

# Commercially formulated glyphosate can kill non-target pollinator bees under laboratory conditions

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## Abstract

The use of glyphosate-based herbicides in agroecosystems has increased over the past few years because of the advent of genetically modified glyphosate-resistant crops and resistant weeds. This is alarming because of potential damaging effects on non-target organisms. In sub-Saharan Africa, for example Ghana, many rural farmers have not received training in the use of glyphosate-based herbicides, thus tend to apply higher than recommended concentrations on farms. Therefore, this study investigated the effect of glyphosate-based herbicides on beneficial insects under laboratory conditions, using *Apis mellifera* L. (Hymenoptera: Apidae, Apini) and *Hypotrigena ruspollii* (Magretti) (Hymenoptera: Apidae, Meliponini) as models. The bees were put in contact for 24 h with the recommended concentration of Sunphosate 360 SL, a glyphosate-based herbicide, 2× the recommended concentration, or distilled water as control. The effect of the herbicide on the bees was compared to the effect of a lambda-cyhalothrin insecticide. Generally, more bees died after contact with plants freshly sprayed with the herbicide than on herbicide-treated filter paper. In both cases, more bees died after contact with the higher concentration of the herbicide. These findings suggest that beneficial insects, specifically *A. mellifera* and *H. ruspollii*, may get killed if they are sprayed upon or come into contact with plants that have been freshly sprayed with (more than) the recommended concentration of glyphosate-based herbicides. Therefore, it is important to restrict access and use of such herbicides to trained personnel who will comply with spraying guidelines, that is, recommended concentrations and timing of spray. Spraying at a time when insects are flying about may be detrimental to beneficial insects such as pollinator bees, parasitoids, and predators.

## Introduction

Herbicides are pesticides formulated to control weeds in agroecological systems (Kraehmer et al., 2014a,b). Among the most common and widely used are glyphosate-based herbicides. It has been the top herbicide in use in Europe, USA, and other parts of the world in recent years (European Commission, 2007; EPA, 2012; Myers et al., 2016). South Africa used about 8 million litres of glyphosate-based herbicides between 2005 and 2012 (African Centre

for Biodiversity, 2012). Elsewhere in sub-Saharan Africa, particularly in Ghana, there are no readily available data on the use of glyphosate-based herbicides. However, farmers in both rural and peri-urban communities rampantly use this chemical purposely for clearing weeds. The farmers opt for glyphosate-based herbicides for land clearance because they find the herbicides cheaper and less laborious to use than the traditional manual or mechanised land clearing methods. In fact, even in cities, many residents use glyphosate-based herbicides to clear weeds around their homes (Howe et al., 2004; J Abraham, pers. obs.).

The rise in the use of commercially formulated glyphosate-based herbicides is alarming owing to several studies pointing to damaging effects on several organisms including frogs (Howe et al., 2004; Meza-Joya et al., 2013), fishes

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(do Carmo Langiano & Martinez, 2008; Marques et al., 2014, 2015; Moreno et al., 2014) and insects (Kale et al., 1995; Kaya et al., 2000). Apparently, the surfactants or adjuvants added to the formulation could contribute to these effects. In fact, it has been demonstrated that polyoxyethylene tallow amine (POEA), a glyphosate surfactant, contributes to the high toxicity of glyphosate-based products (Perkins et al., 2000; Howe et al., 2004; Comstock et al., 2007; Mann et al., 2009) and that there could be a synergistic effect of the surfactant and other ingredients of glyphosate-based products (Comstock et al., 2007). Aside POEA, other surfactants such as nonylphenol ethoxylate (NPE) and alcohol alkoxyolate have been found to be toxic (Mann & Bidwell, 2001). It is even feared that glyphosate is 'probably carcinogenic to humans' because of sufficient evidence for its carcinogenicity in experimental animals, limited evidence for its carcinogenicity in humans, and strong evidence that glyphosate can operate through two key characteristics of known human carcinogens (Guyton et al., 2015; IARC, 2015; Myers et al., 2016). However, this assertion seems not to be conclusive, as several conflicting reports have also been made (European Food Safety Authority, 2015; Vandenberg et al., 2017). Moreover, reports indicate that glyphosate is capable of causing deformities in embryos of frogs and chicken (Paganelli et al., 2010). In areas where glyphosate-based herbicides are heavily applied because glyphosate-resistant crops are cultivated (e.g., Argentina and Paraguay), there have been negative impacts on human health, including birth defects in Argentina (Antoniou et al., 2012).

