271.29	600	0.294
FS 26-20	Gold	1	7/15/10	12	1.821	258.37	239.90	600	0.042
FS 26-20	Black	2	7/15/10	15	3.213	301.34	284.48	600	0.022
FS 6210-34	Black	1	7/15/10	15	2.989	257.32	293.72	600	-0.051
FS 6210-34	Red	2	7/15/10	1.5	0.148	293.72	243.96	600	1.401
PS 6210-24	White	1	7/15/10	8	3.138	257.19	232.70	600	0.033
FS 6210-34	Gold	2	7/15/10	12	3.743	290.73	266.93	600	0.026
PS 26-40	Silver	1	7/15/10	3	0.879	268.85	260.73	600	0.038
FS 6210-14	Black	2	7/15/10	15	8.660	274.14	231.85	600	0.020
PS 26-40	Gold	1	7/15/10	12	4.073	246.85	229.30	600	0.018
PS 26-40	Black	2	7/15/10	15	0.612	275.50	266.93	600	0.058
PS 26-40	White	1	7/15/10	8	1.532	251.04	233.36	600	0.048
FS 26-20	Clear	2	7/15/10	0.5	0.290	287.33	279.04	600	0.119
FS 26-20	Blue	1	7/15/10	5	0.646	248.55	234.80	600	0.089
PS 6210-21	White	1	7/15/10	8	-	249.60	241.48	600	-
PS 6210-21	Gold	2	7/15/10	12	1.890	280.94	261.49	600	0.043
PS 6210-21	Silver	1	7/15/10	3	1.667	246.58	229.17	600	0.044
PS 6210-21	Black	2	7/15/10	15	2.449	269.52	262.58	600	0.012
FS 6210-17	White	1	7/15/10	8	2.540	245.01	221.31	600	0.039
FS 6210-17	Gold	2	7/15/10	12	6.166	263.67	222.62	600	0.028
FS 6210-17	Black	1	7/15/10	15	4.478	251.95	235.19	600	0.016
FS 26-4	Clear	2	7/15/10	0.5	0.076	266.66	259.59	600	0.388
FS 26-4	White	1	7/15/10	8	1.127	247.89	239.51	600	0.031
FS 26-4	Gold	2	7/15/10	12	4.799	270.74	245.31	600	0.022
PS 6210-21	Clear	1	7/15/10	0.5	0.353	247.11	240.95	600	0.073
FS 6210-14	Blue	2	7/15/10	5	1.052	268.43	241.37	600	0.107
FS 26-4	Red	1	7/15/10	1.5	0.258	248.94	250.12	600	-0.019
FS 26-4	Black	2	7/15/10	15	6.455	271.15	253.20	600	0.012
FS 6210-14	Gold	1	7/15/10	12	2.001	245.67	231.92	600	0.029
FS 6210-14	Blue	2	7/15/10	5	1.052	271.15	252.93	600	0.072
FS 6210-14	White	1	7/15/10	8	0.763	259.94	249.33	600	0.058
FS 6210-34	Silver	2	7/15/10	3	0.218	268.02	262.72	600	0.101
PS 26-6	Gold	1	7/15/10	12	2.994	251.30	224.85	600	0.037
PS 26-6	Black	2	7/15/10	15	17.719	265.85	192.14	600	0.017
FS 6210-33	Gold	1	7/15/10	12	1.359	252.48	233.75	600	0.057
FS 26-20	Blue	2	7/15/10	5	2.375	266.39	250.21	600	0.028
FS 6210-33	Red	1	7/15/10	1.5	0.402	247.76	247.37	600	0.004
FS 6210-33	Blue	2	7/15/10	5	0.319	260.95	255.92	600	0.066
PS 6210-19	Green	1	7/16/10	1.5	0.596	300.42	292.08	600	0.058
PS 6210-19	Clear	2	7/16/10	0.5	0.071	317.20	309.28	600	0.465

Table A-5 (cont.)

