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RESEARCH

Lethal concentrations of glyphosate-based herbicide on nymphs of agroecosystem spider predator *Phylloneta impressa* L. Koch 1881

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ABSTRACT

Broad-spectrum herbicides containing glyphosate are one of the most widely used pesticides in the world. They appear to be only slightly toxic to model animals in laboratory experiments. We investigated the lethal effect of the glyphosate-based herbicide to the first nymphal instar of the comb-footed spider *Phylloneta impressa* L. Koch 1881, which is a common spider predator of agroecosystem pests. Lethal concentrations LC_{50} and LC_{90} were calculated 24 h after dorsal application of the recommended herbicide dosage in Potter laboratory spray tower. The concentration recommended by manufacturer killed almost 25% of tested spiders. The concentration that would kill 90% of spiders was calculated to be 2.34 times higher than the highest recommended concentration.

Key words: Beneficial organisms, LC_{50}, LC_{90}, lethal toxicity, Roundup, side effects.

INTRODUCTION

The broad-spectrum herbicide glyphosate (*N*-phosphonomethyl-glycine) is the most widely used herbicide in the world. It is an essential tool to increase agricultural productivity of crops (Baylis, 2000). It has been popular since the 1970s; however, its use is still rising because of the development of technologies combining glyphosate and the crops genetically modified to be resistant to glyphosate (Benbrook, 2012; Powles, 2014; Myers et al., 2016). Glyphosate was considered to be harmless to the environment (Rueppel et al., 1977; Smith and Oehme, 1992), however in March 2015, the International Agency for Research on Cancer retrained the glyphosate from a category "non-toxic" to a category "probably carcinogenic to humans" (Baylis, 2000; Guyton et al., 2015).

The negative effects of glyphosate have been reported across organisms from bacteria through invertebrates to vertebrates, including humans (Gill et al., 2018). In humans, the glyphosate can affect the cardiovascular system, cell physiology, and acts as an endocrine disruptor (Richard et al., 2005; Gress et al., 2015; Martini et al., 2016). In rats, Roundup containing glyphosate may impair fertility of males and form severe degenerative lesions (Owagboriaye et al., 2017). Especially in agricultural areas, glyphosate contaminates drinking water and affects aquatic ecosystems (Myers et al., 2016). In addition to direct mortality, the glyphosate-containing herbicides cause oxidative stress, behavioural changes, and can disrupt the diversity of intestinal microorganisms in aquatic animals (Hong et al., 2017; Talib et al., 2018; Yang et al., 2019). Considering global distribution, these herbicides can significantly affect the operation of terrestrial ecosystems and the role of beneficial animals such as predators and parasitoids (Székács and Darvas, 2012; Bøhn et al., 2014).

Spiders play an important role as predators in agroecosystems. They are generalist predators and, due to their extraintestinal digestion, they overkill prey, among which pests are significantly represented (Foelix, 2010; Pekár, 2012). Therefore, they have become a model group for studying the side effects of pesticides, including glyphosate-based herbicides. Many studies were focused on the sublethal effects of glyphosate. It was found that glyphosate formulation affects the reproduction and development of spiders, for example the ability of *Pardosa milvina* Hentz 1844 males to detect and react to females' pheromones (Benamú et al., 2010; Griesinger et al., 2011). Further, it reduced predation activity in wolf spiders (Korenko et al., 2016; Niedobová et al., 2019b; Lacava et al., 2021).

The foraging of two wolf spiders, *Tigrosa helluo* Walckenaer 1837 and *Pardosa milvina*, was affected contrastingly. While the presence of glyphosate allowed *T. helluo* to hunt prey faster with the same number of lunges, *P. milvina* maintained a uniform hunting time, but with a larger number of lunges (Rittman et al., 2013). "Positive" effect was also shown in the experiment by Behrend and Rypstra (2018), where exposed individuals showed higher activity and hunting success. The negative impact of herbicides on spider communities may not be linked only to their primary toxicity, but also to decline in vegetation height, density, and the increasing representation of dead plants (Haughton et al., 2001; Bell et al., 2002). Glyphosate affects ecosystems and model organisms at a much more complex way than was previously thought. The reaction depends on the model species, the concentration of herbicide and the method of application. Further research on lethal and sublethal effects is therefore still needed.

