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An exposure-based, ecology-driven framework for selection of indicator species for insecticide risk assessment

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ABSTRACT

In the current “tiered” paradigm for evaluating risks of insecticidal products, one of the first decisions that must be made is the selection of indicator species to be used in toxicity assays. However, as yet, no formal system has been developed to determine whether proposed indicator species are relevant to the ecology of the crop system where the product will be released. Here, we propose a protocol that provides information on the ecology and trophic linkages of organisms within agro-ecosystems, and demonstrate its implementation within maize agro-ecosystems, which have been a major focus of recent insecticidal developments. We use molecular gut-content assays and network analysis to identify species that are likely to be exposed to plant-incorporated products, and that likely have important functional roles in interaction webs in the maize ecosystem. The vast majority of arthropod abundance was found in the soil (97% of specimens per m^2 were found in the soil column). Only nine of the 382 morphotaxa met all three of the ecological criteria (high abundance, corn consumption, degree of connectedness within the network) for inclusion as indicator species, only one of which, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), has routinely been considered in risk assessment. Ecological data collected in studies such as this one can be used to ensure that insecticide risk assessments are ecologically relevant. We advocate the use of large-scale field bio-inventories, combined with molecular gut-content assays and ecological network analysis as regular components of the preparation and design phases of all future risk-assessment programs.

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1. Introduction

Ecological risk-assessment programs aim to ensure that biodiversity and ecological dynamics in agricultural landscapes are preserved and protected from unsustainable disturbances by agronomic practices. However, developing and implementing programs that meet this ostensible goal are hampered by the inherent challenges in identifying and measuring important ecological processes, and in identifying which organisms can serve as appropriate indicators for those ecological processes. In most risk-assessment programs, risk is regarded as comprising two elements: hazard and exposure (National Research Council Committee on the Institutional Means for Assessment of Risks to Public Health, 1983). Hazard refers to a defined adverse effect of a product on an organism. Exposure refers to a measure of the amount of contact with the product in the field. Risk is considered to exist where both hazard and exposure can be demonstrated through a series of prescribed tests. Current protocols arrange these tests in a series of “tiers,” in which the hazards of a product for a group of indicator species are evaluated in a succession of tests with increasing levels of ecological complexity (García-Alonso et al., 2006). For example, a risk assessment may begin with isolated organisms in laboratory assays, proceeding to tri-trophic assays in microcosms, before finally concluding with diversity assays in field cages or open field plots. However, later-tier testing is typically only considered necessary if early-tier testing reveals a physiological hazard to non-target organisms (García-Alonso et al., 2006). In effect, ecological dynamics are evaluated mainly for their potential to mitigate observed physiological hazards to indicator species, rather than for the susceptibility of these dynamics to potential disturbances from the product or practice. As a consequence of this, many authors have raised concerns with the ecological relevance of risk-assessment practices (Obrycki et al., 2001; Andow and Hilbeck, 2004; Andow et al., 2013; Lundgren and Duan, 2013). All phases of the risk-assessment process would benefit from being driven by ecological principles, and ensuring that measured indices are ecologically relevant.

Current risk-assessment practices can be enhanced by incorporating ecological principles earlier in the risk-assessment process, even before the first tier of risk-assessment tests are conducted. The first decision point in any risk-assessment protocol is the selection of indicator species to be used for testing. This step is crucial, as hazards are often species-specific, and the likelihood of discovering a hazard is thus contingent on which species are tested. Typical risk-assessment practices use an informal process of species selection based on informal heuristics

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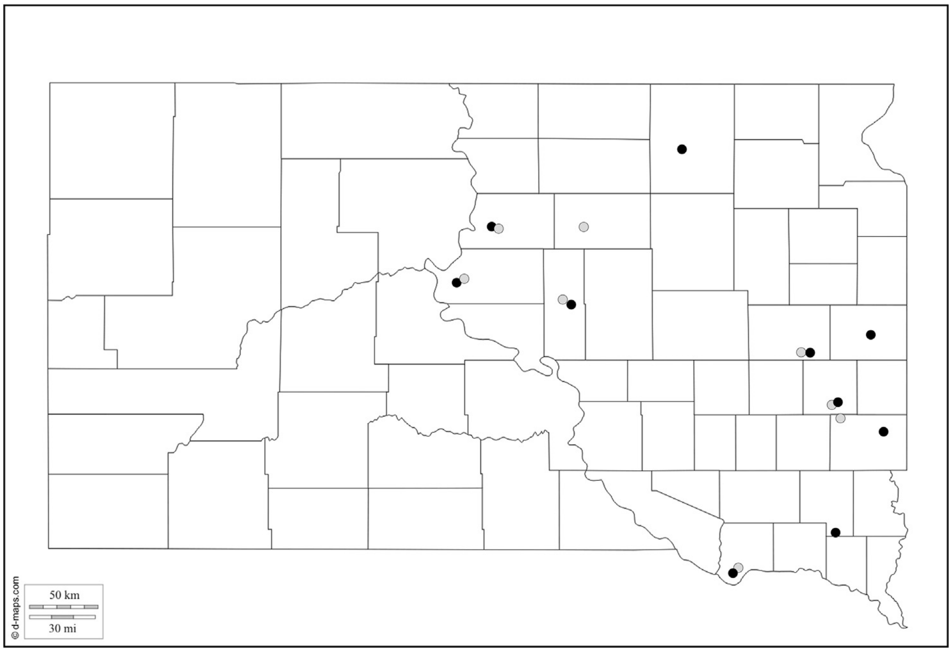