Sample plant	Color	Channel	Sample day	Age (days)	Dry weight (mg)	Manual O2 in	O2 out	Time	Respiration rate (nmol/ mg/sec)
PS 6210-19	Red	1	7/16/10	2.5	0.672	300.00	291.24	600	0.054
PS 6210-19	Black	2	7/16/10	16	10.987	307.66	264.03	600	0.017
PS 6210-19	Blue	1	7/16/10	6	4.159	284.43	260.37	600	0.024
PS 6210-19	Gold	2	7/16/10	13	2.632	304.75	281.16	600	0.037
PS 6210-19	White	1	7/16/10	9	1.012	295.97	288.60	600	0.030
PS 6210-19	Silver	2	7/16/10	4	1.043	298.61	285.68	600	0.052
PS 26-32	Clear	1	7/16/10	0.5	1.596	295.35	271.91	600	0.061
PS 26-32	Green	2	7/16/10	1.5	4.647	311.38	271.47	600	0.036
PS 26-32	White	1	7/16/10	9	1.718	286.10	273.02	600	0.032
PS 26-32	Red	2	7/16/10	2.5	2.210	308.79	283.91	600	0.047
PS 26-32	Silver	1	7/16/10	4	6.023	295.28	274.41	600	0.014
PS 26-32	Gold	2	7/16/10	13	4.550	326.89	282.20	600	0.041
PS 26-32	Blue	1	7/16/10	6	4.575	295.14	289.02	600	0.006
PS 26-32	Black	2	7/16/10	16	9.754	322.21	293.12	600	0.012
PS 6210-2	Black	1	7/16/10	16	4.773	295.28	260.92	600	0.030
PS 6210-2	White	2	7/16/10	9	1.100	315.10	301.20	600	0.053
FS 26-35	Black	1	7/16/10	16	6.300	293.88	277.06	600	0.011
PS 6210-2	Gold	2	7/16/10	13	2.092	318.97	295.22	600	0.047
FS 26-29	White	1	7/16/10	9	0.834	294.44	284.01	600	0.052
FS 26-29	Black	2	7/16/10	16	5.587	321.07	292.15	600	0.022
PS 26-21	Green	1	7/16/10	1.5	1.038	292.91	270.10	600	0.092
FS 26-29	Gold	2	7/16/10	13	1.894	320.43	292.96	600	0.060
PS 26-21	Black	1	7/16/10	16	6.103	281.92	270.10	600	0.008
PS 26-21	Gold	2	7/16/10	13	11.865	324.47	290.37	600	0.012
PS 26-21	Blue	1	7/16/10	6	3.104	299.87	254.80	600	0.060
PS 26-21	White	2	7/16/10	9	2.202	332.06	296.35	600	0.068
FS 6210-25	Blue	1	7/16/10	6	1.253	286.37	273.82	600	0.042
PS 26-21	Silver	2	7/16/10	4	1.116	322.37	307.82	600	0.054
FS 6210-25	Red	1	7/16/10	2.5	0.445	285.54	279.00	600	0.061
FS 6210-25	Gold	2	7/16/10	13	3.374	314.77	285.36	600	0.036
FS 6210-25	White	1	7/16/10	9	4.317	274.27	238.67	600	0.034
FS 6210-25	Silver	2	7/16/10	4	2.612	303.78	248.52	600	0.088
FS 6210-25	Black	1	7/16/10	16	3.821	278.31	261.62	600	0.018
PS 6210-20	Green	2	7/16/10	1.5	0.188	313.64	309.28	600	0.097
PS 6210-20	Gold	1	7/16/10	13	16.861	276.22	195.27	600	0.020
PS 6210-20	Silver	2	7/16/10	4	1.741	312.83	285.52	600	0.065
PS 6210-20	Blue	1	7/16/10	6	1.412	279.00	270.52	600	0.025
PS 6210-20	White	2	7/16/10	9	13.917	310.09	175.32	600	0.040
PS 6210-20	Black	1	7/16/10	16	8.211	298.89	259.25	600	0.020
PS 6210-20	Red	2	7/16/10	2.5	0.075	312.19	311.70	600	0.027
FS 26-8	Silver	1	7/16/10	4	-	288.04	273.02	600	-
FS 26-17	Black	2	7/16/10	16	25.900	315.58	280.35	600	0.006

Table A-5 (cont.)