On the basis of these reports, perceived or real, some countries (e.g., Colombia) have instituted restrictions on the use of glyphosate-based pesticides (African Centre for Biodiversity, 2015; Hansen, 2016), and relevant institutions in Argentina (Sarich, 2015) and Brazil, for instance, have suggested banning the use of glyphosate (African Centre for Biodiversity, 2015). In Bermuda and Sri Lanka, the product is banned, whereas in Germany, there was a call for 'the supply to and use by private persons to be banned for precautionary reasons' by state consumer protection ministers (Hansen, 2016). In spite of this call, the European Union, including Germany, has given approval for the use of glyphosate for the period 16 December 2017 to 15 December 2022 under strict conditions including 'paying particular attention to the risk to diversity and abundance of non-target terrestrial arthropods and vertebrates via trophic interactions' (European Commission, 2018).

Aside all the perceived or real effects of glyphosate-based pesticides, this paper is concerned about the effect commercially formulated glyphosate-based pesticides could have on non-target beneficial insects. Although

earlier studies have demonstrated that ingestion of sub-lethal doses of glyphosate by honeybees impairs their sensitivity to nectar reward, associative learning (Herbert et al., 2014), and cognitive capacities (Balbuena et al., 2015), the effect of commercial formulations on bees is not fully known. It is important to note that, in Ghana, any possible effect on beneficial insects could be aggravated by the users, because many of the rural farmers are not educated on the correct use of the chemical and so seldomly apply the correct concentration (J Abraham, pers. obs.). Beneficial insects are likely to be caught in direct contact with the herbicide at the time of application or immediately after application. It is known that pollinators could be exposed to pesticides through droplets directly on their cuticle or ingestion, and suffer consequences including death (Egan et al., 2014; Sanchez-Bayo & Goka, 2014, 2016). Beneficial insects such as pollinators enhance crop yield, whereas parasitoids and predators protect crops against insect pests. These natural enemies are especially useful for insect pest control in situations where the insect pests are less accessible by insecticides (Thomson, 2012). The increasing number of invasive insect pests – such as *Drosophila suzukii* (Matsumura) in Europe, USA, and parts of South America (Hauser, 2011; Abraham et al., 2015), and *Spodoptera frugiperda* (JE Smith) in Africa recently (Goergen et al., 2016) – makes it important to conserve beneficial insects that could be used for biological control. Continuous use of pesticides (both insecticides and herbicides) in crop production is developing resistant pest populations and reducing the population of beneficial arthropods such as parasitoids, insect predators, insect pollinators, and earthworms (Gill & Garg, 2014).

Several studies have shown that insects (both pests and beneficial) are killed when in direct contact with insecticides or in contact with insecticide residues days after application (Blibech et al., 2015; Ihara et al., 2017; Ullah & Shad, 2017). Although insecticides are formulated to kill obnoxious insects, improper use could result in detrimental effects on non-target organisms (Fishel, 2005). It has been shown that only about 1% of applied insecticides reach their target insect pests, whereas the remaining majority contaminate the environment (Carriger et al., 2006). Moreover, most of the chemicals used for pest control are non-selective and highly toxic thereby killing both pest and beneficial insects. Ideally, insecticides should be toxic only to the target insects, be biodegradable, and eco-friendly (Sanchez-Bayo, 2001; Rosell et al., 2008). A good insecticide should be effective against its target pest but should not have detrimental effects on predators and parasitoids of insect pests (Fernandes et al., 2010).

This study aimed at investigating the effect of a glyphosate-based herbicide on beneficial insects using *Apis mellifera* L. (Hymenoptera: Apidae, Apini) and *Hypotrigona ruspollii* (Magretti) (Hymenoptera: Apidae, Meliponini) as models. The effect of the glyphosate-based herbicide Sunphosate 360 SL on these bees was compared to the effect of a known insect killer, lambda-cyhalothrin, on the bees. *Apis mellifera* and *H. ruspollii* were used as model insects because bees are the most important beneficial insect species offering pollination services in African agroecological systems (Eardley, 2004; Mensah & Kudom, 2010; Mathiasson et al., 2015; Stein et al., 2017). It is important to investigate the effect of pesticides on pollinators because insect pollination accounts for about 67% of all pollinated crops (Park et al., 2012) and honeybees are the most economically valuable pollinators (Klein et al., 2007). Aside pollination services, both *A. mellifera* and *H. ruspollii* produce honey (Eardley & Kwapong, 2013; Mathiasson et al., 2015).

## Materials and methods

### Bees

Worker *A. mellifera* were obtained from the apiary of the College of Agriculture Education, University of Education, Winneba (UEW), Ghana. The apiary is situated in a tropical environment at 23–29 °C, ca. 70 ± 5% r.h., and ca. L12:D12 photoperiod. The bees were collected from their hives and kept in 30 × 30 × 30 cm BugDorm cages (Entomopraxis, Barcelona, Spain) placed in a laboratory at 25–27 °C, 70 ± 5% r.h., and ca. L12:D12 photoperiod. The bees were fed on 10% sucrose soaked in pieces of cotton and placed inside the cages until they were used for the experiments. Likewise, worker *H. ruspollii* were collected from natural hives within the botanic garden of the School of Biological Sciences, College of Agriculture and Natural Sciences, University of Cape Coast (UCC), Ghana. The prevailing environmental conditions in the botanic garden at UCC were similar to those at the apiary at UEW. The *H. ruspollii* were placed under the same laboratory conditions and treated the same as the *A. mellifera* before the experiments.

