



Short communication

Evidence for indirect effects of pesticide seed treatments on weed seed banks in maize and soybean

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ABSTRACT

Use of pesticide seed treatments (coating seeds with insecticides and/or fungicides) is a common practice in conventional maize and soybean production. Despite their widespread use, however, little is known about how this practice affects soil food webs and their associated ecosystem services. We conducted a two-year field experiment in central Pennsylvania, USA in which identical genotypes of maize (2013) and soybean (2014) were planted with and without pesticide seed treatments in a completely randomized design with five replications. We sampled the weed seed bank during both the corn (fall 2013) and soybean (summer 2014) phases of the rotation. After each sampling event, soil samples were transported to a temperature-controlled greenhouse facility and weed seed bank composition and density was assessed over a period of four months via the direct germination method. Seed bank data were analyzed with ANOVA and several multivariate techniques to determine the effect of pesticide seed treatments on seed bank density, diversity, and species composition. We hypothesized that pesticide seed treatments reduce the abundance of natural enemies (e.g., soil-dwelling seed predators and pathogens) that damage or destroy weed seeds in the soil, and therefore seed banks in treated plots would be larger and less diverse than those in untreated plots. In accordance with our hypothesis, weed seed banks were significantly less diverse in treated compared to untreated control plots (Shannon and Simpson's indices of diversity, $p < 0.05$). While not statistically significant, differences in mean germinable weed seed bank density, richness, and evenness were also in the direction that we hypothesized. These data provide the first evidence that we are aware of that weed seed banks, and hence weed populations, may be indirectly affected by pesticide seed treatments. Additional research will be necessary to determine the generality of these responses and their underlying mechanisms.

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1. Introduction

Pesticide seed treatments (seed coatings containing pesticides, hereafter 'PST') are common in large acreage row crops such as maize, soybean, wheat, and cotton (Jeschke et al., 2011). These seed treatments often include both contact and systemic fungicide and systemic neonicotinoid insecticide active ingredients intended to protect crops from soil-borne pathogens and a broad range of soil-inhabiting insect pests. Recently, use of PST, and neonicotinoids in particular, has come under scrutiny due to growing concern over the potential for non-target effects (Hallmann et al., 2014; Krupke et al., 2012) and a dramatic rise in their use as a preemptive pest

management strategy (Douglas and Tooker, 2015). While recent data suggest that over 79% and 34% of hectares planted to maize and soybean, respectively, in the U.S. in 2011 were planted with neonicotinoid-treated seed (Douglas and Tooker, 2015), surprisingly little is known about how PST affect soil food webs and their associated ecosystem services.

One important ecosystem service provided by soil food webs is destruction of weed seeds in the soil seed bank. Soil-dwelling insects and soil-borne fungal pathogens are known to be important sources of weed seed and seedling mortality (Bohan et al., 2011; Chee-Sanford et al., 2006) and their activities can eliminate a substantial portion of the weed seed bank in as little as a year (Mohler, 2001). Given their important role in seed bank dynamics, maintenance and enhancement of soil natural enemy populations and their biological weed control services is seen as a critical component of sustainable weed management (Davis et al., 2008).

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Is it possible then, that PST, intended to protect crop seeds and seedlings from attack by insect and fungal pests, inadvertently also protect weed seeds in the soil seed bank from regulation by soil organisms that provide valuable biological weed control services?

There are at least three reasons to suspect that PST could have the potential to undermine biological control of the weed seed bank. First, cropping systems that rely on chemical pesticides, whether applied as PST or not, often have lower numbers of natural enemies of pests, including enemies of weed seeds, such as ground beetles (Coleoptera: Carabidae), than cropping systems that do not rely on such compounds (MacFadyen et al., 2009; Puech et al., 2014). Second, recent research has shown that neonicotinoid insecticides included in PST can have both direct and indirect negative effects on important components of the soil macrofauna community, including ground beetles (Douglas et al., 2015; Mullin et al., 2005). Finally, many of the causal agents of disease and seed decay in crops, including *Pythium ultimum* and *Fusarium* spp., also have been shown to contribute to disease and decay in seeds and seedlings of some agriculturally-important weed species (Gomez et al., 2014; Mohler et al., 2012), and these organisms are the intended targets of the fungicide component of many PST (Pedersen, 2007).

Here we report the results of a field experiment in which we grew maize and soybean in rotation with and without PST and measured the response of the germinable fraction of the soil weed seed bank community. We hypothesized that pesticide seed treatments reduce the abundance and/or activity of natural enemies (e.g., seed predators and pathogens) that damage or destroy weed seeds in the soil, and therefore seed banks in PST plots would be larger (i.e., higher density of germinable weed seeds) compared to those in untreated (control) plots. Additionally, we hypothesized that by compromising the ability of the natural enemy community to regulate seed survival of dominant weed species, PST would lead to seed bank communities that were less diverse than those in control plots.

