



# Impacts of a pesticide on pollinator species richness at different spatial scales

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<https://doi.org/10.1016/j.baae.2009.11.007> 

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## Abstract

Pesticides are an important potential cause of biodiversity and pollinator decline. Little is known about the impacts of pesticides on wild pollinators in the field. Insect pollinators were sampled in an agricultural system in Italy with the aim of detecting the impacts of pesticide use. The insecticide fenitrothion was over 150 times greater in toxicity than other pesticides used in the area, so sampling was set up around its application. Species richness of wild bees, bumblebees and butterflies were sampled at three spatial scales to assess responses to pesticide application: (i) the 'field' scale along pesticide drift gradients; (ii) the 'landscape' scale sampling in different crops within the area and (iii) the 'regional' scale comparing two river basins with contrasting agricultural intensity. At the field scale, the interaction between the application regime of the insecticide and the point in the season was important for species richness. Wild bee species richness appeared to be unaffected by one insecticide application, but declined after two and three applications. At the landscape scale, the species richness of wild bees declined in vine fields where the insecticide was applied, but did not decline in maize or uncultivated fields. At the regional scale, lower bumblebee and butterfly species richness was found in the more intensively farmed basin with higher pesticide loads. Our results suggest that wild bees are an insect pollinator group at particular risk from pesticide use. Further investigation is needed on how the type, quantity and timing of pesticide application impacts pollinators.

## Zusammenfassung

Pestizide stellen einen möglichen Grund für die Diversitäts- und Bestäuberabnahme dar. Es ist jedoch wenig über die Wirkung von Pestiziden auf wildlebende Bestäuber im Freiland bekannt. Mit dem Ziel die Auswirkungen von Pestizideinsätzen festzustellen wurden Bestäuberinsekten in einem landwirtschaftlichen Systemen in Italien gesammelt. Das Insektizid Fenitrothion ist etwa 150 mal toxischer als die anderen Pestizide, die in der Gegend genutzt werden, und so wurde die Untersuchung rund um seine Nutzung angelegt. Um die Reaktionen auf die Pestizidanwendung abzuschätzen wurde der Artenreichtum der Wildbienen, Hummeln und Schmetterlinge auf drei räumlichen Skalen untersucht: (i) auf der Feldskala entlang von Gradienten der Pestiziddrift, (ii) auf der Landschaftsskala indem verschiedene Feldfrüchte in dem Gebiet beprobt

wurden und (iii) auf der regionalen Skala indem zwei Flusstäler mit unterschiedlicher landwirtschaftlicher Intensität verglichen wurden. Auf der Feldskala war die Interaktion zwischen dem Anwendungsregime des Insektizids und dem Zeitpunkt der Saison für den Artenreichtum wichtig. Der Artenreichtum der Wildbienen schien von einer Insektizidanwendung unbeeinflusst, nahm aber nach zwei- oder dreifacher Anwendung ab. Auf der Landschaftsskala nahm der Artenreichtum der Wildbienen in Weinbergen ab in denen die Insektizide angewendet wurden, aber nicht auf Maisfeldern oder auf nichtkultivierten Flächen. Auf der regionalen Skala wurden geringere Hummel- und Schmetterlingsartenzahlen in dem intensiver bewirtschafteten Flusstal mit den höheren Pestizidbelastungen gefunden. Unsere Ergebnisse zeigen, dass Wildbienen zu einer Bestäuberinsektengruppe gehören, die dem Risiko der Pestizidbelastung besonders ausgesetzt ist. Es sind weitere Untersuchungen darüber notwendig, wie der Typ, die Menge und der Zeitrahmen der Pestizidanwendung die Bestäuber beeinflusst.

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## Introduction

Pollinators are an important component of biodiversity that provide a key ecosystem service through wildflower and crop pollination (Klein, Vaissière, Cane, Steffan-Dewenter, Cunningham et al., 2007; Spira, 2001). They have been proposed as indicators of ecosystem health for assessing the impacts of a pressure such as pesticides (Kevan, 1999). Pollinators increase plant seed set (Morandin & Winston, 2006), fruit set (Klein, Steffan-Dewenter, & Tscharntke, 2003) and fruit quality (Roldan Serrano & Guerra-Sanz, 2006) and their value to agriculture in the European Union is estimated at €14.2 billion per year (Gallai, Salles, Settele, & Vaissière, 2008). There is growing concern and discussion relating to declines found in pollinators around the world (Kluser, & Peduzzi, 2007). A decline in wild bee species richness has been found in the UK and the Netherlands (Biesmeijer, Roberts, Reemer, Ohlemüller, Edwards et al., 2006) and the Committee on the Status of Pollinators in North America (National Research Council, 2007) found downward trends in the abundance of *Apis mellifera* (L.) and a small number of wild pollinators for which they had sufficient data. Though uncertainty remains as to the global extent of this phenomenon, further investigation needs to be undertaken into the scale, magnitude and causes of the decline, and the effects on pollination services (Roubik, 2001).