In our study, we tested the lethal effect of eight concentrations of Roundup on the first nymphal instar of comb-footed spider *Phylloneta impressa* L. Koch 1881. This theridiid spider is often used as a model organism in agroecosystem experiments. Recently, the content of pesticides in the body of adults as well as in the web and cocoon were analysed (Erban et al., 2020). Neonicotinoids negatively affect its ability to disperse and colonize new ecosystems (Řezáč et al., 2019). This spider is also known for its ability due to silken retreat to avoid fresh residues of permethrin and phosalone (Pekár and Haddad, 2005; Michalková and Pekár, 2009). However, previous studies were restricted to the effect of pesticides on adult *Phylloneta impressa*, and nothing is known about the impact of pesticides on supposedly more sensitive ontogenetic stages of this natural pest predator in agroecosystems.

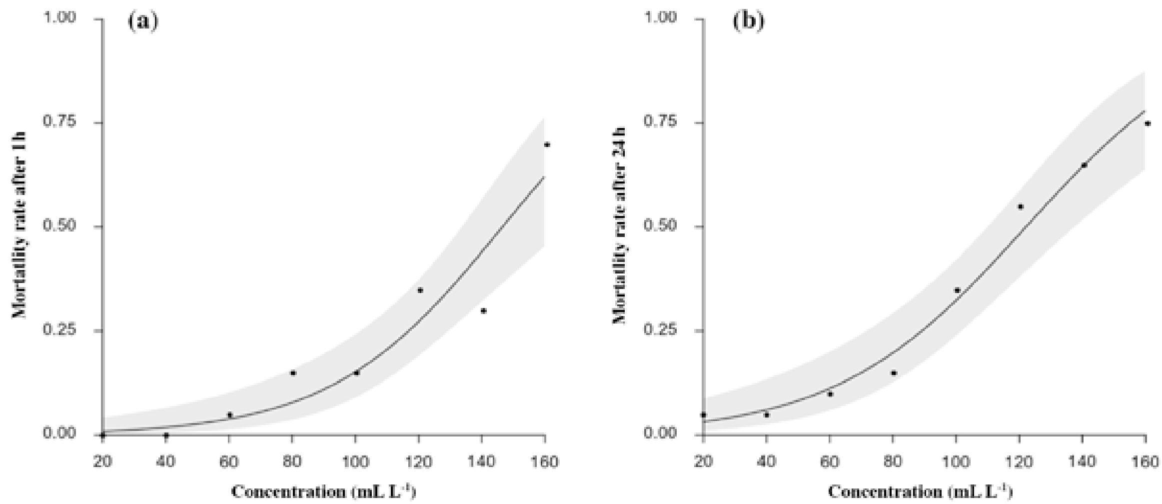
MATERIALS AND METHODS

We studied the effect of eight concentrations of herbicide containing glyphosate on the mortality of nymphs of *Phylloneta impressa*, a very common comb-footed spider in Holarctic agroecosystems. In September 2019 we collected nests with adult females and offspring from flixweed *Descurainia sophia* L. Prantl 1891, in several conventional agroecosystems in the vicinity of Hostivice town (50.074N, 14.217E) near Prague, Czech Republic. We kept nymphs separately in vials in the refrigerator (temperature: 5 °C, humidity: 80%). As glyphosate we used Roundup ACTIVE (Monsanto, Antwerp, Belgium). It contains 229 g propan-2-amine (IPA) salt of *N*-phosphonomethyl-glycine per litre of the solution (1.004 mol dm^{-3}). Eight concentrations of Roundup were tested in this experiment: 20, 40, 60, 80, 100, 120, 140 and 160 mL L^{-1} (molar concentrations: 16.7, 33.5, 50.2, 66.9, 83.6, 100.4, 117.1 and 133.8 mmol L^{-1}). We used distilled water as a control. The highest tested concentration, 160 mL L^{-1}, is double of the maximum recommended concentration by the manufacturer. The experiment was performed in room temperature (25 °C) in well ventilated laboratory. They were placed in a glass Petri dish (5 cm in diameter) with a piece of filter paper soaked in water to provide the humidity. One treatment (one Petri dish) contained five spiders, each from different maternal nest (total n = 180), however, each concentration was replicated four times (four Petri dishes by five individuals, together 20 spiders). The dosage of 400 L ha^{-1} was applied on each Petri dish using a Potter precision laboratory spray tower (Burkard Scientific, Uxbridge, UK) at 3 Pa. We recorded the mortality after 1 and 24 h after treatment. Data were analysed using free statistical software R (version 3.5.3) and packages "ggplot2" and "MASS" (R Core Team; R Foundation for Statistical Computing, Vienna, Austria). Data were analysed by applying generalized linear model (GLM) with binomial error structure and logit link (Pekár and Brabec, 2016).

