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Metabolic composition of apple rootstock rhizodeposits differs in a genotype-specific manner and affects growth of subsequent plantings

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ABSTRACT

Apple replant disease (ARD) negatively impacts apple tree health and reduces crop yield in new orchards established on sites previously grown to the same or related species. Use of tolerant rootstock genotypes can diminish the growth limiting effects of ARD, and while current research characterizes differential root gene expression by ARD tolerance among genotypes, the potential role of genotype-specific rhizodeposits contributing to ARD tolerance has not been intensively examined. A Q-TOF LC/MS metabolic profiling approach targeting phenolic compounds was used to characterize water-soluble phenolic rhizodeposit metabolites collected from water percolated through the rhizosphere of apple rootstocks planted in pasteurized quartz sand. Four rootstock genotypes (two with ARD field tolerance, G935 and G41, and two with ARD susceptibility, M9Nic29 and M26) differed in both rhizodeposit composition of metabolites and quantity over the course of time, with overall quantity of metabolites increasing as leaf area increased. Total metabolite quantity recovered did not differ with relative rootstock tolerance to ARD. Benzoic acid levels were consistently higher in rhizodeposits derived from G935, while rutin was higher in M26. Phloridzin and phloretin, two compounds previously examined in relation to apple root disease pathogenesis, were higher in the ARD-susceptible M9Nic29 at the inception of the experiment, but did not differentiate ARD tolerant from susceptible genotypes at later time assessments. Other untargeted compounds, identified by accurate mass, mass spectral features, and retention time, separated rootstocks according to ARD tolerance, but their chemical identity remains unconfirmed. Orchard soil treated with apple rhizodeposits had lower pH than soil collected from no-tree controls. Seedling growth in rhizodeposit treated soils differed according to rootstock genotype in a subsequent bioassay, but not according to expected replant tolerance. Differences in metabolite composition of rhizodeposits according to rootstock genotype, and temporal dynamics of their production during early stages of rootstock growth following dormancy, offer insight apple rootstock rhizodeposition, and provide the basis to further investigate their impact on soil chemistry, soil microbiology, and plant health.

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1. Introduction

Apple replant disease (ARD) is caused by a pathogen complex, which includes fungi (*Ilyonectria* spp. and *Rhizoctonia solani*), oomycetes (*Phytophthora* spp. and *Pythium* spp.), and plant parasitic nematodes (Mazzola, 1998). Field tolerance to ARD has been reported for certain apple rootstock genotypes (Fazio et al., 2006; Robinson et al., 2015), but specific genetic resistance mechanisms to individual pathogens, although recently proposed (Shin et al., 2016; Zhu et al., 2016), have not been fully elucidated. Furthermore, multitrophic interactions among multiple pathogens and rhizosphere microbial communities with as yet undefined rootstock genotypic preferences for specific environments and soil chemistries complicate full comprehension of disease etiology. Differences in rhizosphere microbial consortia recruited in a rootstock genotype-dependent manner may determine the severity of ARD development in subsequent orchard plantings (Rumberger et al., 2007; St. Laurent et al., 2010); however the specific rootstock attributes regulating composition of these rhizosphere communities have not been explored. An intriguing lead is the insight that genotype-specific root phenolic concentration in the fine distal roots may contribute to ARD tolerance (Emmett et al., 2014), but how this connects with rhizosphere microbiome derived suppression of replant organisms remains unknown.

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Compounds released by roots into the rhizosphere have been termed exudates or rhizodeposits, with exudate implying an active secretion (Weston et al., 2012). "Rhizodeposits" include all metabolites originating from the root that move into the surrounding soil, even passively, and the precise mechanism of release from the root is not strictly defined. Rhizodeposits can be released through active secretion or passive diffusion (Weston et al., 2012), or may emanate from root debris, i.e., root turnover (Leigh et al., 2002), and may include compounds sloughed off from root epidermis or actively growing root tips (McCully and Boyer, 1997). Rhizodeposits consist of a range of metabolites, from simple bicarbonate or hydrogen ions (Marschner and Romheld, 1983; Romheld et al., 1984; Shahbaz et al., 2006) to sugars, organic acids, and amino acids (Sandnes et al., 2005; Chaparro et al., 2013), as well as polymeric compounds such as tannins (Bekkara et al., 1998) and proteins (De-la-Pena and Vivanco, 2011). Metabolites produced by plant roots can have growth promoting or inhibitory effects on soil microbes (Osbourn, 1996; Broeckling et al., 2008), and estimates of photosynthetically derived rhizodeposits range from 5 to 40% of total fixed carbon (Marschner, 1995; Jones et al., 2009).