Agriculture is the primary land use in Europe, so its management has profound consequences for the environment and for biodiversity (Benton, Vickery & Wilson, 2003). Agricultural intensification is widely accepted as a cause of biodiversity decline (Robinson & Sutherland, 2002). Intensification is, however, a broad concept encompassing many factors, such as the loss of semi-natural habitat, fragmentation and increased pesticide input (Tilman, Fargione, Wolff, D'Antonio, Dobson et al., 2001). To understand the causes of biodiversity decline it is important to disentangle the effects of individual components of agricultural intensification. This study focused on pesticides as a driver. Pesticides have been shown to cause declines in non-target beetles (Lee, Menalled, & Landis, 2001), bees (Alston, Tepedino, Bradley, Toler, Griswold et al., 2007), birds (Hart, Milsom, Fisher, Wilkins, Moreby et al., 2006) and aquatic invertebrates (Fairchild & Eidt, 1993). The area to which agro-chemicals are applied in Great Britain has increased since the 1970s, with agricultural intensification (Robinson & Sutherland, 2002) and global pesticide production predicted to continue increasing in the future (Tilman et al., 2001).

This study aimed to isolate the impact of an insecticide on insect pollinators in the field. Most evidence of the impact of pesticides on pollinators comes from laboratory-based toxicity tests, determining LD<sub>50</sub> values for honeybees (*A. mellifera*). Negative effects from sub-lethal doses of insecticide have also been demonstrated (El Hassani, Dacher, Gauthier, & Armengaud, 2005), but field assessments are needed to understand how laboratory-derived toxicity levels relate to real effects observed in pollinator communities (Stark, Jepson, & Mayer, 1995). Information is needed on the impacts of pesticides on the wider pollinator community, such as *Bombus* spp. and butterflies, not just honeybees. Some field- and semi-field-based studies have been undertaken (Gels, Held, & Potter, 2002; Koch & Weisser, 1997). However, most of the field-based studies in this area have

been conducted at the field scale. Given that most systems have many chemical inputs, with varying levels of toxicity to invertebrates and that pollinators are a relatively mobile group, larger scale approaches may be more appropriate and could provide greater insight into the effects of pesticides. This investigation considered the effect of pesticide pressure at different spatial scales on pollinators, using detailed information on pesticide application over an area of 28 km<sup>2</sup>.

As different pollinator taxa have different mobility, life history and feeding strategies, they were expected to interact and respond to pesticide pressure at different scales. Steffan-Dewenter, Münzenberg, Bürger, Thies, & Tschardtke (2002) found that less mobile wild bees were affected by the amount of surrounding semi-natural habitat at smaller scales than more mobile honeybees. We chose to investigate the impact of pesticides on pollinators at multiple spatial scales to be able to detect responses in a range of pollinators. Within part of a river basin in Italy, sampling was set up around the application of the insecticide fenitrothion to vine fields (*Vitis vinifera* L.), as it was by far the most toxic pesticide used in the basin. We conducted our investigation at three spatial scales (Appendix A: Fig. 1): the field scale working around the insecticide sprayed vine fields, the landscape scale sampling throughout 28 km<sup>2</sup> of the river basin, and the regional scale comparing two river basins in the Veneto region of Italy. We hypothesised that pollinator species richness would be negatively related to insecticide pressure at all scales. We expected that pollinators would respond more strongly to insecticide pressure at larger scales as their relatively high mobility may mask patterns at smaller scales.

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## Section snippets

### Field sites and sampling periods

Field work was carried out in Northeast Italy in part of the Meolo basin (45°39'12"N, 12°25'39"E). Information from the farmers was available in this 28 km<sup>2</sup> area on the annual amount and type of pesticides applied to the cultivated fields through the University of Milan Bicocca and the Provincial Consortium for the Protection of Agricultural Activities ([http://www.coditv.it/org\\_01b.htm](http://www.coditv.it/org_01b.htm)). Also, land use had been mapped to the level of individual fields and boundaries in the 28 km<sup>2</sup> area of the

### Results

Species richness and abundance were positively related, and so produced very similar results. Here we focus on the species richness analyses, for the results of the analyses of abundance see Appendix A: Table 3, Table 4.

### Discussion

The lower species richness of wild bees associated with fields sprayed 2 and 3 times may be the result of an accumulation effect. Accumulation effects have been found in soil (Ciglasch, Busche, Amelung, Totrakool & Kaupenjohann, 2006) but there is little information regarding bioaccumulation in bees. A 2nd or 3rd application of insecticide may have a negative effect on pollinators as they are already under stress from an initial application. Earlier in the season, the insecticide's impacts may

### Conclusions

Our results suggest a negative impact of insecticide application on wild bee species richness, though the mechanism requires further investigation. A decline in wild bee species richness could have important consequences for pollination (Klein et al., 2003). With current concerns over the status of pollinators, there is a need to better understand the factors that may be driving pollinator declines (National Research Council, 2007). More information is needed on whether any traits are

## Acknowledgements

Thanks to Oddino Bin, Sara Bonzini, Alessandro Bellia, Stefania Barmaz, Serenella Sala, Roberto Verro for scientific support and the farmers for access to their land. Thanks to Stuart Roberts for assistance with bee identifications, Alain Pauly for Halictidae identifications, Andreas Mueller for Megachilidae identifications. This study was conducted as part of the Integrated Project ALARM (Assessing LARge-scale environmental Risks for biodiversity with tested Methods, Settele et al. (2005),

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