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Sublethal effects of imidacloprid on interactions in a tritrophic system of non-target species

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HIGHLIGHTS

- We investigated short-term sublethal toxicity of imidacloprid in a tritrophic system.
- Low imidacloprid rate only reduced mass gain in crickets.
- High imidacloprid rate reduced crickets' feeding, mass gain, growth and mobility.
- Cricket survival of spider predation was enhanced by low rate treatment.

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ABSTRACT

Imidacloprid is one of the most used insecticides worldwide, but is highly toxic to non-target arthropods. Effects of sublethal imidacloprid intoxication can potentially propagate in food webs, yet little is known about the impact on non-target populations and communities. We investigated short-term sublethal toxicity of imidacloprid in a tritrophic model system of wild strawberry *Fragaria vesca*, wood cricket *Nemobius sylvestris* and nursery web spider *Pisaura mirabilis*. Strawberries were treated two times with 0 mg (control), 1 mg (low rate) and 10 mg (high rate) of Confidor® WG 70 and crickets were allowed to feed on them. In four lab experiments, we quantified the impact of imidacloprid on leaf damage, growth, behaviour and survival of crickets. The high imidacloprid rate reduced feeding, mass gain, thorax growth and mobility in crickets compared to the control, while mortality was similarly low in all treatments. The low rate reduced mass gain only. Cricket survival of spider predation was higher in the low rate treatment than in the control.

Overall, herbivory and predation were reduced at sublethal imidacloprid rates in a non-target organism, three-level food chain, which demonstrates possible propagation of sublethal effects through trophic interactions.

1. Introduction

Systemic insecticides are taken up and distributed inside the plant and are intended to kill herbivore pests (Stenersen, 2004). The systemic neonicotinoid insecticides are the most rapidly expanding chemical class of insecticides with a market share of 28.5% in 2011 (Jeschke et al., 2013). They can be used as plant protection products for a large variety of plants ranging from crops to trees. For example, the neonicotinoid insecticide imidacloprid is marketed world-wide for the use in over 140 agricultural crops (Jeschke et al., 2011) and in forestry (Kreutzweiser et al., 2009; Szczepaniec et al., 2013).

Imidacloprid is used to control a variety of insect pests including plant- and leafhoppers, aphids, termites, whiteflies and thrips (Jeschke et al., 2011). However, imidacloprid is highly toxic to many beneficial insects, e.g. the honey bee *Apis mellifera* (Cox, 2001). Imidacloprid has been demonstrated to adversely affect pollinators at sublethal concentrations (Gill et al., 2012; Whitehorn et al., 2012). Recent findings have resulted in the restriction of imidacloprid use in the European Union (European Commission, 2013) and a public debate about the need for a refined ecological risk assessment that puts stronger emphasis on pollinator taxa and examines a wider range of ecological endpoints, e.g. acute and chronic effects on behaviour and learning (Vanbergen and the Insect Pollinators Initiative, 2013). Sublethal imidacloprid effects have been reported for several terrestrial arthropod species other than pollinators. Feeding reduction was observed in studies on sap-sucking pests (Devine et al., 1996;

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Boina et al., 2009) and leaf-shredding non-target insects (Kreutzweiser et al., 2009). Adverse development effects of imidacloprid have been shown in the pest citrus root weevil *Diaprepes abbreviatus* (Quintela and McCoy, 1997). Moreover, Smith and Krischik (1999) demonstrated behavioural effects in the form of reduced mobility of the beneficial spotted lady beetle *Coleomegilla maculata*. However, most terrestrial studies on sublethal effects of direct imidacloprid exposure have been done with pest or beneficial species. There is a paucity of studies on the environmental fate of neonicotinoids regarding their uptake by non-target plants and subsequent persistence therein. Indeed, due to their accumulation and persistence in soils, non-crop vegetation has been predicted to take up neonicotinoids (Goulson, 2013). Therefore, imidacloprid has the potential to affect crop-adjacent ecosystems and their inherent trophic relationships.

Trophic interactions can transmit effects between multiple trophic levels, e.g. a predator decimating herbivore abundance (density-mediated effect) leading to increased plant growth. However, effects can also be non-lethal, e.g. avoidance behaviour by herbivores as a reaction to predator cues (trait-mediated effect). The propagation of such trait-mediated effects along the food chain can have similar or even greater impacts on communities than the propagation of density-mediated effects (Preisser et al., 2005; Werner and Peacor, 2006). This may also apply to pesticide impact on trophic systems. In analogy to trait-mediated effects, the propagation of sublethal pesticide effects through trophic interactions may be pervasive though few studies have been conducted (Relyea and Hoverman, 2006; Rohr et al., 2006). In a tritrophic system composed of plant, herbivore and predator two interactions can be affected by the insecticide: Plant-herbivore and herbivore-predator (Fig. 3). Most previous ecotoxicological studies on trophic interactions investigated two-species systems in an agricultural context, i.e. crop and pest species or pest and beneficial species. Data on the toxicity of imidacloprid towards agriculturally non-beneficial, terrestrial non-target organisms is scarce especially for multitrophic systems.