### Pesticides

Sunphosate 360 SL, a commercially formulated glyphosate-based herbicide containing 360 g of glyphosate l<sup>-1</sup> in the form of 480 g l<sup>-1</sup> isopropylamine salt of soluble liquid (Zhejiang Xinan Chemical Industrial Group, Jiande, Zhejiang, China), was used at the concentration recommended by the manufacturer (10 ml l<sup>-1</sup> of water) and 2× the recommended concentration. The experiment on *H. ruspollii* was repeated with a commercially formulated

insecticide, PAWA 2.5 EC, containing 25 g of lambda-cyhalothrin l<sup>-1</sup> (Chemico, Tema, Ghana) as a positive control, to compare the toxicity of the herbicide. Therefore, only the recommended concentration (4 ml l<sup>-1</sup> of water) was used. Distilled water was used as a negative control.

### Effect of freshly sprayed glyphosate-based herbicide on bees

Flower-bearing branches of *Senna siamea* (Lam.) Irwin et Barneby (Fabaceae) were cut and potted following a modified design from Cowles et al. (2015). Each potted plant was placed in a 30 × 30 × 30 cm BugDorm cage (n = 9). Using a handheld 450-ml sprayer bottle (Taizhou Youfeng Plastics, Taizhou, Zhejiang, China), the plants were sprayed manually with the recommended concentration of the glyphosate-based herbicide until run-off following Blibech et al. (2015). Ten worker *A. mellifera* were placed inside each cage and allowed to interact with the freshly sprayed plants for 24 h following Cowles et al. (2015). A piece of cotton soaked in 10% sucrose was placed inside each cage to prevent the bees from starving. Dead bees were confirmed after another 24 h. Through interactions with the freshly sprayed plants, the bees contaminated themselves topically and/or orally with droplets of the herbicide. The same experimental set-up was repeated with 2× the concentration of the glyphosate-based herbicide. As control, the plants (n = 9) were sprayed with distilled water until run-off and 10 *A. mellifera* each were placed in each cage as described above. A similar experimental set-up was used with *H. ruspollii* for control, recommended concentration, and 2× the recommended concentration (n = 10 per treatment).

### Effect of pesticide (herbicide and insecticide) residues on *Hypotrigona ruspollii*

In another experiment, we tested the effect of residues of the glyphosate-based herbicide on *H. ruspollii*. In this experiment, pieces of filter paper (Hangzhou Special Paper Industry, Hangzhou, Zhejiang, China) were cut to 2 × 2 cm (n = 10) and soaked in the herbicide following modified designs from Ksentini et al. (2010) and Roubos et al. (2014). The filter papers were air-dried in the laboratory. They were then placed singly in 50-ml fly vials (DD Biolab, Barcelona, Spain) with the vials lying by their sides. In each vial, 10 workers of *H. ruspollii* were placed and covered with a cotton stopper. A piece of cotton soaked with 10% sucrose was placed inside the vial to prevent the stingless bees from starving. The stingless bees were left in contact with the soaked filter paper for 24 h. This was repeated 10× (n = 10). The experiment was repeated with filter paper soaked in distilled water as control (n = 10).

Dead stingless bees were confirmed after another 24 h. The same experimental set-up was used with lambda-cyhalothrin at the recommended concentration (4 ml l<sup>-1</sup> of water).

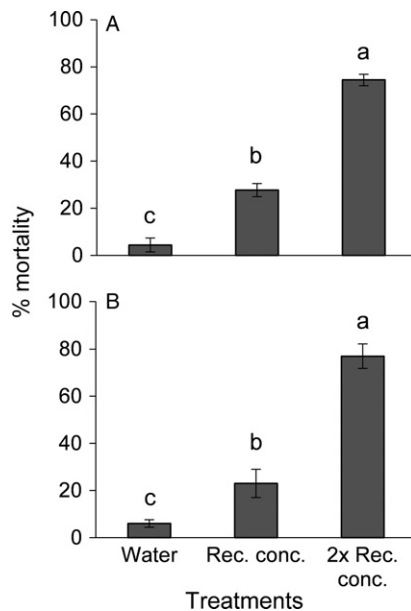
#### Data analysis

Mortality rates (%) of the bees at the two herbicide concentrations and water were subjected to ANOVA. Two-sample t-tests were conducted where significant differences were obtained. Mortality (%) of *H. ruspolti* was analysed by one-sample t-test, as the insecticide caused 100% mean mortality. Analyses were performed with Minitab v.16 (Minitab, State College, PA, USA).

