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Research Paper

Biodiversity analyses for risk assessment of genetically modified potato

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ABSTRACT

An environmental risk assessment for the introduction of genetically modified crops includes assessing the consequences for biodiversity. In this study arthropod biodiversity was measured using pitfall traps in potato agro-ecosystems in Ireland and The Netherlands over two years. We tested the impact of site, year, potato genotype, and fungicide management regime on arthropod community composition. Three potato genotypes were compared: the cultivar Désirée, susceptible to the late blight pathogen *Phytophthora infestans*, a genetically modified cisgenic clone of Désirée resistant to *P. infestans* and the cultivar Sarpo Mira, also resistant to late blight. We aimed to test several ways to measure biodiversity in the context of risk assessment by using both univariate biodiversity indices and multivariate ordination methods, categorizing the pitfall trap catch by taxonomic or functional category. The Shannon-Wiener and Simpson biodiversity indices both showed strong differences between sites, years and potato genotypes, but showed no effects of the fungicide management regime. The effect of genotype was due to cultivar differences between Désirée and Sarpo Mira rather than between the GM-event (A15-31) and its isogenic comparator Désirée. Multivariate permutation analyses and RDA ordination confirmed these findings and also showed interactions between year, site and either genotype or treatment. The added value of the multivariate analysis was that it provided information on the specific arthropod groups or taxa that contributed to community structure. Multivariate analyses are recommended for use as a sensitive method to compare functionally important arthropod groups driving community structure within the framework of environmental risk assessments, or for the process of indicator species selection.

1. Introduction

The value of biodiversity for ecosystem functioning has been demonstrated in many environments (Hooper et al., 2005; Reiss et al., 2009; Tilman et al., 2014). Greater biodiversity is commonly associated with higher agricultural yields, better biological control (Aquilino et al., 2005), more efficient land use, and many other ecosystem services (Swift and Anderson, 1994; Benayas et al., 2009; Tilman et al., 2014). Therefore, when it has been decided that environmental risks need to be assessed under field conditions (EFSA, 2010), monitoring of changes in biodiversity is essential.

Methods for assessing environmental risks of genetically modified (GM) crops are still being developed and debated (Johnson et al., 2007; Stirling, 2007; Todt and Luján, 2014; Devos et al., 2016). Although the guidance documents of the European Food Safety Authority (EFSA) emphasize the importance of conservation and protection of biodiversity in the European Union (EFSA, 2007, 2010), there are no uniform guidelines for assessing effects of GM crops on biodiversity. Quantifying biodiversity is a prerequisite for being able to reach set targets. The European biodiversity targets for 2020 aim to "halt biodiversity loss and the degradation of ecosystem services… [by] protecting and restoring biodiversity and associated ecosystem services, enhancing the positive contribution of agriculture and forestry and reducing key pressures on EU biodiversity, [thereby] stepping up the EU's contribution to global biodiversity" (European-Commission, 2011). While setting biodiversity targets is essential to make progress towards sustainability of agriculture, we are still hindered by the lack of consensus about how to measure biodiversity for environmental risk assessment (ERA). There are many ways to measure biodiversity (Magurran, 2004; Balvanera et al., 2006), all of which may provide different information about the species assemblages in a given community. We aim to compare several ways of measuring biodiversity, and to evaluate advantages and disadvantages of using these different measures in the context of risk assessment.

There are several guidance articles about choosing focal, indicator or surrogate species for ERA (Hilbeck et al., 2006, 2008, 2014; Arpaia, 2010; EFSA, 2010). There are also very concrete directives for setting limits of concern for endpoints (EFSA, 2010), which in practice can be

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interpreted as total counts of the individuals belonging to focal or indicator species. Single-species counts are unsuitable for representing community structure, nor do they take into account any trophic links between species or groups present. Single-variable biodiversity indices, however, were also deemed insufficient for accurately measuring effects on biodiversity (EFSA, 2010; Perry et al., 2009). For this reason, multivariate analyses are considered useful in the guidance documents (EFSA, 2010; Perry et al., 2009). In both cases, there is no concrete advice given to the manner in which biodiversity in the receiving environments of GM crops should be quantified, yet some recommendations have been recently published on this topic (Dolezel et al., 2017).

In 2012, the European Union commissioned the four-year project "Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems" (AMIGA) (Arpaia et al., 2014). This project made use of EFSA guidance documents for testing environmental impacts of GM crops (EFSA, 2007, 2010), and developed methodology and executed proof-of-concept studies for testing non-target effects of GM potato and maize. Non-target organisms (NTOs) are defined as "all those species directly and/or indirectly exposed to the GM plant, and which are not the targets of the newly expressed metabolite(s) in these plants" (Arpaia, 2010). For testing of GM potato modified for resistance to late blight (*Phytophthora infestans*), experimental fields were set up from which arthropod biodiversity data were collected. We have conducted field experiments with GM potatoes in Ireland and The Netherlands, two countries in the Atlantic biogeographic zone as defined by the European Commission (Pinborg and Larsson, 2016).