RESULTS AND DISCUSSION

In laboratory conditions, glyphosate-based herbicide Roundup ACTIVE showed acute toxicity to comb-footed spider *Phylloneta impressa* juveniles. The 50% and 90% mortality concentrations were calculated from the mortalities of eight tested concentrations after 1 h (GLM, p = 2.297e-11; Chi-squared test) and after 24 h after exposure (GLM, p = 1.524e-13). After 1 h, the 50% mortality value was set at 145.9 (SE = 7.44) mL L^{-1} and 90% mortality at 204.9 (SE = 16.74) mL L^{-1}, while after 24 h the 50% mortality was only at 121.62 (SE = 6.4) mL L^{-1} and 90% mortality at 187.2 (SE = 14.35) mL L^{-1} (Figure 1). After 24 h the overall mortality rate increased by 13%. A clear concentration-response relationship was observed only after 24 h. Therefore, we conclude that 1 h is not an efficient time to evaluate the impact of glyphosate on the mortality of our model organism, 24 h appeared to be a more appropriate time lapse.

Figure 1. Concentration-response curves for eight concentrations of glyphosate-based herbicide and mortalities of juveniles of the spider *Phylloneta impressa*, recorded after 1 h (a) and 24 h (b).



Our study shows the negative impact of glyphosate-containing herbicide on juveniles of the comb-footed spider *P. impressa*, a natural enemy of insect pests in agroecosystems (Mader et al., 2016). However, the maximum concentration recommended by manufacturer killed less than 25% of tested spiders. The model allowed us to predict the concentration that would kill 90% of individuals, this value is 2.34 times higher than the highest recommended concentration.

The herbicides have been shown to have many sub-lethal effects on spiders. Godfrey and Rypstra (2018; 2019) showed, that different concentrations of the herbicide atrazine may significantly decrease the probability of egg-sac production of wolf spiders (Lycosidae) and may act as stressor for individuals. No changes were detected in predatory behaviour of lycosid spiders under exposure to the herbicide Roundup Biaktiv (Michalková and Pekár, 2009) or Roundup klasik Pro (Niedobová et al., 2019a). On the other side, solution of the glyphosate-based herbicide Glifoglex affected almost all aspects of life of orb-weaver spider *Alpaida veniliae* Keyserling 1865, from web building and fertility to prey consumption (Benamú et al., 2010). On the contrary, when exposed to a glyphosate-based Buccaneer plus solution, the wolf spider *Pardosa milvina* displayed higher activity and prey capture success (Behrend and Rypstra, 2018).

Concerning the lethal effects of glyphosate-based herbicides on spiders, they have not been recorded yet (Benamú et al., 2010; Behrend and Rypstra, 2018; Niedobová et al., 2019b; Lacava et al., 2021). However, the long-term mortality caused by residual exposure of glyphosate was observed on lycosid spider *P. milvina* (Evans et al., 2010).

Although we recorded mortality, it was relatively low. One of the possible explanations of this discrepancy with our observation could be that different model organisms were used in those studies, in particular adult females used in previous experiments are probably more resistant compared to juveniles. We consider that it is important to study the impact of pesticides on the most sensitive stages. However, we expect our results to differ from the mortality that would occur in the natural environment. In agroecosystems, nymphs are protected by a three-dimensional web with bell shaped retreat, which provides them with a shelter from spraying (Pekár, 2012).

CONCLUSIONS

Our research focused on the effect of eight different concentrations of broad-spectrum glyphosate-based herbicide on first nymphal instar of important spider predator in agroecosystems - *Phylloneta impressa*. This herbicide is considered to be only slightly toxic to the environment. In our experiment we detected the mortality caused by the formula of the Roundup ACTIVE herbicide. However, the manufacturer-recommended dose of herbicide caused less than 25% mortality. We recommend to test the effects of pesticides on juveniles that may be more sensitive to toxins than adults.