## Results

#### Effect of freshly sprayed glyphosate-based herbicide on bees

The glyphosate-based herbicide had a strong effect on *A. mellifera* mortality ( $F_{2,24} = 171.50$ ,  $P < 0.001$ ; Figure 1A). On plants freshly sprayed with 2× the recommended concentration, mortality was higher than on plants sprayed with the recommended concentration (74 vs. 28%;  $t = 12.66$ ,  $P < 0.001$ ; Figure 1A). In comparison,



**Figure 1** Mean ( $\pm$  SE) mortality (%) of (A) *Apis mellifera* ( $n = 9$  replicates with 10 bees each) and (B) *Hypotrigena ruspolti* ( $n = 10$  replicates with 10 bees each) after direct contact for 24 h in a cage with flower-bearing branches of *Senna siamea* freshly sprayed with a commercially formulated glyphosate-based herbicide at 1 or 2× the recommended concentration, or water as control. Means capped with different letters are significantly different (two-sample t-test:  $P < 0.05$ ). Rec. conc. = Recommended concentration.

mean mortality on water-treated control plants (4%) was negligible (Figure 1A).

The herbicide had a similar effect on the mortality of *H. ruspolti* ( $F_{2,27} = 63.32$ ,  $P < 0.001$ ; Figure 1B). When in contact with 2× the recommended concentration, mortality was higher than when in contact with the recommended concentration (77 vs. 23%;  $t = 6.83$ ,  $P < 0.001$ ) and control mortality was very low (6%; Figure 1B).

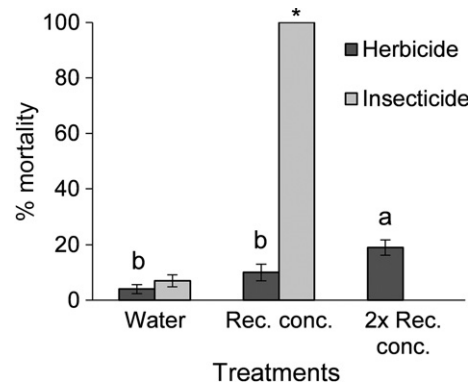
#### Effect of herbicide and insecticide residues on *Hypotrigena ruspolti*

When *H. ruspolti* were in contact with air-dried filter paper soaked with Sunphosate 360 SL or distilled water as control, there were differences in mortality ( $F_{2,27} = 8.90$ ,  $P = 0.001$ ; Figure 2). Mortality was higher when in contact with 2× the recommended concentration than when in contact with the recommended concentration (19 vs. 10%;  $t = 2.21$ ,  $P = 0.04$ ). Control mortality was 4%, which was not significantly lower than the mortality at the recommended concentration ( $t = 1.77$ ,  $P = 0.10$ ; Figure 2).

All bees died (100% mortality) when in contact with filter paper treated with lambda-cyhalothrin at the recommended concentration (Figure 2). In this experiment, control mortality was 7% (Figure 2).

## Discussion

High demands for food by the growing world population of man have led to an increase in the use of pesticides to



**Figure 2** Mean ( $\pm$  SE) mortality (%) of *Hypotrigena ruspolti* after contact for 24 h in fly tubes with air-dried filter papers treated with a glyphosate-based herbicide at 1 or 2× the recommended concentration, and a lambda-cyhalothrin insecticide at the recommended concentration ( $n = 10$  replicates with 10 bees each). Water was used as control. Means capped with different letters are significantly different (two-sample t-test:  $P < 0.05$ ); the mean capped with the asterisk is significantly different from all others (t-test:  $P < 0.001$ ). Rec. conc. = Recommended concentration.

enhance crop production. Pesticide use has become an indispensable component of agriculture for eradicating arthropod pests and weeds, and for protecting stored crops (Gill & Garg, 2014). Pesticide production is ever increasing and its use may continue to increase (Migheli, 2017; Damalas & Koutroubas, 2018). For instance, the use of glyphosate-based herbicides has increased due to the advent of genetically modified glyphosate-resistant crops (Howe et al., 2004, African Centre for Biodiversity, 2012, 2015; Myers et al., 2016) and this has led to the development of glyphosate-resistant weeds (Myers et al., 2016). In many sub-Saharan African countries, for instance Ghana, agricultural land preparation increasingly depends on glyphosate-based herbicides. The high demand for these herbicides is likely driving their high production. The rapidly increasing production of pesticides and their associated problems have, however, triggered efforts at minimising the use of pesticides in agriculture without reducing yield (Lechenet et al., 2014; Pretty & Bharucha, 2015; Sgolastra et al., 2017; Shuqin & Fang, 2018).