Sample plant	Color	Channel	Sample day	Age (days)	Dry weight (mg)	Manual O2 in	O2 out	Time	Respiration rate (nmol/ mg/sec)
FS 26-17	Gold	1	7/16/10	13	1.881	275.11	271.35	600	0.008
FS 26-17	Green	2	7/16/10	1.5	4.609	297.64	242.70	600	0.050
FS 26-17	Red	1	7/16/10	2.5	4.289	278.17	253.69	600	0.024
FS 26-17	White	2	7/16/10	9	1.187	306.37	298.13	600	0.029
FS 26-17	Silver	1	7/16/10	4	6.068	268.29	243.68	600	0.017
FS 26-8	Black	2	7/16/10	16	5.802	312.51	280.68	600	0.023
FS 6210-18	Black	1	7/16/10	16	9.840	271.35	247.57	600	0.010
FS 26-8	White	2	7/16/10	9	0.468	302.81	291.67	600	0.099
FS 6210-18	White	1	7/16/10	9	2.816	269.13	241.03	600	0.042
FS 6210-18	Silver	2	7/16/10	4	0.481	299.26	289.56	600	0.084
FS 6210-18	Red	1	7/16/10	2.5	0.394	268.57	263.56	600	0.053
FS 6210-18	Gold	2	7/16/10	13	0.809	297.97	288.11	600	0.051
FS 6210-5	Red	1	7/16/10	2.5	0.715	270.38	255.78	600	0.085
FS 6210-5	Silver	2	7/16/10	4	1.660	296.67	269.54	600	0.068
FS 6210-5	Blue	1	7/16/10	6	3.101	270.10	248.68	600	0.029
FS 6210-5	White	2	7/16/10	9	2.050	275.83	236.73	600	0.079
FS 6210-5	Gold	1	7/16/10	13	4.504	248.82	212.80	600	0.033
PS 26-3	White	2	7/16/10	9	0.380	275.02	267.75	600	0.080
PS 6210-24	Blue	1	7/16/10	6	1.386	247.01	228.52	600	0.056
PS 26-3	Blue	2	7/16/10	6	2.036	272.76	227.96	600	0.092
FS 6210-3	Green	1	7/16/10	1.5	0.735	246.18	235.19	600	0.062
FS 6210-3	Silver	2	7/16/10	4	1.084	274.86	261.77	600	0.050
PS 6210-9	Silver	1	7/16/10	4	1.679	244.09	230.18	600	0.035
PS 6210-9	Green	2	7/16/10	1.5	0.640	240.44	234.79	600	0.037
FS 6210-3	Blue	1	7/16/10	6	1.540	241.87	223.09	600	0.051
PS 26-10	Green	1	7/16/10	1.5	0.399	246.04	237.69	600	0.087
PS 26-10	Gold	1	7/16/10	13	3.652	247.99	224.62	600	0.027
PS 26-10	Silver	2	7/16/10	4	2.193	244.31	221.24	600	0.044
PS 26-10	Red	1	7/16/10	2.5	0.393	244.51	240.48	600	0.043
FS 6210-24	Green	2	7/16/10	1.5	0.908	237.33	230.50	600	0.031
FS 26-11	Black	2	7/15/10	15	9.439	189.44	160.35	600	0.013
PS 26-17	Blue	1	7/15/10	5	0.992	258.76	242.26	600	0.069
PS 26-17	White	2	7/15/10	8	6.348	283.25	239.33	600	0.029

