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UV-irradiation and leaching in water reduce the toxicity of imidacloprid-contaminated leaves to the aquatic leaf-shredding amphipod *Gammarus fossarum*☆

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ABSTRACT

Systemic neonicotinoid insecticides such as imidacloprid are increasingly applied against insect pest infestations on forest trees. However, leaves falling from treated trees may reach nearby surface waters and potentially represent a neonicotinoid exposure source for aquatic invertebrates. Given imidacloprid's susceptibility towards photolysis and high water solubility, it was hypothesized that the leaves' toxicity might be modulated by UV-irradiation during decay on the forest floor, or by leaching and re-mobilization of the insecticide from leaves within the aquatic ecosystem. To test these hypotheses, the amphipod shredder *Gammarus fossarum* was fed (over 7 d; $n = 30$) with imidacloprid-contaminated black alder (*Alnus glutinosa*) leaves that had either been pre-treated (i.e., leached) in water for up to 7 d or UV-irradiated for 1 d (at intensities relevant during autumn in Central Europe) followed by a leaching duration of 1 d. Gammarids' feeding rate, serving as sublethal response variable, was reduced by up to 80% when consuming non-pretreated imidacloprid-contaminated leaves compared to imidacloprid-free leaves. Moreover, both leaching of imidacloprid from leaves (for 7 d) as well as UV-irradiation reduced the leaves' imidacloprid load (by 46 and 90%) thereby mitigating the effects on gammarids' feeding rate to levels comparable to the respective imidacloprid-free controls. Therefore, natural processes, such as UV-irradiation and re-mobilization of foliar insecticide residues in water, might be considered when evaluating the risks systemic insecticide applications in forests might pose for aquatic organisms in nearby streams.

1. Introduction

Neonicotinoids currently constitute the most widely used class of insecticides worldwide (Simon-Delso et al., 2015). In contrast to traditional, broad-spectrum insecticides (such as carbamates and organophosphates) neonicotinoids are virtually non-toxic to mammals. Still, they exhibit high acute toxicity towards insects by selectively binding to their nicotinic acetylcholine receptors (Tomizawa and Casida, 2003). Due to their physico-chemical properties, neonicotinoids are rapidly taken up by roots or leaves and distributed in all plant parts ensuring protection against herbivorous insects (mainly sap feeders; Jeschke et al., 2011). Depending on the plant species being treated and the neonicotinoid compound used, this period of protection may last from weeks to month for agricultural crops (Alford and Krupke, 2017; Donnarumma et al., 2011; Laurent and Rathahao, 2003) and deciduous trees (Mota-Sanchez et al., 2009; Poland et al., 2006; Tattar et al., 1998), and up to several years for conifers (Benton et al., 2016a; Eisenback et al., 2014).

In recent years, neonicotinoids have attracted public attention due to their suspected role in the decline of pollinators (Forster, 2009). This incident resulted in a re-evaluation of neonicotinoids by the European Food Safety Authority and ultimately led to a

Abbreviations: CI, confidence interval; DBH, trunk diameter at breast height; IMI, imidacloprid; LOQ, limit of quantification; SE, standard error; UV, ultraviolet.

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European Union wide temporary ban of three neonicotinoids (imidacloprid (IMI), clothianidin and thiamethoxam) and their application on pollinator-attracting crops (European Commission, 2013). Following this sanction, several countries, including the United States and Canada, also re-evaluated the risks of these insecticides to pollinators and the aquatic environment (USEPA, 2017; Health Canada, 2016). These reviews covered a variety of scenarios mostly concerning the effects of neonicotinoids when applied on crops via seed treatment and foliar sprays. Although the application of neonicotinoids (primarily of IMI) to deciduous and coniferous forest trees has increased in recent years (Benton et al., 2016b; Eisenback et al., 2010), the potential exposure of non-target invertebrate species due to such use has received little attention. When trees are treated via soil drenching, neonicotinoid concentrations can leach to nearby streams (Cowles, 2009). Moreover, leaves have been shown to accumulate vast amounts of the systemic insecticides regardless of the application method (i.e., soil or trunk application; e.g., Tattar et al., 1998). During autumn leaf fall, such neonicotinoid containing leaves can enter non-target ecosystems (Kreutzweiser et al., 2007), a factor rarely considered in neonicotinoid risk assessment. Consequently, forest floor dwelling and aquatic invertebrates that consume fallen leaf litter may be indirectly exposed to neonicotinoids in treated forest ecosystems (cf. Kreutzweiser et al., 2008).

