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Influence of pesticide seed treatments on rhizosphere fungal and bacterial communities and leaf fungal endophyte communities in maize and soybean

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ABSTRACT

Insecticide and fungicide seed treatments are commonly used to control pests and pathogens in conventional maize and soybean culture, but little is known about their effects on the communities of non-target microbes that inhabit the rhizospheres or leaves of these crops. Because rhizosphere bacterial and fungal communities influence carbon and nutrient turnover, nutrient transformation, nutrient uptake and disease suppression, and because leaf endophyte fungal communities influence many aspects of stress tolerance in plants, any effect of a pesticide seed treatment on these microbial communities could have unintended and possibly adverse effects on seedling performance. We conducted a three-year field experiment in which maize (2013, 2015) and soybean (2014) were grown in rotation from seeds that were either coated or not coated with common pesticide treatments, which included contact and systemic fungicides and systemic insecticides. We sampled seedling rhizosphere soil (maize in 2013, soybean in 2014) and seedling leaves (soybean in 2014, maize in 2015) and characterized their microbial communities. For maize, the rhizosphere fungal and bacterial communities were significantly affected by the seed treatment, but leaf endophytic fungal communities were not. For soybean, the rhizosphere fungal community was significantly affected, as was the leaf endophytic fungal community, but not the rhizosphere bacterial community. These results show that pesticide seed treatments may affect rhizosphere soil microbial communities and endophytic leaf fungal communities more than one month after planting and, therefore, may have significant, unintended effects on non-target organisms. Additional research must determine the consequences of these effects and the nature of their context dependency.

1. Introduction

The seeds of agriculturally important plant species have been coated with various materials at least since the 1920s (Lambert et al., 1926). Seed treatments are now commonly used for maize (*Zea mays* L.), soybean (*Glycine max* L.) Merr., wheat (*Triticum aestivum* L.) and cotton (*Gossypium spp.* L.) (Jeschke et al., 2011; Douglas and Tooker, 2015) in order to deliver chemicals such as fungicides and insecticides that target pathogens and other pests of seeds or seedlings (Taylor and Harman, 1990).

While pesticides applied to seeds may enter the air during planting and have potentially negative consequences on above-ground insects (Hallmann et al., 2014; Krupke et al., 2012; Marzaro et al., 2011), most of the chemicals in seed coatings are delivered to the region of the soil in which the roots of the seedling develop (Thompson, 2010), the rhizosphere. While these pesticides are frequently effective against targeted, soil-borne pathogens and herbivores (Baird et al., 1994), they are not species-specific and, unfortunately, we currently know little about their effects on communities of non-target microorganisms.

Roots interact with complex communities of non-pathogenic fungi and bacteria, each member of which may benefit the plant in

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direct or indirect ways. Microorganisms that may be negatively impacted by pesticide seed treatments include those that are directly beneficial to plants such as those involved in nutrient mineralization, nutrient transformation, N-fixation, P-solubilization, plant nutrient uptake, and plant hormone production (Lugtenberg and Kamilova, 2009). Other potentially impacted microorganisms include those that indirectly improve crop vigor, such as competitors of pathogens or those that produce antibiotics (Weller, 1988; Mendes et al., 2011). Still others include those that promote the formation of beneficial symbioses (Hameeda et al., 2007).

Because most fungicides in seed treatments are systemic, it is possible for them to influence endophytic fungi. Complex communities of endophytic fungi occur in the living tissues of every plant species investigated thus far (Arnold et al., 2003; Arnold and Lutzoni, 2007; Rodriguez et al., 2009). Endophytic fungi have been shown to confer on their hosts increased resistance to herbivory (Carroll, 1988; Cheplick and Clay, 1988; Clay, 1987, 1988), disease (Carroll, 1988; Arnold et al., 2003), heat stress (Rodriguez et al., 2004; Márquez and Redman, 2007) and water stress (Clay and Schardl, 2002; Rodriguez et al., 2004; Peñuelas et al., 2012), as well as increased rates of photosynthesis, growth, reproduction and seed germination (Clay, 1987, 1988). In stressful environments endophytic fungi may even be essential for plant survival (Rodriguez et al., 2004).

Thus, while the net effect of pesticide seed treatments on crop vigor is frequently positive due to their impact on targeted pathogens, parasites and herbivores, unplanned adverse effects on beneficial microorganisms may attenuate their net benefit. We, therefore, documented the effects of commonly used pesticide seed treatments (mixtures of insecticides and fungicides applied prophylactically) on rhizosphere fungal and bacterial communities and leaf endophytic fungal communities of maize and soybean. Because beneficial microorganisms may exert impacts on plants via their effects on soil enzymes, which influence nutrient transformation, litter mineralization and nutrient uptake by roots, we also determined the influence of the seed treatments on rhizosphere enzyme activities, including an enzyme that influences P mineralization (acid phosphatase), one that influences N mineralization (β-N-acetylglucosaminidase), and one that influences C mineralization (1,4-β-cellobiohydrolase). We further documented the effects of the pesticide seed treatments on shoot N and P concentrations and shoot weight. Both maize and soybean form associations with arbuscular mycorrhizal fungi that may strongly influence the P economy of their hosts (Koide, 1991). A number of fungicides can significantly retard the development of mycorrhizal fungi (Menge, 1982) but it is unclear whether any fungicide delivered as a seed treatment significantly affects the colonization of roots by arbuscular mycorrhizal fungi. Therefore, we also documented the effect of seed treatment on colonization by mycorrhizal fungi.