Table A-6: Respiration of CG.6210 and M.26 roots by age, August 2010

Sample plant	Color	Channel	Sample day	Age	Dry weight (mg)	Manual O2 in	O2 out	Time	Respiration rate (nol/mg/sec)
FS 6210-20	Black	1	8/25/10	15	31.937	228.24	184.66	600	0.006
FS 6210-20	White	1	8/25/10	3	0.834	263.13	247.27	600	0.079
FS 6210-20	Green	1	8/25/10	6.5	0.377	267.27	257.48	600	0.108
FS 6210-20	Blue	1	8/25/10	8.5	0.284	252.79	250.58	600	0.032
FS 6210-20	Red	1	8/25/10	4.5	0.172	256.1	253.48	600	0.063
PS 26-1	Green Circle	1	8/25/10	1.5	0.149	260.1	253.06	600	0.197
PS 26-1	Yellow	2	8/25/10	5.5	0.189	335.19	310.74	600	0.539
PS 26-1	Black	1	8/25/10	15	5.763	227.27	190.87	600	0.026
PS 26-1	Silver	2	8/25/10	12.5	0.667	319.39	296.45	600	0.143
PS 26-1	Blue	1	8/25/10	8.5	0.182	246.99	276.88	600	-0.684
PS 6210-8	White	2	8/25/10	3	2.908	300.96	243.68	600	0.082
PS 6210-8	Green	1	8/25/10	6.5	2.89	247.68	215	600	0.047
PS 6210-8	Blue	2	8/25/10	8.5	2.052	304.35	289.3	600	0.031
PS 6210-8	Green Circle	1	8/25/10	1.5	0.545	240.38	229.76	600	0.081
PS 6210-8	Black	1	8/25/10	15	8.542	243.82	233.76	600	0.005
PS 6210-8	Red	2	8/25/10	4.5	-	243.27	235.88	600	-
PS 6210-8	Silver	1	8/25/10	12.5	0.393	243.82	241.62	600	0.023
PS 6210-8	Clear	2	8/25/10	0.5	0.481	215.29	202.05	600	0.115
FS 26-6	Black	1	8/25/10	15	11.398	241.25	199	600	0.015
FS 26-6	Silver	2	8/25/10	12.5	1.174	211	196.35	600	0.052
FS 6210-26	White	1	8/25/10	3	4.696	240.24	203.97	600	0.032
FS 6210-26	Silver	2	8/25/10	12.5	10.186	199.48	166.75	600	0.013
FS 6210-26	Black	1	8/25/10	15	13.856	242.03	217.07	600	0.008
FS 6210-26	Green Circle	2	8/25/10	1.5	0.722	193.41	188.63	600	0.028
FS 26-3	White	1	8/25/10	3	1.541	237.89	211.83	600	0.070
FS 26-3	Green	2	8/25/10	6.5	-	188.81	161.97	600	-
FS 26-3	Black	1	8/25/10	15	10.858	240.24	221.76	600	0.007
FS 26-3	Silver	2	8/25/10	12.5	7.867	174.29	137.89	600	0.019
FS 26-3	Blue	1	8/25/10	8.5	0.124	237.48	234.72	600	0.093
PS 26-14	Silver	2	8/25/10	12.5	0.574	167.12	164.36	600	0.020
PS 6210-3	Black	1	8/25/10	15	2.005	235.69	220.93	600	0.031
PS 26-14	Blue	2	8/25/10	8.5	0.387	173.37	168.77	600	0.050
PS 6210-3	Green	1	8/25/10	6.5	0.135	238.17	236.51	600	0.051
PS 6210-3	Blue	2	8/25/10	8.5	0.413	170.24	164.54	600	0.058
PS 6210-3	White	1	8/25/10	3	0.126	236.24	234.03	600	0.073
FS 26-14	Black	2	8/25/10	15	5.384	163.62	152.04	600	0.009
PS 6210-3	Silver	1	8/25/10	12.5	0.377	236.51	233.48	600	0.033
PS 6210-3	Yellow	2	8/25/10	5.5	0.6	162.34	157.93	600	0.031
FS 6210-30	Black	1	8/25/10	15	7.605	237.62	226.03	600	0.006

Table A-6 (cont.)

