



The effects of insecticides on butterflies – A review[☆]

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ABSTRACT

Pesticides, in particular insecticides, can be very beneficial but have also been found to have harmful side effects on non-target insects. Butterflies play an important role in ecosystems, are well monitored and are recognised as good indicators of environmental health. The amount of information already known about butterfly ecology and the increased availability of genomes make them a very valuable model for the study of non-target effects of pesticide usage. The effects of pesticides are not simply linear, but complex through their interactions with a large variety of biotic and abiotic factors. Furthermore, these effects manifest themselves at a variety of levels, from the molecular to metapopulation level. Research should therefore aim to dissect these complex effects at a number of levels, but as we discuss in this review, this is seldom if ever done in butterflies. We suggest that in order to dissect the complex effects of pesticides on butterflies we need to integrate detailed molecular studies, including characterising sequence variability of relevant target genes, with more classical evolutionary ecology; from direct toxicity tests on individual larvae in the laboratory to field studies that consider the potentiation of pesticides by ecologically relevant environmental biotic and abiotic stressors. Such integration would better inform population-level responses across broad geographical scales and provide more in-depth information about the non-target impacts of pesticides.

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1. Introduction

1.1. Non-target effects pesticides

There is no doubt that pesticides can be enormously beneficial in both agriculture and preventive medicine, for example to increase (the quality of) crop yields, to maintain healthy livestock and to prevent the spread of diseases (Oerke, 2006; Cooper and Dobson, 2007; Aktar et al., 2009; Benelli and Mehlhorn, 2016; Guedes et al., 2016). However, due care is needed for their use in an effective manner. Not only do we need to carefully establish the mode of action of pesticides, but also the effects of pesticides on both their intended targets and non-target species. It is clear that where innocent bystanders of pesticides find their natural habitat replaced or reduced by agricultural practices they are doubly affected (Potts et al., 2016). One such group of insects are Lepidoptera which may comprise good indicator species for the non-

target impacts of pesticides. Our relationship with Lepidoptera is a complex one. On the one hand they are the focus of considerable conservation efforts, predominantly butterflies (Brereton et al., 2011; Potts et al., 2016), but on the other hand 70% of agricultural pests are Lepidoptera, in particular many moth species and a few butterflies. Various studies on pest moth species have identified genes that could be targeted for pest control, either through pesticides, or genome editing techniques (Guan et al., 2018). While there is a substantial body of literature on pesticide use and effects on moths (e.g. Shakeel et al. (2017)), a comprehensive overview for butterflies is lacking (Pisa et al., 2015). Furthermore, although numerous studies have addressed the effects of land use *per se* on butterfly population dynamics and life-history strategies, very few have taken pesticide use into account (Lebeau et al., 2016; Hallmann et al., 2017; Malcolm, 2018). In this review we will therefore provide a comprehensive overview of what is known about the effects of pesticide use on butterflies, provide novel insights, highlights gaps in our knowledge, and propose future directions of study. Finally, it is hoped that although the focus will be on butterflies, extrapolation will be possible to those benign moth species that have seen their numbers reduced, not least due to

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indiscriminate effects of pesticides (Fox, 2012).

Benefits of using pesticides in agriculture range from nutritional health and/or increased diversity of viable crops, to more derived secondary benefits such as a reduced migration by humans to cities and a better educated population (Cooper and Dobson, 2007; Aktar et al., 2009). On the other hand, the increased use of pesticides can also result in harmful side-effects for wildlife (Boutin et al., 1999; Bell et al., 2001; Mineau, 2005). While such negative impacts of modern, intensive agriculture on biodiversity have been widely recognised, the contribution that agricultural pesticides make to this overall impact has largely been neglected (Gibbs et al., 2009; Gilburn et al., 2015). Insecticides are one of the biggest classes of pesticides used in the world (Aktar et al., 2009), and this review reflects that insecticides are also the class of pesticides predominantly investigated in butterflies. Although insecticides are produced as a pest preventative method, the vast spectrum of their toxicity inadvertently leads to the suppression of non-target insects and organisms inhabiting the same niche or environment. Affected, non-target organisms might include pollinators, natural predators and parasites (Johansen, 1977).

The main focus of research on non-target pesticide effects has been the European honey bee (*Apis mellifera*) (Sanchez-Bayo and Goka, 2014). The honey bee is the most economically valuable pollinator of crop monocultures and their absence could cause a decrease in yield of up to 90% in some crops (Southwick and Southwick, 1992; Winfree et al., 2007; Arena and Sgolastra, 2014). In recent years many (managed) bee colonies suddenly died over winter, through a phenomena named Colony Collapse Disorder (CCD) (van Engelsdorp et al., 2009). The cause of CCD is unknown and is probably the result of a complex interaction between multiple factors. One of the factors implicated in CCD are pesticides, especially neonicotinoids (Ratnieks and Carreck, 2010; van der Sluijs et al., 2013; Lu et al., 2014; Pisa et al., 2015). Neonicotinoids are the most used class of pesticides in the world. They are widely applied as seed dressing and work systemically throughout the plant. Neonicotinoids mimic the acetylcholine neurotransmitter and are highly neurotoxic to insects (Goulson, 2013; van der Sluijs et al., 2013; Crossthwaite et al., 2017). The indication of their role in CCD caused the European Union to ban three pesticides in the class of neonicotinoids in 2013, namely clothianidin, thiamethoxam and imidacloprid (European-Commission, 2013). The observation of CCD and the consequent neonicotinoid ban renewed and intensified the interest and research into the (non-target) effects of neonicotinoids in particular and pesticides in general (e.g. Pisa et al., 2015; Woodcock et al., 2016, 2017; Wood and Goulson (2017)).