Here we present the results from four experiments on the effects of sublethal imidacloprid rates on a tritrophic model system consisting of wild strawberry *Fragaria vesca* L. (imidacloprid-treated), wood cricket *Nemobius sylvestris* (Bosc) and nursery web spider *Pisaura mirabilis* (Clerck). *N. sylvestris* can be a dominant herbivore on wild strawberry, and *P. mirabilis* is a dominant plant-dwelling predator in various habitat types. This model system represents a potentially exposed food chain on a forest edge adjacent to a crop field. Our aim was to test if sublethal effects of imidacloprid directly affect intermediate species in a non-target, three-level model food chain and whether effects are transmitted from thereon through trophic interactions. Due to the scarcity of data on sublethal pesticide effects and trophic effect propagation we chose a simple model system independent from agricultural purposes and concentrated on basic parameters of species interactions.

Based on the toxicological properties and adverse effects of imidacloprid found in previous studies we developed 3 hypotheses. Feeding reduction, as well as development effects, were reported in several studies (Devine et al., 1996; Quintela and McCoy, 1997; Boina et al., 2009; Kreutzweiser et al., 2009). Therefore, we predicted that (1) *Nemobius sylvestris* feeding and growth decreases because of imidacloprid exposure from contaminated plant material. Imidacloprid disrupts transmission of nerve impulses and has been shown to impair mobility (Smith and Krischik, 1999; Stenersen, 2004). Hence, we predicted that (2) the behaviour, i.e. locomotive parameters, of *N. sylvestris* is adversely affected. The ability to sense predator attacks is imperative for *N. sylvestris'* probability to escape, as well as fleeing rapidly (i.e. jumping) (Dangles et al., 2006). Thus, we hypothesize that (3) behavioural changes and sensory impairment in *N. sylvestris* lead to increased predation success of *P. mirabilis*.

2. Materials & methods

2.1. Test organisms and insecticide

Immature crickets were collected from the field in summer/fall 2012 at the edge of a mixed forest near Bellheim, Germany (49°11′45″N/8°19′11″E). Immature spiders were retrieved from the same site, except for fall where they were retrieved from a semi-natural grassland near Landau, Germany (49°11′4″N/8°7′51″E) because of too low abundances at the Bellheim site. *P. mirabilis* is a known predator of *N. sylvestris* (Gabbutt, 1959; Bucher et al., 2014). Strawberry plants were collected from the Bellheim site and kept in an environmental chamber for the experiments until early December. Outside of the natural growing season they were reared from seeds (Templiner Kräutergarten, Templin, Germany) (see Appendix Fig. A.1 in Online Supplementary Material). Crickets were observed prevalently sitting on strawberry plants and displayed elevated abundance in their vicinity at the Bellheim site. The studied species represent a non-target food chain, unlikely to occur in agricultural crops. However, they may be affected by the contamination of non-target areas due to in-field pesticide use or by the application against insect pests in forests. Imidacloprid was applied as formulation (Confidor® WG 70, 700 g a.i kg^{-1}, water dispersible granules, Bayer CropScience AG, Monheim, Germany).

2.2. Microcosm preparation

Plastic cups (7.3 and 10.1 cm bottom and top diameter, 10.3 cm height) were each filled with 40 g potting soil and one strawberry plant (hereafter termed microcosms, Appendix Figs. A.3 and A.4). The maximum number of leaves was limited to three by cutting excess leaves close to stem basis. Microcosms were randomly assigned to three (or four) treatments (Table 1):

- Control(s): two subsequent applications of 10 mL distilled water per microcosm with a pipette directly to the soil surface in one spot.
- Low imidacloprid rate treatment (hereafter LRT): two subsequent applications of 1 mg Confidor® WG 70 (700 g kg^{-1} imidacloprid) dissolved in 10 mL distilled water per microcosm (0.24 g m^{-2}). This treatment is in the range of expected rates in field margins and is corresponding to low exposure in forests.
- High imidacloprid rate treatment (hereafter HRT): two subsequent applications of 10 mg Confidor® WG 70 dissolved in 10 mL distilled water per microcosm (2.39 g m^{-2}). This treatment represents a worst-case direct exposure scenario in field margins and is corresponding to intermediate exposure in forests.

Treatment rates and number of applications were selected based on preliminary results (causing sublethal intoxication in crickets) and are in the range of application rates allowed by German authorities for application in lettuce crops (1.3 g m^{-2}) (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, 2012), as well as expected exposure in forests (for more details see Appendix Section A.1).

Applications were done on day-10 and -3, before the start of each experiment. During the experiment the microcosms were placed in an environmental chamber at 20 °C temperature, 65% humidity and 12/12 h day/night rhythm. Before inserting crickets, the soil surface was covered with silica sand to minimize contact exposure.