Sample plant	Color	Channel	Sample day	Age	Dry weight (mg)	Manual O2 in	O2 out	Time	Respiration rate (nol/mg/sec)
FS 6210-30	Blue	2	8/25/10	8.5	2.615	161.23	140.83	600	0.033
FS 6210-30	White	1	8/25/10	3	2.264	235	190.73	600	0.081
FS 6210-30	Green Circle	2	8/25/10	1.5	0.049	155.35	152.59	600	0.235
FS 6210-30	Silver	1	8/25/10	12.5	2.016	233.62	212.79	600	0.043
FS 6210-30	Green	2	8/25/10	6.5	1.032	155.54	148.92	600	0.027
PS 26-37	Green	1	8/25/10	6.5	0.764	236.38	229.62	600	0.037
PS 26-37	Black	2	8/25/10	15	4.109	153.7	152.59	600	0.001
PS 26-37	White	1	8/25/10	3	2.727	236.51	210.59	600	0.040
PS 26-37	Black	2	8/25/10	15	4.109	157.37	152.59	600	0.005
PS 26-39	Green	1	8/25/10	6.5	3.134	234.45	204.93	600	0.039
PS 26-39	White	2	8/25/10	3	3.035	154.43	145.61	600	0.012
PS 26-39	Black	1	8/25/10	15	1.773	233.62	228.38	600	0.012
FS 26-30	White	2	8/25/10	3	0.189	152.78	151.49	600	0.028
FS 26-30	Black	1	8/25/10	15	11.957	227.55	214.86	600	0.004
FS 26-30	Silver	2	8/25/10	12.5	1.013	156.45	152.23	600	0.017
PS 6210-32	Clear	1	8/25/10	0.5	0.113	234.86	233.2	600	0.061
FS 26-30	Green Circle	2	8/25/10	1.5	0.162	156.64	152.23	600	0.113
PS 6210-32	Green Circle	1	8/25/10	1.5	0.198	234.17	231.69	600	0.052
PS 6210-32	Silver	2	8/25/10	12.5	5.9	155.54	127.04	600	0.020
PS 6210-32	White	1	8/25/10	3	0.39	233.34	222.17	600	0.119
PS 6210-32	Black	2	8/25/10	15	4.645	166.93	159.95	600	0.006
PS 6210-32	Blue	1	8/25/10	8.5	3.051	230.86	199.28	600	0.043
FS 6210-36	Silver	2	8/25/10	12.5	0.175	164.91	160.68	600	0.101
PS 6210-36	Silver	1	8/25/10	12.5	8.63	235.41	185.76	600	0.024
FS 6210-36	Black	2	8/25/10	15	5.415	157.01	153.7	600	0.003
PS 6210-36	Blue	1	8/25/10	8.5	1.588	233.2	220.79	600	0.033
PS 6210-36	White	2	8/25/10	3	2.789	151.49	130.53	600	0.031
PS 6210-36	Clear	1	8/25/10	0.5	0.255	230.31	219.96	600	0.169
PS 6210-36	Green Circle	2	8/25/10	1.5	0.143	149.28	148	600	0.037
PS 6210-36	Black	1	8/25/10	15	2.421	234.86	225.89	600	0.015
PS 26-8	Blue	1	8/26/10	9.5	0.578	274.16	259.06	600	0.109
FS 6210-16	Blue	1	8/26/10	9.5	0.352	277.15	276.6	600	0.007
PS 26-8	Silver	2	8/26/10	13.5	6.006	266.3	234.36	600	0.022
PS 26-8	Green	1	8/26/10	7.5	0.097	284.22	286.8	600	-0.111
PS 6210-13	Red	2	8/26/10	5.5	0.182	262.36	259.97	600	0.055
FS 26-15	Blue	1	8/26/10	9.5	0.252	290.2	288.98	600	0.020
PS 26-2	Yellow	2	8/26/10	6.5	1.119	265.18	251.58	600	0.051

Table A-6 (cont.)

