



# The effects of agrochemicals on Lepidoptera, with a focus on moths, and their pollination service in field margin habitats



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## ABSTRACT

In agricultural landscapes, field margins are potential habitats for moths and butterflies (Lepidoptera). However, because of their proximity to agricultural sites, field margins can be affected by inputs of pesticides and fertilizers. In the present study, we assessed the use of field margins by caterpillars as habitat. Furthermore, the effects of realistic field margin input rates of various agrochemicals on moths, especially on their caterpillar stages, were studied in field, semi-field, and laboratory experiments. Our monitoring results indicate that, although caterpillars were found in field margins, their mean abundance was 35–60% lower compared to meadows. In a field experiment, the insecticide treatment (pyrethroid, lambda-cyhalothrin) significantly reduced the number of caterpillars and only 15% of the sampled caterpillars occurred in the insecticide-treated plots. Furthermore, the insecticide affected the community composition of the caterpillars, whereas the fertilizer treatment slightly increased the caterpillar abundance. In laboratory experiments, *Mamestra brassicae* caterpillars were shown to be very sensitive when exposed to insecticide-treated leaves (rate that kills 50% of the test caterpillars (LR50) after 48 h: 0.78% of the recommended field rate; this rate corresponds to the arable spray drift input in field margins at a distance of 3–4 m from the crop), and the caterpillars also appeared to avoid feeding on the treated leaves. In addition, in a semi-field study, 40% fewer eggs of *Hadena bicruris* moths were found on *Silene latifolia* plants sprayed with the insecticide compared to control plants and the flowers of insecticide-treated plants were less likely to be pollinated by moths. Overall, these studies illustrate that moths use field margins as habitats and that they can be affected by realistic input rates of agrochemicals. As caterpillars are important prey organisms and adult moths can act as pollinators, inputs of agrochemicals in field margins should be reduced to maintain biodiversity in agricultural landscapes.

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## 1. Introduction

Agriculture is the most common form of land use in Europe (Stoate et al., 2009). As a result, a large portion of European biodiversity can now be found in agricultural landscapes (Robinson and Sutherland, 2002). Modern agricultural landscapes are often subject to intensified use, which is characterized by, for example, increased field sizes, decreased crop diversity, a reduced availability of semi-natural habitats, and high inputs of agrochemicals (pesticides and fertilizers) in fields (Stoate et al., 2001; Robinson and Sutherland, 2002). This intensified management of agricultural sites has negative effects on biodiversity, such as plants, birds, and invertebrates (Wilson et al., 1999; Stoate et al., 2001). The loss and degradation of semi-natural habitats in agricultural

landscapes and the intensification of agricultural management are thought to be major reasons for declines in the abundances of moths (Fox, 2012). For instance, agricultural intensification has been shown to decrease species richness of moths and abundance of nationally declining moth species in the UK (Merckx et al., 2012).

Moths and butterflies belong to the Lepidoptera, a species-rich insect order. Although a large portion of research on Lepidoptera has focused on butterflies (New, 2004), the majority of Lepidoptera (approximately 90%; Shields, 1989) are classified as moths. Field margins are common semi-natural habitats (Marshall and Moonen, 2002) that are often vegetated with grasses and herbs. Because the large majority of caterpillars are herbivores, and a majority of adult moths (and butterflies) visit flowering plants, field margins are a potential habitat for Lepidoptera, especially in agriculture-intensive regions in which these elements represent a majority of semi-natural habitats (Hahn et al., 2014b). Adult moths have been found to benefit from extended-width field margins in terms of the overall species richness (Merckx et al., 2012) and the

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abundance of certain species (Merckx et al., 2009; Merckx et al., 2010), possibly because of an increased host and nectar plant availability (e.g., the results of Pywell et al., 2004 for butterflies). Furthermore, field margins can increase the connectivity of ‘stepping stone’ habitats for moths (e.g., solitary trees) which may mitigate the negative consequences of habitat fragmentation (Slade et al., 2013). However, field margins can receive substantial inputs from agrochemicals that are applied on adjacent agricultural sites via spray drift or direct overspray (Rautmann et al., 2001; de Jong et al., 2008; Otto et al., 2013; Schmitz et al., 2013) and that might be detrimental to Lepidoptera (Sinha et al., 1990; Davis et al., 1991; Cilgi and Jepson, 1995; de Jong et al., 2008).

Herbicides and fertilizers may influence Lepidoptera via changes in host plant abundance, diversity (Longley and Sotherton, 1997; Fox, 2012), or quality (Hahn et al., 2014a). Insecticides can directly target juvenile and adult Lepidoptera and cause lethal effects (Sinha et al., 1990; Davis et al., 1991; Cilgi and Jepson, 1995; Abivardi et al., 1998). Furthermore, insecticides can also cause sublethal effects or act as a repellent to moths. These effects include, for example, avoidance of oviposition on sprayed surfaces by the adults (Kumar and Chapman, 1984; Gist and Pless, 1985; Abivardi et al., 1998; Seljasen and Meadow, 2006) or antifeedant effects against caterpillars (Kumar and Chapman, 1984).