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REFERENCES

Baylis, A.D. 2000. Why glyphosate is a global herbicide: strengths, weaknesses and prospects. Pest Management Science 56:299-308.

Behrend, J.E., and Rypstra, A.L. 2018. Contact with a glyphosate-based herbicide has long-term effects on the activity and foraging of an agrobiont wolf spider. Chemosphere 194:714-721.

Bell, J.R., Haughton, A.J., Boatman, N.D., and Wilcox, A. 2002. Do incremental increases of the herbicide glyphosate have indirect consequences for spider communities? The Journal of Arachnology 30:288-297.

Benamú, M.A., Schneider, M.I., and Sánchez, N.E. 2010. Effects of the herbicide glyphosate on biological attributes of *Alpaida veniliae* (Araneae, Araneidae), in laboratory. Chemosphere 78:871-876.

Benbrook, C.M. 2012. Impacts of genetically engineered crops on pesticide use in the U.S. - the first sixteen years. Environmental Sciences Europe 24:24.

Bøhn, T., Cuhra, M., Traavik, T., Sanden, M., Fagan, J., and Primicerio, R. 2014. Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans. Food Chemistry 153:207-215.

Erban, T., Sopko, B., Vaclavikova, M., Tomesova, D., Halesova, T., and Rezac, M. 2020. Pesticide comparison of *Phylloneta impressa* (Araneae: Theridiidae) females, cocoons and webs with prey remnants collected from a rape field before the harvest. Pest Management Science 76:1128-1133.

Evans, S.C., Shaw, E.M., and Rypstra, A.L. 2010. Exposure to a glyphosate-based herbicide affects agrobiont predatory arthropod behaviour and long-term survival. Ecotoxicology 19:1249-1257.

Foelix, R.F. 2010. Biology of spiders. 3rd ed. Oxford University Press, New York, USA.

Gill, J.P.K., Sethi, N., Mohan, A., Datta, S., and Girdhar, M. 2018. Glyphosate toxicity for animals. Environmental Chemistry Letters 16:401-426.

Godfrey, J.A., and Rypstra, A.L. 2018. Impact of an atrazine-based herbicide on an agrobiont wolf spider. Chemosphere 201:459-465.

Godfrey, J.A., and Rypstra, A.L. 2019. Atrazine exposure shifts activity but has minimal effects on courtship in an agrobiont spider. Ecotoxicology 28:499-506.

Gress, S., Lemoine, S., Séralini, G.E., and Puddu, P.E. 2015. Glyphosate-based herbicides potently affect cardiovascular system in mammals: Review of the literature. Cardiovascular Toxicology 15:117-126.

Griesinger, L.M., Evans, S.C., and Rypstra, A.L. 2011. Effects of a glyphosate-based herbicide on mate location in a wolf spider that inhabits agroecosystems. Chemosphere 84:1461-1466.

Guyton, K.Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., et al. 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. The Lancet Oncology 16(5):490-491.

Haughton, A.J., Bell, J.R., Boatman, N.D., and Wilcox, A. 2001. The effect of the herbicide glyphosate on non-target spiders: Part II. Indirect effects on *Lepthyphantes tenuis* in field margins. Pest Management Science 57:1037-1042.

Hong, Y., Yang, X., Yan, G., Huang, Y., Zuo, F., Shen, Y., et al. 2017. Effects of glyphosate on immune responses and haemocyte DNA damage of Chinese mitten crab, *Eriocheir sinensis*. Fish and Shellfish Immunology 71:19-27.

Korenko, S., Niedobová, J., Kolářová, M., Hamouzová, K., Kysilková, K., and Michalko, R. 2016. The effect of eight common herbicides on the predatory activity of the agrobiont spider *Pardosa agrestis*. BioControl 61:507-517.

Lacava, M., García, L.F., Viera, C., and Michalko, R. 2021. The pest-specific effects of glyphosate on functional response of a wolf spider. Chemosphere 262:127785.