Sample plant	Color	Channel	Sample day	Age	Dry weight (mg)	Manual O2 in	O2 out	Time	Respiration rate (nol/mg/sec)
PS 26-2	Silver	1	8/26/10	13.5	1.32	294.96	281.91	600	0.041
PS 6210-23	Blue	2	8/26/10	9.5	1.345	260.96	246.89	600	0.044
PS 6210-23	Silver	1	8/26/10	13.5	5.688	291.84	259.33	600	0.024
FS 6210-28	Blue	2	8/26/10	9.5	2.264	264.63	230.57	600	0.063
FS 6210-28	Yellow Circle	1	8/26/10	1.5	0.777	286.13	266.68	600	0.104
FS 6210-28	Green Circle	2	8/26/10	2.5	0.637	260.53	235.49	600	0.164
FS 6210-28	White	1	8/26/10	4	4.86	289.39	272.8	600	0.014
FS 6210-28	Silver	2	8/26/10	13.5	4.947	260.54	200.04	600	0.051
FS 26-19	Blue	1	8/26/10	9.5	0.771	286.26	274.56	600	0.063
PS 26-33	White	2	8/26/10	4	2.304	250.26	223.16	600	0.049
PS 6210-35	Green	1	8/26/10	7.5	0.485	288.03	281.77	600	0.054

Table A-7: Elemental analysis of CG.6210 and M.26 roots by age

Well	Plant	Age group	Age (days)	Harvest month	Weight	%tN	%C	%S	CN ratio
A1	FS 6210-5	2	5-11	July	4.31	1.15	45.07	0.30	39.05
A2	FS 6210-5	3	12+	July	4.03	0.94	44.44	0.27	47.39
A3	FS 6210-14	3	12+	July	10.17	0.99	40.76	0.16	41.30
A4	FS 6210-17	3	12+	July	7.62	1.02	41.47	0.17	40.69
A5	FS 6210-18	3	12+	July	8.16	1.04	40.45	0.15	38.86
A6	FS 6210-24	2	5-11	July	3.80	1.01	39.43	0.24	39.21
A7	FS 6210-25	3	12+	July	5.54	0.99	37.22	0.21	37.59
A8	FS 6210-33	3	12+	July	6.12	1.18	40.33	0.22	34.19
A9	FS 6210-34	3	12+	July	5.54	1.20	40.85	0.20	33.98
A10	FS 26-9	2	5-11	July	2.97	1.71	42.37	0.27	24.74
A11	FS 26-9	3	12+	July	6.40	1.15	45.44	0.17	39.53
B1	FS 26-11	2	5-11	July	9.97	1.91	42.47	0.23	22.23
B2	FS 26-11	3	12+	July	11.13	1.56	45.55	0.19	29.11
B3	FS 26-17	1	0-4	July	13.75	1.64	33.15	0.13	20.17
B4	FS 26-17	3	12+	July	24.37	0.93	37.15	0.12	40.16
B5	FS 26-20	3	12+	July	4.19	1.34	35.04	0.20	26.12
B6	FS 26-29	3	12+	July	6.21	1.33	39.21	0.21	29.49
B7	FS 26-35	3	12+	July	5.22	1.04	37.13	0.21	35.64
B8	PS 26-6	3	12+	July	17.89	1.87	36.38	0.18	19.46
B9	PS 26-17	2	5-11	July	6.76	0.88	42.13	0.20	47.71
B10	PS 26-17	3	12+	July	9.95	1.10	40.06	0.15	36.43
B11	PS 26-21	2	5-11	July	4.32	1.06	42.44	0.19	40.21
B12	PS 26-21	3	12+	July	15.82	0.96	42.13	0.15	44.04
C1	PS 26-32	1	0-4	July	14.25	1.79	40.82	0.21	22.84
C2	PS 26-32	2	5-11	July	5.88	1.47	33.93	0.18	23.13
C3	PS 26-32	3	12+	July	6.71	1.33	40.64	0.17	30.47
C4	PS 26-40	3	12+	July	3.93	0.76	42.56	0.19	56.04
C5	PS 6210-1	3	12+	July	10.00	1.12	37.03	0.15	32.96
C6	PS 6210-2	3	12+	July	5.58	0.94	42.01	0.17	44.90
C7	PS 6210-9	2	5-11	July	3.56	1.07	35.25	0.19	33.09
C8	PS 6210-19	2	5-11	July	4.34	1.04	39.59	0.21	38.22
C9	PS 6210-20	2	5-11	July	13.88	0.98	38.20	0.13	39.16
C10	PS 6210-20	3	12+	July	22.51	1.09	40.36	0.12	37.08
C11	PS 6210-21	3	12+	July	3.95	1.09	38.28	0.16	35.18
C12	PS 6210-24	2	5-11	July	3.44	0.86	35.42	0.21	41.36
D1	PS 6210-24	3	12+	July	3.59	0.49	30.34	0.14	61.95
D2	PS 6210-29	3	12+	July	8.31	1.41	37.55	0.19	26.62
D3	PS 26-22	3	12+	July	4.05	1.54	43.84	0.18	28.41
D4	FS 6210-11,18,3	1	0-4	July	3.05	2.84	42.65	0.27	15.03
G9	FS 6210-26	3	12+	July	21.51	0.77	44.18	0.15	57.60
D5	FS 6210-34,33,5	1	0-4	July	2.30	1.88	45.24	0.29	24.02
D6	FS 6210-14,11	2	5-11	July	2.34	1.20	42.88	0.27	35.67
D7	FS 6210-25,18	2	5-11	July	3.52	1.14	37.68	0.24	32.96
D8	FS 6210-25,24	1	0-4	July	3.56	1.51	41.37	0.23	27.38
D9	FS 6210-33,34	2	5-11	July	2.89	1.12	41.91	0.25	37.52
D10	FS 26-20,8,4	1	0-4	July	0.70	1.68	45.48	0.80	27.03