In this laboratory study, when workers of *A. mellifera* and *H. ruspolti* had direct contact with plants freshly sprayed with Sunphosate 360 SL at the manufacturer's recommended concentration (10 ml l<sup>-1</sup>), about 25% of the bees died within 24 h. At 2× the recommended concentration, about 3× as many bees (ca. 75%) were killed within 24 h. This double concentration resembles the concentration applied by many rural farmers in Ghana who have no education on the use of glyphosate-based herbicides. The mortality at the recommended concentration is considerable, compared to the ca. 5% mortality when in contact with the water control. It is important to note that the mortality of the stingless bees was lower when in contact with air-dried herbicide-treated filter paper than when in contact with freshly sprayed plants. As the filter paper contained only residues, about 19% of *H. ruspolti* died after contact with double the recommended concentration of the herbicide, and even fewer died after contact with the recommended concentration or with the control. This suggests that if bees get into contact with dry residue of glyphosate-based herbicides, they may not be killed. Therefore, the timing of application is important – spraying should be done when insects are not flying around. Residue of the insecticide, lambda-cyhalothrin, was still highly toxic: all stingless bees died after contact with filter paper treated at the recommended concentration. Lambda-cyhalothrin is a potent pyrethroid contact insecticide, disrupting the normal functioning of the nervous system (National Pesticide Information Center, 2001). *Hypotrigona ruspolti* were observed trembling before death.

The mechanism by which the bees died from the glyphosate-based herbicide is unknown. As the bees were in contact with the herbicide over a period of 24 h, they may have died from contact and/or ingestion toxicity. The study was conducted in confinement (fly tubes and cages) in a laboratory and so mortality may differ under field conditions. Studies with the fruitfly *Drosophila melanogaster* Meigen exposed to 0.169 mg l<sup>-1</sup> of glyphosate showed that a mutation was induced (Kaya et al., 2000), whereas a glyphosate-based formulation caused sex-linked recessive lethal mutations in *Drosophila* sp. (Kale et al., 1995). It is not known whether such lethal mutation could cause the death of adult bees within 24 h. The cause of death of bees upon contact with the glyphosate-based herbicide would be an interesting subject for further investigations.

Although the toxicity of glyphosate is not targeted at insects (Mesnage et al., 2015; Myers et al., 2016; Vandenberg et al., 2017), data from Thompson et al. (2014) suggest that between 6% and 18% of eggs or larvae in glyphosate-treated broods of *A. mellifera* died after dosing with ca. 1 l of 75–300 mg l<sup>-1</sup> of technical grade glyphosate for 5 days. This was about 2–3% above mortality in control experiments (Thompson et al., 2014). In our study, mortality after contact with the recommended concentration was about 10–28% and about 6–24% above mortality in control experiments. On the basis of these mortality rates, the glyphosate-based herbicide falls in the category of pesticides classified as being 'slightly harmful' to beneficial insects if the recommended concentrations are applied (Thomson, 2012). It becomes 'harmful' if concentrations higher than the recommended are used (Thomson, 2012). In 1994, the World Health Organization classified technical grade glyphosate and glyphosate formulation (Roundup) as 'slightly toxic' to bees when applied either orally or topically (WHO, 1994). The observed mortality on contact with the recommended concentration appears to be low and may not be biologically significant, but the effect of repeated exposure over a long term on bees has not been explored exhaustively (Herbert et al., 2014; Balbuena et al., 2015).

In conclusion, a key message of our study is that glyphosate-based herbicides have the potential to kill beneficial insects especially at concentrations higher than recommended. Relevant authorities should regulate access to and use of glyphosate and glyphosate-based herbicides to prevent potential damages to the environment. Beneficial insects are important for food (honey) production, provision of pollination services, and pest control – without them the world faces food insecurity. It is important to protect all beneficial insects from any possible harm from pesticides.