2. Materials and methods

The experiment was conducted at The Pennsylvania State University's Russell E. Larson Agricultural Research Center near Rock Springs, PA (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). For several years before the experiment the site had been under a conventionally managed maize and soybean rotation, a practice representative of the region.

The experiment was established in spring 2013 and conducted over two growing seasons (2013–2014). The experiment involved ten experimental units (plots) each randomly assigned to one of two treatments: maize (2013) and soybean (2014) planted with PST (PST) or the identical genotype planted without PST (control). Each plot measured 6 m by 3 m. Treatment assignments to the plots were the same in both growing seasons; thus, the two-year study represented a typical maize–soybean rotation. In spring 2013, prior to planting maize, the field was tilled (chisel plow), disked, and cultimulched. Maize was planted on 16 May 2013 with a John Deere no-till planter. Soybean was no-till planted into the maize residue on 27 May 2014 with a John Deere no-till planter. Planting and management for both crops followed standard agronomic practices for the region. Maize (2013) and soybean (2014) were seeded with 76 cm row spacing at rates of 78,299 and 432,250 seeds ha⁻¹, respectively. Synthetic fertilizer (urea) was applied to maize and weed control in maize and soybean was achieved with herbicide (glyphosate and 2,4-D).

We used identical crop genotypes for control and PST treatments in both the maize and soybean growing seasons; therefore, the only difference between the control and PST treatments was whether or not the seeds were coated with PST. In 2013 (maize, hybrid TA51-18, TA Seeds, Jersey Shore, PA, USA) the PST was CruiserMaxx[®] Corn 250 (Syngenta Crop Protection, LLC, Greensboro, NC, USA). Cruiser Maxx[®] 250 contains the systemic neonicotinoid insecticide thiamethoxam, one contact fungicide (fludioxonil), and three systemic fungicides (mefenoxam, azoxystrobin, and thiabendazole) (Syngenta, 2010). In 2014 (soybean, TS2849R2S, TA Seeds), the PST was CruiserMaxx Beans[®] with Vibrance[™] (Syngenta Crop Protection, LLC, Greensboro, NC, USA) + Gaucho[®] (Bayer Crop Science LP, Research Triangle Park, NC, USA), which is a mixture of the two systemic neonicotinoid insecticides thiamethoxam and imidacloprid, and the fungicides fludioxonil, mefenoxam, and sedaxane. Each treatment (PST and control) was replicated five times.

2.1. Seed bank sampling

Soil seed banks were sampled once each season by taking soil cores (7 cm diameter) to a depth of 5 cm in early fall 2013 (maize) and late summer 2014 (soybean). These sampling times were chosen to maximize the time that the PST would potentially interact with the soil food web community, but occur before the timing of peak weed biomass and seed shed, which could potentially obscure the seed bank signal with fresh weed seed inputs. Each plot was sampled in four (2013) and three (2014) locations within the inner portion of each plot in order to minimize the potential for edge effects. After each sampling event, soil cores were transported to the University of New Hampshire MacFarlane Greenhouse Facility in Durham, NH and subjected to an assay of the germinable fraction of the weed seed bank via the direct germination method described in Smith and Gross (2006). Each soil core was spread on a flat containing soilless growth medium (Pro Mix[®], Premier Tech Ltd., Rivière-du-Loup, Quebec, Canada) and watered daily from above with a mist sprayer to keep the soil surface moist. Flats were monitored for germination approximately twice a week over a period of four months. Weed seedlings that emerged from the soil samples were identified and removed. Seedlings that could not be identified immediately were transplanted and grown until identification was possible. We employed a 'blind' census approach, in which flat labels contained the plot ID, but no treatment information, which minimized the chance that observer bias could inadvertently influence the data collection.

For each soil seed bank sample collected from the experiment we calculated the density (total seedlings emerged), species richness (number of species), evenness, and several indices of diversity. Data for each of the samples taken from a plot were averaged and plot means were used as the unit of replication ($n=5$). We chose to evaluate several different measures of weed seed bank diversity, as each combines information about both species richness and evenness but emphasize different components of community composition. The Shannon index (H) emphasizes the richness component of diversity, whereas Simpson's index of diversity (D) gives more weight to the more abundant species in a sample and therefore emphasizes the evenness component (Nagendra, 2002).

2.2. Data analyses

Seed bank density, richness, evenness, and diversity data were analyzed with repeated-measures ANOVA in SAS (Version 9.4, SAS Institute, Cary, NC) using the MIXED procedure. In all analyses, replicate was considered a random factor. To account for the

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