Although honey bees are cheap, versatile, easy to manage and create their own economically valuable product they are not the most effective pollinator for a lot of crops (Klein et al., 2007). Furthermore, honey bees are not the only non-target species affected. A recent review by Pisa et al. (2015) assessing the impact of pesticides on non-target species, identified a need for studies investigating the effect of pesticides on Lepidoptera, in particular butterflies (see also Wood and Goulson (2017)).

1.2. Butterflies as models for non-target effects of pesticides

Butterflies play an important role in ecosystems as plant pollinators (Feber et al., 1997; Potts et al., 2016) and as prey for other organisms (Strong et al., 2000). Well-known to the general public, they are well monitored, recognised as indicators of environmental health (Whitworth et al., 2018) and as such they have been used to measure impact of factors such as climate change (Schweiger et al., 2012) and landscape fragmentation (Scriven et al., 2017). Comparatively, their ecology and abundance is much better known than any other invertebrate taxa (New, 1997). This allows the possibility

to investigate the impact of pesticides across a large ecological range (Fontaine et al., 2016). Butterfly species diversity and abundance has already been shown to be influenced by landscape complexity and type of farming (Rundlöf and Smith, 2006), quality of habitat (Pocewicz et al., 2009) and habitat management (Marini et al., 2009). Obviously some butterfly species are agricultural pests, such as the cabbage white species (*Pieris* sp.), but nothing like the scale and species diversity observed for moths (Feber et al., 1997). Understanding butterflies' sensitivity and responses to pesticide exposure more fully might help assess the overall risk of pesticide use (Pisa et al., 2015). The availability of genomic data for an ever-increasing number of butterfly species allows one to investigate the observed sensitivity and responses at the underlying molecular level (Shen et al., 2016; Liu et al., 2018), but also how they may adapt to agricultural environments (Sikkink et al., 2017). Research at the level of such integration in butterflies is far behind that of moths, and thus the detailed studies on pesticide development, usage and effects on pest moths can provide valuable starting points for such an approach (Trocza et al., 2017).

The habitat of many butterfly species consists of hedgerows or the fragmented areas between arable lands (Warren et al., 2001; Krauss et al., 2003). Butterflies can therefore come into contact with pesticide treated plants and areas through foraging or translocation. Butterflies inhabiting hedgerows are susceptible to spray drift from insecticides (Davis et al., 1991a,b; Çilgi and Jepson, 1995; Kjær et al., 2014). Numbers of widespread butterflies on monitored farm land have declined by 58% between 2000 and 2009 (Brereton et al., 2011), and a number of species are under threat. Some pesticides are applied in the form of a coating around seeds, this coating leaves a residue in the soil, and if water-soluble this residue can enter the ground water (Bonmatin et al., 2015; Schaafsma et al., 2015). Uptake from soil and soil water by non-target plants, particularly those in hedgerows and field margins is another potential route of (sub)lethal exposure in non-target species (Goulson, 2013). Butterflies that engage in mud puddling behaviour can also be exposed to pesticide residues or run-off in soil water (Still et al., 2015). Pesticides, such as neonicotinoids, that have systemic properties can translocate to pollen, nectar and guttation droplets, and become other potential routes of exposure (van der Sluijs et al., 2013). For example, via plant surfaces, as butterflies may collect honey dew/sap from trunks and leaves. However, little is known about the presence of pesticides in honey dew, but Corke (1999) suggested that 15 different species of honey dew/sap feeding UK butterfly species may have been negatively affected by exposure to particulate air pollution via this route. Therefore, there is the potential for these butterfly species to also be adversely affected by exposure to systemic pesticides, such as neonicotinoids, via honey dew/sap feeding. Adult feeding also has the potential to result in transovarial transport of pesticides from mothers to offspring, including bio pesticides (Paula et al., 2014). Insect growth regulators such as juvenile hormone analogues and chitin synthesis inhibitors are particularly amenable to transovarial transport (Campbell et al., 2016). However, much more work is required to explore the full range of potential routes by which butterflies may be exposed to pesticides in nature.

2. Data source and study selection

Here we provide a comprehensive review of research on the effects of pesticides on butterflies. The number of published studies on pesticide use and effects on butterflies is very small in comparison to that of moths, and we have set out to review every single study in this overview, making it therefore unique in its depth. We have identified three main approaches to pesticide research on butterflies, each of which will be discussed in turn in this review.

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