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## References

- Abraham J, Zhang A, Angeli S, Abubeker S, Michel C et al. (2015) Behavioral and antennal responses of *Drosophila suzukii* (Diptera: Drosophilidae) to volatiles from fruit extracts. *Environmental Entomology* 44: 356–367.
- African Centre for Biodiversity (2012) Glyphosate in SA: Risky pesticide at large and unregulated in our soil and water. Available at: <https://acbio.org.za/en/media-release/2012/glyphosate-sa-unregulated-and-contaminating-our-water-and-soil-acb-calls> (accessed 18 November 2017).
- African Centre for Biodiversity (2015) What next after a ban on glyphosate – more toxic chemicals and GM crops? Or the transformation of global food systems? Available at: <https://acbio.org.za/wp-content/uploads/2015/06/Glyphosate-report-2015.pdf> (accessed 17 November 2017).
- Antoniou M, Robinson C & Fagan J (2012) GMO Myths and Truths: An Evidence-Based Examination of the Claims Made for the Safety and Efficacy of Genetically Modified Crops, v.1.3b. Earth Open Source, London, UK. Available at: [https://thebigcarrot.ca/fileadmin/pdfs/GMO\\_Myths\\_and\\_Truths\\_1.3b.pdf](https://thebigcarrot.ca/fileadmin/pdfs/GMO_Myths_and_Truths_1.3b.pdf) (accessed 21 February 2018).
- Balbuena MS, Tison L, Hahn M-L, Greggers U, Menzel R & Farina WM (2015) Effects of sublethal doses of glyphosate on honeybee navigation. *Journal of Experimental Biology* 218: 2799–2805.
- Blibech I, Ksantini M, Jardak T & Bouaziz M (2015) Effect of insecticides on *Trichogramma* parasitoids used in biological control against *Prays oleae* insect pest. *Advances in Chemical Engineering and Science* 5: 362–372.
- do Carmo Langiano V & Martinez CBR (2008) Toxicity and effects of a glyphosate-based herbicide on the Neotropical fish *Prochilodus lineatus*. *Comparative Biochemistry and Physiology C* 147: 222–231.
- Carriger JF, Rand GM, Gardinali PR, Perry WB, Tompkins MS & Fernandez AM (2006) Pesticides of potential ecological concern in sediment from South Florida canals: an ecological risk prioritization for aquatic arthropods. *Soil and Sediment Contamination* 15: 21–45.
- Comstock BA, Sprinkle SL & Smith GR (2007) Acute toxic effects of round-up herbicide on wood frog tadpoles (*Rana sylvatica*). *Journal of Freshwater Ecology* 22: 705–708.
- Cowles RS, Rodriguez-Saona C, Holdcraft R, Loeb GM, Elsensohn JE & Hesler SP (2015) Sucrose improves insecticide activity against *Drosophila suzukii* (Diptera: Drosophilidae). *Journal of Economic Entomology* 108: 640–653.
- Damalas CA & Koutroubas SD (2018) Current status and recent developments in biopesticide use. *Agriculture* 8: 1–6.
- Eardley CD (2004) Taxonomic revision of the African stingless bees (Apoidea: Apidae: Apinae: Meliponini). *African Plant Protection* 10: 63–96.
- Eardley C & Kwapong P (2013) Taxonomy as a tool for conservation of African stingless bees and their honey. *Pot-Honey: A Legacy of Stingless Bees* (ed. by P Vit, SRM Pedro & DW Roubik), pp. 261–268. Springer, New York, NY, USA.
- Egan JF, Bohnenblust E, Goslee S, Mortensen D & Tooker J (2014) Herbicide drift can affect plant and arthropod communities. *Agriculture, Ecosystems and Environment* 185: 77–87.
- EPA (2012) Pesticide industry sales and usage: 2008-2012 market estimates. Available at: [https://www.epa.gov/sites/production/files/2017-01/documents/pesticides-industry-sales-usage-2016\\_0.pdf](https://www.epa.gov/sites/production/files/2017-01/documents/pesticides-industry-sales-usage-2016_0.pdf) (accessed 17 November 2017).
- European Commission (2007) The use of plant protection products in the European Union. Available at: <http://ec.europa.eu/eurostat/documents/3217494/5611788/KS-76-06-669-EN.pdf> (accessed 17 November 2017).
- European Commission (2018) Plants: Glyphosate. Available at: [https://ec.europa.eu/food/plant/pesticides/glyphosate\\_en](https://ec.europa.eu/food/plant/pesticides/glyphosate_en) (accessed 22 February 2018).
- European Food Safety Authority (2015) Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. *EFSA Journal* 13: 4302.
- Fernandes FL, Bacci L & Fernandes MS (2010) Impact and selectivity of insecticides to predators and parasitoids. *Entomobrasiliis* 3: 1–10.
- Fishel FM (2005) Pesticides Effects on Non-Target Organisms. Pesticide Information Office, PI-85. Florida Cooperative Extension Service, UF/IFAS, Gainesville, FL, USA.
- Gill HK & Garg H (2014) Pesticides: environmental impacts and management strategies. *Pesticides-Toxic Effects* (ed. by S Solonieski), pp. 187–230. Intech, Rijeka, Croatia.
- Goergen G, Kumar PL, Sankung SB, Togola A & Tamò M (2016) First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS ONE* 11: e0165632.
- Guyton KZ, Loomis D, Grosse Y, El Ghissassi F, Benbrahim-Talaa L et al. (2015) Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *Lancet Oncology* 16: 490–491.
- Hansen M (2016) Monsanto, RoundUp and Junk Science. Huffington Post, The Blog. Available at: [https://www.huffingtonpost.com/michael-hansen-phd/monsanto-roundup-and-junk-science\\_b\\_7473736.html](https://www.huffingtonpost.com/michael-hansen-phd/monsanto-roundup-and-junk-science_b_7473736.html) (accessed 18 November 2017).
- Hauser M (2011) A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. *Pest Management Science* 67: 1352–1357.
- Herbert LT, Vázquez DE, Arenas A & Farina WM (2014) Effects of field-realistic doses of glyphosate on honeybee appetitive behaviour. *Journal of Experimental Biology* 217: 3457–3464.