Table A-7 (cont.)

Well	Plant	Age group	Age (days)	Harvest month	Weight	%tN	%C	%S	CN ratio
D11	FS 26-29,17,8,4	2	5-11	July	3.41	1.34	43.85	0.23	32.82
D12	FS M26-20	2	5-11	July	3.97	1.46	39.74	0.21	27.29
E1	PS M26-17,10	1	0-4	July	1.88	1.99	45.05	0.29	22.61
E2	PS M26-10	3	12+	July	3.10	1.18	42.57	0.24	36.03
E3	PS 6210-1,19	1	0-4	July	2.15	1.59	43.78	0.35	27.49
E4	PS 6210-1,10	2	5-11	July	3.30	1.45	40.35	0.24	27.83
E5	PS M26-40,3	2	5-11	July	3.88	1.09	39.45	0.20	36.07
E6	PS 6210-20,10	1	0-4	July	2.94	1.48	42.05	0.32	28.50
E7	PS 6210-37,10	3	12+	July	4.57	0.97	40.95	0.16	42.02
E8	PS 6210-37,21	1	0-4	July	2.25	1.66	38.29	0.28	23.10
E9	PS 6210-37,29,2	2	5-11	July	3.66	1.32	43.72	0.27	33.03
E10	FS M26-4	3	12+	July	4.30	1.18	41.15	0.15	34.97
E11	FS 6210-17,3	2	5-11	July	3.32	1.11	38.01	0.20	34.12
F1	PS M26-1,39	1	0-4	August	2.97	1.74	31.36	0.21	18.04
F2	PS M26-1	3	12+	August	5.68	1.75	42.00	0.25	24.04
F3	PS M26-8	3	12+	August	5.09	1.82	40.02	0.18	22.01
F4	PS M26-33,37	1	0-4	August	4.51	1.68	33.21	0.18	19.79
F5	PS M26-1,2,14,37	2	5-11	August	2.15	1.55	40.39	0.26	26.07
F6	PS M26-37	3	12+	August	3.09	0.71	30.20	0.17	42.67
F7	PS M26-39,8	2	5-11	August	4.06	3.35	39.39	0.28	11.75
F8	PS M26-2,14,33,39	3	12+	August	4.21	1.16	36.19	0.19	31.24
F9	PS 6210-8	1	0-4	August	3.40	1.96	41.25	0.27	21.09
F10	PS 6210-8	2	5-11	August	4.46	1.16	44.13	0.16	37.96
F11	PS 6210-8	3	12+	August	7.78	0.75	43.09	0.10	57.32
F12	PS 6210-23	1	0-4	August	5.37	0.80	20.08	0.11	25.14
G1	PS 6210-23	3	12+	August	4.85	0.95	41.21	0.14	43.58
G2	PS 6210-23,32	2	5-11	August	4.01	1.34	39.50	0.20	29.49
G3	PS 6210-32	3	12+	August	9.29	0.95	45.14	0.12	47.47
G4	PS 6210-3,32,36	1	0-4	August	3.79	1.83	44.14	0.18	24.15
G5	PS 6210-3	3	12+	August	2.07	0.80	42.77	0.24	53.24
G6	PS 6210-36	3	12+	August	9.93	0.81	38.74	0.14	47.61
G7*	FS 6210-20	3	12+	August	30.90	0.00	0.01	0.01	0.00
G8*	FS 6210-26	1	0-4	August	4.73	6.69	348.30	0.98	52.06
G10	FS 6210-28	1	0-4	August	6.00	1.28	42.20	0.22	32.94
G11	FS 6210-28	3	12+	August	4.55	1.82	43.44	0.22	23.93
G12	FS 6210-16,20,26	2	5-11	August	3.44	1.38	42.42	0.21	30.67
H1	PS 6210-3,35,36	2	5-11	August	4.21	0.90	35.15	0.12	39.02
H2	FS 6210-6,20,30	1	0-4	August	4.85	1.48	33.08	0.19	22.37
H3	FS 6210-28,30	2	5-11	August	5.51	1.68	46.47	0.21	27.73
H4	FS 6210-30	3	12+	August	9.28	0.85	42.43	0.13	50.04
H5	FS 6210-36	3	12+	August	5.16	0.71	38.06	0.13	53.84
H6	FS M26-3,30	1	0-4	August	1.75	1.56	44.14	0.27	28.29
H7	FS M26-2,3,15,19	2	5-11	August	1.17	1.39	44.27	0.44	31.80
H8	FS M26-6	3	12+	August	11.19	1.33	38.12	0.17	28.64
H9	FS M26-3	3	12+	August	17.62	1.10	44.26	0.17	40.06