Fig. 1. A map of the U.S. state of South Dakota, with all collection sites for this study marked. Dark circles = sites sampled in 2013; light circles = sites sampled in 2014. The map is borrowed from http://d-maps.com/carte.php?num_car=19977&lang=en (used with permission).

and general information about a species' ecology, phylogeny and interest to society (García-Alonso et al., 2006) (Bachman et al., 2013). This usually involves qualitatively selecting organisms that represent different functional groups (e.g., pollinators, predators, and detritivores) or that have special relevance to producers or society (e.g., honey bees and aquatic insects). Additionally, we know of at least one attempt that has been made to institute a more rigorous system of risk assessment based on large-scale ecological data (Andow et al., 2013). Here, we propose a straightforward, data-driven method for quantifying various ecological aspects of organisms within an agro-ecosystem where transgenic or other plant-incorporated insecticidal products will be used. Our proposal builds on these previous systems by (1) formalizing a simple, practical process that can be integrated into the existing risk-assessment apparatus with minimal alteration, and (2) incorporating modern, high-throughput analytical techniques to generate multifaceted matrices of ecological attributes that can aid in the identification of organisms of primary relevance to the function of ecosystems and the movement of insecticidal products within them. This method will allow scientists to increase the transparency with which indicator species are selected for risk-assessment studies based on their importance within the target ecosystem, and improve the ecological relevance of the tests conducted.

Current risk-assessment protocols have resulted in somewhat uneven coverage of different components of agro-ecosystems. While pollinators and natural enemies have featured prominently in risk-assessment studies, some groups of organisms, such as detritivores and other soil-dwelling organisms, have been less thoroughly investigated from an ecological perspective (Wagg et al., 2014), and are consequently under-represented in the risk-assessment literature. Recent trends in agriculture and ecology are increasingly recognizing the importance of soil health for crop production (Bender and van der Heijden, 2015) and ecosystem functioning (Gessner et al., 2010). The major role that soil food webs play in cycling nutrients and maintaining soil health, combined with their high sensitivity to land-management practices (de Vries et al., 2013), make it essential that soil dynamics be investigated more thoroughly, and their interactions with insecticidal products be evaluated. However, soil communities are inherently difficult to study, and researchers must rely on novel approaches to collect and analyze data from these communities.

The emergence of modern research methodologies and analytical tools makes possible an evaluation of ecosystems with detail and efficiency. Molecular gut-content methodologies, such as polymerase chain reaction (PCR) and enzyme-linked immuno-sorbent assay (ELISA), provide access to previously inaccessible data on the diets of organisms in the field, thereby accommodating large-scale evaluation of trophic webs (Sheppard and Harwood, 2005; Juen and Traugott, 2007; Weber and Lundgren, 2009), which are important components of organisms' ecology and a primary vehicle for transmission of insecticides through ecological communities. Additionally, techniques for network analysis that were pioneered for use in sociological and economic studies are increasingly being used to characterize associations among organisms in ecological networks (Proulx et al., 2005; Fath and Grant, 2007; Blüthgen, 2010; Lundgren and Fausti, in press). This facilitates visualization of the large-scale properties of entire ecosystems, and identification of species that play significant ecological roles via the frequency and strength of network interactions with other organisms. These two modern ecological techniques in combination with traditional approaches for creating bio-inventories can provide the foundation for a robust framework to guide risk-assessment for plant-incorporated insecticidal agents.

Here, we demonstrate the fundamentals of ecology-based indicator-species selection, using a common agroecosystem from the Great Plains region of the United States. Maize, *Zea mays* L. (Poales: Poaceae), is one of the most widely-grown crops in the world, and its primary pest, the Western corn rootworm, *Diabrotica virgifera* LeConte (Coleoptera: Chrysomelidae) (hereafter "rootworm"), has been a major focus of commercial insecticidal transgenic products (e.g., Rice, 2003; Baum et al., 2007; Bolognesi et al., 2012). We collect field data on the distributions and trophic links of arthropod taxa associated with maize fields over a 95,000 km^2 area in one U.S. state, South Dakota. Although we focus on one example system, the principles outlined here can be readily adapted to other crop systems, regions, and other types of insecticidal agents.

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