- Howe CM, Berrill M, Bruce DP, Caren CH, Werry K & Veldhoen N (2004) Toxicity of glyphosate-based pesticides to four North American frog species. *Environmental Toxicology and Chemistry* 23: 1928–1938.
- IARC [International Agency for Research on Cancer] (2015) Monograph 112: Glyphosate. Available at: <http://monographs.iarc.fr/ENG/Monographs/vol112/mono112-10.pdf> (accessed 18 November 2017).
- Ihara M, Buckingham SD, Matsuda K & Sattelle DB (2017) Toxicity of insecticides targeting nicotinic acetylcholine receptors. *Current Medicinal Chemistry* 24: 2925–2934.
- Kale PG, Petty BT Jr, Walker S, Ford JB, Dehkordi N et al. (1995) Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. *Environmental and Molecular Mutagenesis* 25: 148–153.
- Kaya B, Creus A, Yanikoğlu A, Cabré O & Marcos R (2000) Use of the *Drosophila* wing spot test in the genotoxicity testing of different herbicides. *Environmental and Molecular Mutagenesis* 36: 40–46.
- Klein A-M, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA et al. (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B* 274: 303–313.
- Kraehmer H, Laber B, Rosinger C & Schulz A (2014a) Herbicides as weed control agents: state of the art. I. Weed control research and safener technology: the path to modern agriculture. *Plant Physiology* 166: 1119–1131.
- Kraehmer H, van Almsick A, Beffa R, Dietrich H, Eckes P et al. (2014b) Herbicides as weed control agents: state of the art. II. Recent achievements. *Plant Physiology* 166: 1132–1148.
- Ksentini I, Jardak T & Zeghal N (2010) *Bacillus thuringiensis*, deltamethrin and spinosad side-effects on three *Trichogramma* species. *Bulletin of Insectology* 63: 31–37.
- Lechenet M, Bretagnolle V, Bockstaller C, Boissinot F, Petit M-S et al. (2014) Reconciling pesticide reduction with economic and environmental sustainability in arable farming. *PLoS ONE* 9: e97922.
- Mann RM & Bidwell JR (2001) The acute toxicity of agricultural surfactants to tadpoles of four Australian and two exotic frogs. *Environmental Pollution* 114: 195–205.
- Mann RM, Hyne RV, Choung CB & Wilson SP (2009) Amphibians and agricultural chemicals: review of the risks in a complex environment. *Environmental Pollution* 157: 2903–2927.
- Marques A, Guilherme S, Gaivão I, Santos MA & Pacheco M (2014) Progression of DNA damage induced by a glyphosate-based herbicide in fish (*Anguilla anguilla*) upon exposure and post-exposure periods—insights into the mechanisms of genotoxicity and DNA repair. *Comparative Biochemistry and Physiology C* 166: 126–133.
- Marques A, Guilherme S, Gaivão I, Santos MA & Pacheco M (2015) Erratum. *Comparative Biochemistry and Physiology C* 168: 1.
- Mathiasson ME, Kwapong PK, Wubah DA & Wubah JA (2015) Early colony development of an Equatorial Afrotropical stingless bee (*Hypotrigena* sp.) in a new habitat. *Journal of Young Investigators* 29: 11–17.
- Mensah BA & Kudom A (2010) Relating bee activity to pollination of *Luffa aegyptiaca* Mill. in Ghana. *Journal of Apicultural Research* 49: 192–196.
- Mesnager R, Defarge N, Spiroux de Vendômois J & Séralini GE (2015) Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. *Food and Chemical Toxicology* 84: 133–153.
- Meza-Joya FL, Ramírez-Pinilla MP & Fuentes-Lorenzo JL (2013) Toxic, cytotoxic, and genotoxic effects of a glyphosate formulation (Roundup®SL-Cosmoflux®411F) in the direct-developing frog *Eleutherodactylus johnstonei*. *Environmental and Molecular Mutagenesis* 54: 362–373.
- Migheli M (2017) Land ownership and use of pesticides. Evidence from the Mekong Delta. *Journal of Cleaner Production* 145: 188–198.
- Moreno NC, Sofia SH & Martinez CB (2014) Genotoxic effects of the herbicide Roundup Transorb and its active ingredient glyphosate on the fish *Prochilodus lineatus*. *Environmental Toxicology and Pharmacology* 37: 448–454.
- Myers JP, Antoniou MN, Blumberg B, Carroll L, Colborn T et al. (2016) Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. *Environmental Health* 15: 19.
- National Pesticide Information Center (2001) Lambda Cyhalothrin. General Fact Sheet. Available at: [http://npic.orst.edu/fact\\_sheets/l\\_cyhalogen.pdf](http://npic.orst.edu/fact_sheets/l_cyhalogen.pdf) (accessed 20 November 2017).
- Paganelli A, Gnazzo V, Acosta H, Lopez SL & Carrasco AE (2010) Glyphosate-based herbicides produce teratogenic effects on vertebrates by impairing retinoic acid signaling. *Chemical Research in Toxicology* 23: 1586–1595.
- Park M, Danforth B, Losey J, Biddinger D, Vaughan M et al. (2012) Wild Pollinators of Eastern Apple Orchards and How to Conserve Them. Cornell University, Penn State University, and The Xerces Society. Available at: <http://www.northeastipm.org/park2012> (accessed 14 June 2013).
- Perkins PJ, Boermans HJ & Stephenson GR (2000) Toxicity of glyphosate and triclopyr using the frog embryo teratogenesis assay – *Xenopus*. *Environmental Toxicology and Chemistry* 19: 940–945.
- Pretty J & Bharucha ZP (2015) Integrated pest management for sustainable intensification of agriculture in Asia and Africa. *Insects* 6: 152–182.
- Rosell G, Quero C, Coll J & Guerrero A (2008) Biorational insecticides in pest management. *Journal of Pesticide Science* 33: 103–121.
- Roubos CR, Rodriguez-Saona C, Holdcraft R, Mason KS & Isaacs R (2014) Relative toxicity and residual activity of insecticides used in blueberry pest management: mortality of natural enemies. *Journal of Economic Entomology* 107: 277–285.
- Sanchez-Bayo F (2001) Impacts of agricultural pesticides on terrestrial ecosystems. *Ecological Impacts of Toxic Chemicals* (ed. by F Sánchez-Bayo, PJ van den Brink & RM Mann), pp. 63–87. Bentham Science Publishers, Sharjah, United Arab Emirates.