2. Materials and methods

2.1. Site description

This study was conducted at the Pennsylvania State University R.A. Larson Agricultural Research Center at Rock Springs, PA, USA (40°43′ N, 77°55′ W, 350 m elevation). Soils at the site are shallow, well-drained lithic Hapludalfs formed from limestone residuum, and the dominant soil type is a Hagerstown silt loam (fine, mixed, semiactive, mesic Typic Hapludalf) (Braker, 1981). The soil is characterized by a silt loam surface texture and subsurface textures of silty clay loam and silty clay. In the five years preceding this study the field was planted with the following crops: no-till maize for grain in 2008 and 2009, no-till soybean in 2010, no-till spring oats in 2011, and barley and wheat crops in 2012. The field was plowed prior to planting in fall of 2011. For the purposes of this study, the field was divided into ten plots, each 3 m wide (encompassing four experimental crop rows) by 6 m long and each randomly assigned to one of the two treatments (treated or untreated seeds, see below). Plot treatment assignments were maintained throughout the 3-year duration of this study.

2.2. (maize) methods

On 26 April 2013 1520 g ha^{-1} glyphosate (in the form of the potassium salt) and 1400 g ha^{-1} dichlorophenoxyacetic acid (2,4-D) was applied for weed control. On 14 May 2013 the field was S-tined. On 15 May 2013 the field was disked and cultimulched. On 16 May 2013 the field was planted with maize hybrid TA510-18 (TA Seeds, Jersey Shore, PA, USA) in 76 cm-spaced rows at a seed density of 78,300 seeds ha^{-1}. Untreated seeds were planted in five randomly selected plots, and treated seeds were planted in the other five plots. The seed treatment was CruiserMaxx Corn 250 (Syngenta, Greensboro, NC, USA), which is a mixture of the systemic insecticide thiamethoxam (class neonicotinoid), the contact fungicide fludioxonil, and the systemic fungicides mefenoxam, azoxystrobin, and thiabendazole. The same maize genotype was used for both treatments. On 31 May 2013 the field was fertilized using urea with Agrotain® at the rate of 358 kg N ha^{-1}. On 20 June 2013 a post-emergence application of 1390 g ha^{-1} of glyphosate was made to control emerged weeds.

Two pooled rhizosphere soil samples were taken from each plot between 30 May and 3 June 2013, resulting in a total of ten pooled rhizosphere soil samples per treatment. For each pooled sample from each plot, 5 seedlings were randomly chosen and removed from the soil while attempting to recover as much of the root system as possible. The root system was then manually shaken to remove excess soil. The soil that remained adhering to the root system was considered rhizosphere soil. Rhizosphere soil was removed manually from the root system. Rhizosphere soil samples from each of the five seedlings were pooled and sealed in a plastic bag and stored overnight at 5 °C until they were used for enzyme analyses and for DNA extraction on the next day.

Following soil collection, root systems were separated from shoots, pooled by plot, and stored in 50% ethanol. Later, roots were cleared in 10% KOH (w:v), acidified in dilute HCl and stained in a solution of acetic acid and trypan blue (Sharda and Koide, 2008). Mycorrhizal colonization was quantified by line-intercept method as in Koide and Mooney (1987). Shoots were rinsed under distilled water and dried at 70 °C until they attained a constant weight.

The day after collection, three soil subsamples per plot were used for the analyses of acid phosphatase (Pase, EC 3.1.3.2), 1,4-β-cellobiohydrolase (CBase, EC 3.2.1.91) and β-N-acetylglucosaminidase (NAGase, EC 2.4.1.255) according to Peoples and Koide (2012). On the same day, genomic DNA was extracted from a fourth subsample of each soil sample. To extract genomic DNA, approximately 0.25 g of soil from each sample were placed in extraction tubes from the PowerSoil DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA). Extraction tubes were vortexed for a total of 15 min. All other steps were completed as outlined in the PowerSoil protocol. Extracted genomic DNA was then stored at −20C until amplification by PCR.

To amplify the bacterial and fungal genomic DNA, Sigma Jumpstart Taq (Sigma–Aldrich, St. Louis, MO, USA) was used. Fungal DNA was amplified using ITS1F[Hex] and ITS4 primers (Gardes and Bruns, 1993), while bacterial DNA was amplified using 1406F[FAM] and 23SR primers (Fisher and Triplett, 1999). The thermal cycling program used to amplify fungal DNA was: 30 cycles of 95 °C for 40 s, 49 °C for 30 s, and 72 °C for 48 s with final elongation at 72 °C for 7 min. The thermal cycling program used to