Rhizodeposition is a dynamic process that is influenced by microbial activity, which cycles carbon and nutrients back into the plant (Jones et al., 2009), in turn affecting rhizodeposition (Dessaux et al., 2016). Rhizodeposit composition and quantity may vary in a temporal manner and changes can correlate to functional gene expression by corresponding microbes (Chaparro et al., 2013). Other classes of compounds, specifically phenolics, which in Arabidopsis constitute 84% of the secondary metabolites exuded from the roots (Narasimhan et al., 2003), can inhibit microbial growth (Niro et al., 2016), and were purportedly an important determinant of the rhizobiome community composition (Chaparro et al., 2013). Specific classes of compounds, i.e., benzoxazinoids, can attract Pseudomonads to the rhizosphere (Neal et al., 2012). Benefits of both chemical changes due to rhizodeposit release as well as corresponding microbial activity to plants include nutrient sequestration and solubilization through changes in pH and H_2CO_3 (carbonic acid), with the intriguing feedback of microbe released CO_2 increase also having plant growth promotional effects (Glenn and Welker, 1997). Plant species (Hartmann et al., 2009) and genotype have demonstrated effects on the rhizosphere microbiome and may even influence genotypic composition of functional traits including antibiotic production (Mazzola et al., 2004). Apple rootstock genotype influences microbial community composition (St. Laurent et al., 2010), but the cultivar specific metabolic composition of rhizodeposits driving these differences has not been explored.

In apples, numerous research articles note that phloridzin, a dihydrochalcone glycoside, is a component of apple root exudates (Hoffman et al., 2009) and is present in high concentration within the roots (Emmett et al., 2014). Levels of phloridzin vary in response to pathogen infection of roots, although the quantity does not alter the level of pathogen damage to the root system (Hoffman et al., 2009). Hoffman et al. (2009) suggested phloridzin levels correlate positively with apple host susceptibility to pathogens in ARD, but other research indicates phloridzin's aglycone, phloretin, can suppress growth of plant pathogenic oomycetes or fungi (*Phytophthora capsici*, *Rhizocotonia solani* AG4, and others) (Shim et al., 2010). A variety of phenolic compounds have been detected in apple orchard soil (Jinshui et al., 2014), although root origin was not ascertained.

Our fundamental objective was to contrast and describe rhizodeposition in apple rootstocks with differing field tolerance to ARD using targeted and untargeted metabolic profiling approaches, and to then determine the effects of these rhizodeposits on soil chemistry (pH) and the next generation of trees grown in rhizodeposit treated soil. Targeted compounds included phenolic metabolites previously found in association with apple roots (detected in root extracts and orchard soil), and untargeted compounds included other metabolites compatible with a phenolic metabolite Q-TOF LC/MS analysis solvent system. The primary hypothesis was that the metabolic composition and quantity of rhizodeposits would differ among apple rootstock cultivars relative to ARD tolerance, and that specific metabolites potentially driving soil microbial community differences, including pathogens, could be defined. As the initial experimental results were considered, follow-up sub-hypotheses were also defined, specifically that subsequent tree growth in this soil would be affected by microbial and chemical changes induced by rhizodeposits, and that attributes of microbial populations found to be present in rhizodeposits corresponded to leaf area. A validation experiment using axenically propagated trees was also performed to affirm that rhizodeposits were of tree origin versus potential microbial origin when rhizodeposits were generated and collected in a non-sterile environment.

2. Material and methods

A primary experiment was performed twice followed by several validation experiments (Fig. 1). The main experimental goal was to determine the apple rootstock genotype specific composition and quantity of rhizodeposits and assess their impact on soil. The intent of the study was to gain insight into the relative effects of rhizodeposits from disease tolerant and susceptible rootstock genotypes on soil chemistry and biology. In response to results from the primary experiments, several sub-experiments or follow-up tests were conducted including 1) performing a bioassay assessing the impacts of rhizodeposits on growth of next-generation trees, 2) quantifying microbes in greenhouse rhizodeposits in tandem with measuring tree leaf area, and 3) validating the composition of tree-originating rhizodeposits obtained in greenhouse experiments with axenically grown micropropagated trees.

2.1. Rootstock selection

Four apple rootstocks (M9Nic29, M26, G41, G935) were selected on the basis of their relative field tolerance to ARD (Robinson et al., 2012), with M9Nic29 and M26 representing highly susceptible genotypes, while G41 and G935 exhibit superior performance in soil with a history of ARD, and specifically are less susceptible to *Pythium* spp. and *Pratylenchus* spp. in WA (Mazzola et al., 2009).

2.2. Greenhouse experiments

0.95 cm diameter (sold as 3/8 inch liners) dormant rootstocks (G.935, G.41, M.26, M.9Nic29 [Willow Drive Nursery, Ephrata, WA]) were planted in 30 mesh Lane Mountain Sand (Valley, WA) in D40 Deepots (25.4 cm [10 inch] long x 6.4 cm [2.5 inch] wide; Greenhouse Megastore, Danville, IL). Soil or sand was added to obtain a growth medium depth in the pot of 20.4 cm. Six trees were planted for each genotype in Experiment 1, and 8 trees for Experiment 2; further specifics to each experiment are detailed below. Sand was pasteurized at 80 °C for 8 h on two successive days prior to rootstock planting, with 12 h between each heating session. Pots were surface sterilized in a solution of 10% Clorox bleach (Oakland, CA, USA) [v/v in water] (active ingredient, 8.25% NaCLO) for 10 min and a piece of sterilized fabric mesh was inserted into the bottom of the pot to prevent sand or fine soil loss. Trees were rinsed clean of dirt and debris and roots were surface sterilized by submersion for 5 min in 10% bleach (as above) and rinsed with distilled water prior to planting. No additional nutrients were applied to the trees