Table A-7 (cont.)

Well	Plant	Age group	Age (days)	Harvest month	Weight	%tN	%C	%S	CN ratio
H10	FS M26-30	3	12+	August	12.53	1.04	45.70	0.12	43.97
H11	FS M26-14	3	12+	August	4.71	0.79	38.48	0.13	48.78
H12	FS M26-25	3	12+	August	4.28	0.94	38.68	0.21	41.16
A12	FS M26-11	1	0-4	August	4.45	2.96	42.33	0.46	14.31

- G7 and G8 did not run properly in autosampler

Table A-8: Isolates recovered from 1st and 2nd order root segments of M.26 and CG.6210 rootstocks. Taxonomic identity assigned based on greater than 95% sequence coverage and 97% sequence identity with GeneBank entries.

Isolate	M.26	CG.210
Alternaria	1	1
Apiospora sp.		1
Aureobasidium	1	
Ceptospheria		
Cladosporum	1	1
Cryptosporiopsis	3	
Epicoccum nigrum	1	
Fusarium oxysproum	1	
Fusarium solani		2
*Ilyonectria robusta (*anamorph*: Cylindrocarpon)*	1	
Ischnoderma benzoinum	1	
Lepotodontidium orchidicola		
Leptosphaerulina	1	
Mortierella elongata	1	
Myrmecridium schulzeri		1
Nectria	1	
Phaeosphaeriopsis		2
Phlebia tremellosa		1
Phoma radicina		1
Phytophthora cactorum	1	
Pythium attrantheridium	1	
Pythium macrosporum	1	
Pythium sylvaticum	2	5
Rhizoctonia AG-G	7	6
Schizophyllum commune		1
Tulasnella sp.		1
Xylariales	1	
Zalerion varium		1
Total	25	24

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