- Sanchez-Bayo F & Goka K (2014) Pesticide residues and bees – a risk assessment. *PLoS ONE* 9: e94482.
- Sanchez-Bayo F & Goka K (2016) Impacts of pesticides on honey bees. *Beekeeping and Bee Conservation – Advances in Research* (ed. by E Chambo), pp. 77–97. InTech, Rijeka, Croatia.
- Sarich C (2015) 30,000 doctors in Argentina demand that glyphosate be banned. *Natural Society*. Available at: <http://naturalsociety.com/30000-doctors-in-argentina-demand-that-glyphosate-be-banned/> (accessed 18 November 2017).
- Sgolastra F, Porrini C, Maini S, Bortolotti L, Medrzycki P et al. (2017) Healthy honey bees and sustainable maize production: why not? *Bulletin of Insectology* 70: 156–160.
- Shuqin J & Fang Z (2018) Zero growth of chemical fertilizer and pesticide use: China's objectives, progress and challenges. *Journal of Resources and Ecology* 9: 50–58.
- Stein K, Coulibaly D, Stenchly K, Goetze D, Porembski S et al. (2017) Bee pollination increases yield quantity and quality of cash crops in Burkina Faso, West Africa. *Scientific Reports* 7: 17691.
- Thompson HM, Levine SL, Doering J, Norman S, Manson P et al. (2014) Evaluating exposure and potential effects on honeybee brood (*Apis mellifera*) development using glyphosate as an example. *Integrated Environmental Assessment and Management* 10: 463–470.
- Thomson L (2012) Pesticide Impacts on Beneficial Species. Fact-sheet May 2012, Grape and Wine Research and Development Corporation, Adelaide, Australia. Available at: <http://www.mvwi.com.au/items/511/2012-05-FS-Pesticide-Impacts2.pdf> (accessed 20 November 2017).
- Ullah S & Shad SA (2017) Toxicity of insecticides, cross-resistance and stability of chlorfenapyr resistance in different strains of *Oxycarenus hyalinipennis* Costa (Hemiptera: Lygaeidae). *Crop Protection* 99: 132–136.
- Vandenberg LN, Blumberg B, Antoniou MN, Benbrook CM, Carroll L et al. (2017) Is it time to reassess current safety standards for glyphosate-based herbicides? *Journal of Epidemiology and Community Health* 71: 613–618.
- WHO (1994) Glyphosate. *Environmental Health Criteria* 159, United Nations Environment Programme, International Labour Organisation, and World Health Organization, Geneva, Switzerland. Available at: <http://apps.who.int/iris/bitstream/10665/40044/1/9241571594-eng.pdf> (accessed 20 November 2017).