The negative effects of agrochemicals on Lepidoptera might affect other organisms as well. For example, adults contribute to the transport of pollen as they visit flowers (Clinebell et al., 2004; Alarcon et al., 2008; Devoto et al., 2011) and hence can provide pollination services. In addition, both caterpillars and adults are important prey for various organisms such as birds (Wilson et al., 1999) and bats (Vaughan, 1997).

We hypothesized that agrochemicals, especially insecticides, affect Lepidoptera in various ways. One of the most commonly used insecticide in winter wheat in Germany (Freier et al., 2008) is Karate Zeon (Syngenta; active ingredient: lambda-cyhalothrin), a pyrethroid with contact, stomach action and repellent properties. We assumed that this insecticide could reduce the number of caterpillars in field margins due to toxic and antifeedant effects. Furthermore, synthetic pyrethroids have been found to act as ovipositional repellent for a moth species (Gist and Press, 1985), and we presumed that such an effect would reduce the pollination service of a specialized moth pollinator (*Hadena bicruris*) whose females pollinate *Silene latifolia* flowers during their oviposition.

To assess if field margins are used as habitats by caterpillars and to determine whether moths and their pollination services are affected by agrochemical inputs in field margins, we conducted four studies: First, we surveyed the occurrence of caterpillars in actual field margins. Second, we analyzed the effects of realistic input rates of an insecticide, an herbicide, and fertilizer in field margins on caterpillars in a field experiment. Third, we applied realistic field margin insecticide rates on host plants and assessed the survival and feeding behavior of *Mamestra brassicae* caterpillars in laboratory experiments. Fourth, we evaluated the avoidance of insecticide-treated flowers by moths regarding pollination and oviposition in a semi-field experiment.

## 2. Methods

The methods section is divided into four chapters that describe the design and statistics of each of the four experiments. The aim of the first study (Section 2.1) was to assess whether caterpillars use field margins as habitats. It was assumed that agrochemical inputs would have a negative effect on caterpillar abundance, and we therefore also sampled meadows for comparison that received no agrochemical inputs. The subsequent experiments focused on the effects of agrochemicals on caterpillars (Section 2.2, field

experiment; Section 2.3, laboratory experiments) and adult moths (Section 2.4, semi-field study).

### 2.1. Caterpillars in field margins

#### 2.1.1. Study design and sampling methods

Caterpillars were surveyed in cereal field margins and meadows in the area surrounding Landau, Germany, using sweep nets (300 sweeps per site and transect length of approximately 180 m) on sunny to partly cloudy days when the vegetation was dry. Overall, 14 field margins and twelve meadows were sampled for caterpillars during an initial sampling phase in May (18–26 May 2011). In addition, caterpillar abundances were assessed in nine cereal field margins and eleven meadows during a second sampling period in June (9–17 June 2011). The surveyed meadows had a size of approximately 1–1.5 ha. The field margins were between 1–2 m wide, which is a common margin width in the study area (Hahn et al., 2014b), and were vegetated with grasses and herbs. In Germany, field margins less than 3 m in width can receive high inputs of pesticides from overspray and spray drift because farmers are not forced to maintain a certain distance from such narrow elements during pesticide applications (Schmitz et al., 2013; Hahn et al., 2014b).

If possible, the same field margins and meadows were surveyed for caterpillars in both sampling phases (i.e., = six field margins and seven meadows). However, if a study site was mown between the first and the second sampling period and, hence, the vegetation height was inadequate (<30–40 cm) for appropriate sampling with sweep nets, another unmown site was chosen.

The sampled caterpillars were identified at the family level (Carter and Hargreaves, 1987; Porter, 1997; Rennwald and Rode-land, 2004; Bellmann, 2009). If a clear identification of a caterpillar was not possible, it was reared to an adult state.

In addition to caterpillars, the vegetation of the sampling sites was also assessed. The details of the identified plant species are presented in the supplementary data (part 1).

#### 2.1.2. Statistics

Data for caterpillars in field margins and in meadows were compared for each phase using the Primer (Version 6) statistical program and the PERMANOVA+ add-on (Anderson et al., 2008). We conducted permutational analyses of variance for the analysis of caterpillar abundance (PerAnova, univariate data, resemblance matrices: Euclidean distance) and the caterpillar communities at family level (PerManova, multivariate data, resemblance matrices: Bray Curtis distance). Each analysis was based on 999 permutations.

### 2.2. Effects of agrochemicals on caterpillars in a field experiment

#### 2.2.1. Study design

Caterpillars were sampled during the course of a field experiment with the aim of identifying the individual and combined effects of repeated agrochemical applications (duration of the experiment: 2010–2012) on the flora (Schmitz et al., 2013b; Schmitz et al., 2014a,b) and fauna of field margins. In the experiment, 64 plots (each 8 m × 8 m) were created within an extensively managed hay meadow located near Landau, Germany. The plots were assigned to one of seven treatments (either a single application of fertilizer (F), herbicide (H), or insecticide (I), or a combination of these treatments (F+I, H+I, F+H, F+H+I)), or the control (C). Each treatment and the control were replicated eight times within a randomized block design (see Schmitz et al., 2013 for more details on the experimental design).

The applications of the agrochemicals and their application sequences mimicked the field management of winter wheat fields

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