While numerous studies have assessed the effectiveness of neonicotinoid application on trees to suppress insect pests (e.g., Coleman et al., 2017; Cowles et al., 2006) as well as indirect effects on their predators (e.g., Eisenback et al., 2010; Szczepaniec et al., 2011), only a few studies have investigated the implications of neonicotinoids on non-target terrestrial and aquatic decomposers exposed to these compounds through the consumption of contaminated leaves (Englert et al., 2017b; Kreutzweiser et al., 2007, 2008, 2009). Further, little to no research has been completed regarding the fate of neonicotinoids in fallen leaves during decay under ambient environmental conditions. For instance, neonicotinoids in leaf tissues may undergo photolytic degradation during sunlight exposure (i.e., ultraviolet (UV) irradiation) on the forest floor. Moreover, within stream ecosystems, the high water solubility of neonicotinoids may result in their remobilization from fallen leaves (Kreutzweiser et al., 2007) thereby reducing their potential toxicity to aquatic decomposers. In this context, the present study aimed at assessing these hypothesized consequences of UV-irradiation and leaching duration on the toxicity of IMI-contaminated black alder leaves (*Alnus glutinosa*) to the key shredder *Gammarus fossarum* (Koch; Dangles et al., 2004) – an amphipod frequently used in non-standard aquatic toxicity studies (Kunz et al., 2010). Therefore, IMI-contaminated leaves, submerged in water for different durations of time or irradiated with UV, were offered as food to *G. fossarum* during laboratory experiments while its feeding rate served as response variable.

2. Materials & methods

2.1. Test organisms

Gammarus fossarum was chosen as the test organism for the present study as this species is widely distributed in European headwater streams (Westram et al., 2011) and is also an important prey resource for many fish and invertebrate predators (e.g., MacNeil et al., 1999). Moreover, due to their high abundances and efficiency in shredding coarse particulate organic matter (such as leaves), gammarids are considered a key species in nutrient recycling (i.e., leaf litter breakdown; Dangles et al., 2004) and are frequently used in non-standard toxicity tests (Kunz et al., 2010).

One week prior to the start of each experiment, *G. fossarum* were kick-sampled from the Hainbach stream (49°14′ N; 8°03' E) located in the Palatinate Forest. This *G. fossarum* population is exclusively composed of cryptic lineage B (Feckler et al., 2012). As the sampling site was located upstream of any settlement and agricultural activity, and neonicotinoids compounds are not applied to trees by the local forestry office, previous exposure of test organisms to neonicotinoids was unlikely. Adult male gammarids of 6–8 mm body length and visibly free of macro-parasites were selected as per Bundschuh et al. (2011). Prior to testing, organisms were acclimated for 7 d in laboratory aquaria containing well-aerated stream water collected from the sampling site and maintained at 16 ± 1 °C followed by a gradual transition to SAM-S5 medium (i.e., test medium; Borgmann, 1996). During this time, organisms were fed *ad libitum* with black alder leaves that had been conditioned with a near-natural microbial community consisting of fungi and bacteria as described in Bundschuh et al. (2011).

2.2. Source of plant material, imidacloprid application and preparation of leaf discs

For the present study, IMI-free and IMI-contaminated black alder leaves were prepared as described in detail in Englert et al. (2017a). In brief, black alder trees were soil drenched once in June 2014 with either 500 mL tap water or with 500 mL of tap water spiked with the neonicotinoid formulation ConfidorWG70 (70% IMI, Bayer CropScience; dose: 0.15 g IMI/cm trunk diameter at breast height (DBH)). Due to the relatively small trees size (mean DBH: 7.5 ± 0.2 mm; Englert et al., 2017a), soil drenching instead of trunk injection was used as application method. The amount of IMI applied to trees represented 25% of the highest dose recommended for soil application (e.g., for the product Merit75WP; Bayer CropScience). Shortly before leaf fall in October 2014, all leaves were collected from trees and stored at −20 °C until further use. To minimize the variation of IMI residues in leaves that can occur among treated trees (e.g., Englert et al., 2017a), only leaves collected from a single tree were used for this study. Leaf discs (diameter = 2.0 cm) were cut from leaves using a cork borer, freeze-dried (for 24 h) and subsequently weighed to the nearest 0.01 mg to determine their initial dry weight.

To simulate leaching of the IMI dose from treated leaves, preweighed IMI treated leaf discs were placed into plastic beakers (two discs per beaker) filled with 150 mL of the test medium and leached for 1, 3, or 7 d prior to the start of exposures. During this period, the test medium was renewed daily to remove any IMI that may have accumulated in the test medium. IMI-contaminated leaf discs that were not subjected to simulated leaching were used as positive control. IMI-free leaf discs were subjected to the same leaching process (i.e., for 0, 1, 3 and 7 d) and used in the corresponding controls to account for any changes in leaf condition as associated with the different leaching times.

For the experiment assessing the influence of UV-irradiation on the toxicity of IMI-contaminated leaves, IMI-contaminated leaf discs were UV-irradiated for 1 d using a UV fluorescent lamp (Magic Sun 20/160R; Heraeus Holding GmbH; Hanau, Germany) at an intensity (mean ± standard error (SE)) of 4.15 ± 0.09 and 0.15 ± 0.01 W/m^2 for UV-A and UV-B, respectively (measured with a RM12 radiometer; Dr. Gröbel UV-Elektronik GmbH, Ettlingen Germany). This is ~90% below peak intensities measured for UV-A and UV-B, respectively, under clear skies during summer in Central Europe (Häder et al., 2007). The dose generated during the 24 h UV exposure period (UV-A: ~360; UV-B: 13 kJ/m^2) represents the approximate cumulative dose measured over 1.5 and 4 d for UV-A and UV-B, respectively, during October in Central Germany (Häder et al., 2001). Irradiated leaf discs were placed into plastic beakers filled with 150 mL test medium and allowed to leach for 1 d