Mader, V., Birkhofer, K., Fiedler, D., Thorn, S., Wolters, V., and Diehl, E. 2016. Land use at different spatial scales alters the functional role of web-building spiders in arthropod food webs. Agriculture, Ecosystems & Environment 219:152-162.

Martini, C.N., Gabrielli, M., Codesido, M.M., and Del Vila, M.C. 2016. Glyphosate-based herbicides with different adjuvants are more potent inhibitors of 3T3-L1 fibroblast proliferation and differentiation to adipocytes than glyphosate alone. Comparative Clinical Pathology 25:607-613.

Michalková, V., and Pekár, S. 2009. How glyphosate altered the behaviour of agrobiont spiders (Araneae: Lycosidae) and beetles (Coleoptera: Carabidae). Biological Control 51:444-449.

Myers, J.P., Antoniou, M.N., Blumberg, B., Carroll, L., Colborn, T., Everett, L.G., et al. 2016. Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement. Environmental Health 15:19.

Niedobová, J., Krištofová, L., Michalko, R., Hula, V., Kýnický, J., and Brtnický, M. 2019a. Effects of glufosinate-ammonium herbicide and pod sealant on spider *Pardosa agrestis*. Journal of Applied Entomology 143:196-203.

Niedobová, J., Skalský, M., Ouředníčková, J., Michalko, R., and Bartošková, A. 2019b. Synergistic effects of glyphosate formulation herbicide and tank-mixing adjuvants on *Pardosa spiders*. Environmental Pollution 249:338-344.

Owagboriaye, F.O., Dedeke, G.A., Ademolu, K.O., Olujimi, O.O., Ashidi, J.S., and Adeyinka, A.A. 2017. Reproductive toxicity of Roundup herbicide exposure in male albino rat. Experimental and Toxicologic Pathology 69:461-468.

Pekár, S. 2012. Spiders (Araneae) in the pesticide world: an ecotoxicological review. Pest Management Science 68:1438-1446.

Pekár, S., and Brabec, M. 2016. Modern analysis of biological data: generalized linear models in R. Masaryk University Press, Brno, Czech Republic.

Pekár, S., and Haddad, C.R. 2005. Can agrobiont spiders (Araneae) avoid a surface with pesticide residues? Pest Management Science 61:1179-1185.

Powles, S. 2014. Global herbicide resistance challenge. Pest Management Science: formerly Pesticide Science 70:1305.

Řezáč, M., Řezáčová, V., and Heneberg, P. 2019. Neonicotinoid insecticides limit the potential of spiders to re-colonize disturbed agroecosystems when using silk-mediated dispersal. Scientific Reports 9:12272.

Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., and Seralini, G.E. 2005. Differential effects of glyphosate and roundup on human placental cells and aromatase. Environmental Health Perspectives 113:716-720.

Rittman, S., Wrinn, K.M., Evans, S.C., Webb, A.W., and Rypstra, A.L. 2013. Glyphosate-based herbicide has contrasting effects on prey capture by two co-occurring wolf spider species. Journal of Chemical Ecology 39:1247-1253.

Rueppel, M.L., Brightwell, B.B., Schaefer, J., and Marvel, J.T. 1977. Metabolism and degradation of glyphosate in soil and water. Journal of Agricultural and Food Chemistry 25:517-528.

Smith, E.A., and Oehme, F.W. 1992. The biological activity of glyphosate to plants and animals: a literature review. Veterinary and Human Toxicology 34:531-543.

Székács, A., and Darvas, B. 2012. Forty years with glyphosate. p. 247-284. In Hasaneen, M.N. (ed.) Herbicides-properties, synthesis and control of weeds. IntechOpen, London, UK. doi:10.5772/2511.

Talib, A.H., AL-Rudainy, A.J., Gathwan, M.A., Thakir, B.M., and Abdulfattah, R.K. 2018. The acute toxicity of herbicide roundup ultra in mosquito fish *Gambusia affinis*. Journal of Biodiversity and Environmental Science 13:9-15.

Yang, X., Song, Y., Zhang, C., Pang, Y., Song, X., Wu, M., et al. 2019. Effects of the glyphosate-based herbicide roundup on the survival, immune response, digestive activities and gut microbiota of the Chinese mitten crab, *Eriocheir sinensis*. Aquatic Toxicology 214:105243.