This study has two main aims: first, to investigate the importance of collection site and year in predicting arthropod biodiversity; second, to assess within each site how fungicide spraying regime (none, weekly or IPM 2.0 (Kessel et al., 2016)) and genotype (the susceptible potato cultivar Désirée, resistant GM Désirée (transformed with a gene conferring resistance to *P. infestans*), and the resistant non-GM cultivar Sarpo Mira) influence the assemblage of arthropods.

We analyzed biodiversity at the two sites using both univariate (linear mixed effects model) and multivariate analyses: Nonmetric Multidimentional Scaling (NMDS) and redundancy analysis (RDA) with permutation tests; either using taxonomic (family level) or functional grouping. We used relative diversity (total richness divided by total abundance); and two well-known biodiversity indices: Shannon-Wiener (H') and Simpson (1-D). The Shannon-Wiener index takes into account species richness and diversity such that unique species and higher evenness increase the value. The Simpson index indicates the chance that two random draws from a population represent individuals of the same type (in this case taxon or functional group), and subtraction from 1 ensures that the index increases with diversity. These different statistical approaches were evaluated for their sensitivity to reveal differences by comparing the significance of explanatory factors and comparing them for consistency of biological conclusions made from the results of each approach. We then discuss the feasibility of these approaches and usefulness of each grouping method in ecological risk assessment. We aim to provide advice for monitoring biodiversity as part of the risk assessment of genetically modified crops, and more generally for cases where biodiversity is deemed an important trait to be assessed.

2. Methods

2.1. Plant material and experimental design

Two potato cultivars and one GM event were used in the field trials: the highly susceptible cultivar Désirée, the highly resistant cultivar Sarpo Mira and the highly resistant Désirée-derived, cisgenically modified event A15-31 (detailed description in Haesaert et al. (2015), Haverkort et al. (2016)). Jacobsen and Schouten (2007) generated A15-31 through cisgenic modification of the Désirée cultivar through the transfer of an R-gene coding for resistance to *P. infestans: Rpi-Vnt1.1* (Pel et al., 2009), originally obtained from *Solanum venturii*. The GM event in this study was created at the Laboratory of Plant Breeding of Wageningen University and Research.

Table 1
Experimental field designs in The Netherlands and in Ireland.

The Netherlands		
Potato genotypes	3	Désirée, A15-31 and non-GM resistant cultivar: Sarpo Mira
Fungicide regimes	3	None, Weekly, IPM 2.0
Blocks	7	Total of 3 * 3 * 7 = 63 plots
Plot size	6 × 6 m	
Ireland		
Potato genotypes	3	Désirée, A15-31 and non-GM resistant cultivar: Sarpo Mira
Fungicide regimes	3	None, Weekly, IPM 2.0
Blocks	6	Total of 3 * 3 * 6 = 54 plots
Plot size	3 × 3 m	

Field trials were carried out in 2013 and 2014 in The Netherlands (Valthermond; GPS coordinates 52.871829846N and 6.942662896E) and in Ireland (Oak Park, Carlow; GPS coordinates 52.8560667N and 6.9121167W). These trials were carried out under permit IM10-006 for The Netherlands and in Ireland were licensed by the Environmental Protection Agency as per Notification No. B/IE/12/01. Valthermond has predominantly reclaimed peat soil (90.1% sand, 9.9% organic matter, pH = 5.1). The Oak Park campus in Carlow has a mix of light textured gravelly and heavy textured soils derived from limestone till, commonly known as boulder clay. At both sites, glyphosate was applied pre-planting. In Ireland weeding was done manually; in the Netherlands, Challenge and Lineron were applied pre-emergence and Titus was applied after emergence before final ridging. Herbicide applications were all at least 3 weeks before the first collection, and weed biomass was comparable at the two sites. The experimental design at both sites is described in Table 1. Plots (6 × 6 m in The Netherlands and 3 × 3 m in Ireland) were 6 m apart at both sites with grass in between. The nine plots per replicate (block) were randomly assigned to one of the nine combinations of genotype and management regime (no fungicide, weekly fungicide spraying or management using IPM2.0). Plot management regimes and specific qualities of each site are described in detail by Kessel et al. (unpublished).

At both sites, two pitfall traps were placed in the center of each plot, 1 m apart and connected by 10 cm high plastic edging pressed into the soil, to facilitate insect edging behaviour. Pitfall traps were 1 L plastic containers with an opening of 10 cm diameter, each containing 100 mL 70% ethylene glycol. Both traps were covered with an aluminum cover about 2 cm off the ground to protect the trap from rain, leaving room for ground dwellers to enter. Traps were left in the plots for one week, three times throughout the field season, with about four weeks between two trapping sessions.

2.2. Identification of species to family and to functional groups

The identification of arthropods, mollusks or oligochaetes from pitfall trap samples was done using appropriate dichotomous taxonomic keys (Goulet and Huber, 1993; Triplehorn et al., 2005). Family level was used by default for taxonomic grouping, though when identification to family was not feasible, the order, sub-order or super-family was used (for example the super-family Aphidoidea) or Entomobryomorpha (sub-order of Collembola). Each family grouping was assigned to one or two of the following ecological functional groups: predators, detritivores, parasitoids, fungivores, herbivores, hyperparasitoids or unknown (see Appendix A in Supplementary material). This means that in some cases, functional groups may contain the same family, exclusivity per functional group could not be achieved with family level identification.