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Systematic Review of the Effects of Chemical Insecticides on Four Common Butterfly Families

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Safeguarding crop productivity by protecting crops from pest attacks entails the wide use of plant protection products that provide a quick, easy and cheap solution. The objective of this study is to understand the effects of insecticides used in agriculture on non-target butterflies, specifically on the families Lycaenidae, Nymphalidae, Hesperiidae, and Papilionidae. To achieve this goal, a formal systematic review was performed according to European Food Safety Authority (EFSA) guidelines, by entering a combination of keywords on 3 online databases. Three reviewers independently extracted information on study characteristics and quality. The main results were collected and grouped by the insecticide used, butterflies species and family, and endpoints. The output was valuable but heterogeneous as the endpoints and methodologies of the studies reviewed were different. Few experimental studies on the effects of insecticides on the most common butterfly families have been published. Naled and permethrin are the most commonly used insecticides in the experiments, whilst the target organisms of these studies are *Vanessa cardui*, *Danaus plexippus*, *Heliconius charitonius*, belonging to the Nymphalidae family, and *Eumaeus atala*, belonging to the Lycaenidae family; the effects were evaluated on all developmental stages, with special attention to the larval phase. This systematic review highlights the need for more studies on the effects of chemical insecticides on non-target Lepidoptera in light of their ecological importance and the extensive use of these chemical products.

Keywords: Lepidoptera, plant protection products, non-target, risk assessment, pesticides

INTRODUCTION

Agriculture is the most common form of land use in Europe. Modern agricultural lands are often subject to intensified use, characterized by increased field sizes, decreased crop diversity and reduced availability of semi-natural habitats. Moreover, they are subject to high inputs of agrochemicals, mainly Plant Protection Products (PPPs) (Hahn et al., 2015), used to safeguard agricultural production from pests (Sciarra et al., 2015). Globally, agricultural producers apply approximately 3 million tons of pesticides per annum, worth around USD 40 billion (Popp et al., 2012); insecticide use reached 12.2 billion in 2015, and the market is projected to reach more than16.4 billion by 2019 (AAVV, 2015). Insecticides are widely used to control insect pests, but a number of concerns have arisen regarding their environmental safety.

Insecticides are found almost everywhere, and this contamination puts the environment and non-target organisms, ranging from beneficial soil microorganisms, to insects, fishes and birds, at increased risk (Aktar et al., 2009). The use of insecticides in agriculture is well documented as one cause of pollinator declines, especially when spraying times coincide with flowering times (Nicholls and Altieri, 2013). Several studies have suggested that butterflies are key taxa and good indicators for the monitoring of anthropogenic disturbance, including the effect of xenobiotics, and habitat quality (Bonebrake et al., 2010; EEA, 2013). In addition, it has been estimated that approximately 70% of butterfly species (Papilionidae and Hesperiidae) occur in arable land (Boriani et al., 2005; Fileccia et al., 2015), potentially exposing them to various insecticide intensities, depending on their spatial and temporal overlap with applications.

Butterfly populations, both larvae and adults, are at risk of exposure to single and multiple insecticide applications coming from direct spraying or indirect residual deposits on plant tissue, especially as larval periods can coincide with the timing of insecticide applications (Hoang et al., 2011). Considering the enormous quantity of insecticides applied in agriculture, the importance of butterflies as bioindicators and the global decline of several butterflies species, understanding the impact of insecticides on this taxa has become paramount.

The objective of this study was to follow European Food Safety Authority (EFSA) guidelines (EFSA, 2010) in order to carry out a systematic review of published studies to gauge the extent of current knowledge regarding the effects of agricultural insecticide use on non-target butterflies. There are a number of reasons for choosing butterflies as a case study. Butterflies are certainly among the most popular insects for their attractive appearance, and many species play important roles in the ecosystem as pollinators of many wild and cultivated plants. They are also key taxa for biodiversity monitoring because they reflect changes in climatic conditions as well as seasonal and other ecological changes (Fileccia et al., 2015). In addition, butterflies are small, have high reproductive rates and a low trophic level which allows them to quickly respond to environmental stress (Griffis et al., 2001). In this study, we specifically focus our attention on the Lycaenidae, Nymphalidae, Hesperiidae, and Papilionidae, chosen for their sensitivity to stress and presence in a large number of habitats, especially agro-ecosystems.

Lycaenidae is the second-largest family of butterflies (Fiedler, 1996). The majority of lycaenids have associations with ants, which can be facultative or obligate and range from mutualism to parasitism (Pierce et al., 2002). Nymphalidae is the largest family of butterflies (Fiedler, 1998) and includes popular species such as the Monarch butterfly, which has received a lot of attention because it is a migratory, charismatic species (Gullan and Cranston, 2008). The Hesperiidae family, commonly known as "skipper butterflies," are recognized by their quick, darting flight habits (Wang et al., 2013). Finally, Papilionidae includes some of the most spectacular and magnificent of all insects (Collins and Morris, 1985), and they are recognized as model organisms in ecology, evolutionary biology, genetics, and conservation biology (Zakharov et al., 2004).

MATERIALS AND METHODS

Search Criteria

This systematic review was performed following the steps of the EFSA guidelines (EFSA, 2010) as closely as possible. Two research questions were asked: (1) Do agricultural insecticides cause negative effects on non-target butterflies?; and (2) If so, what are they? To answer these questions, systematic research of the available literature in three databases [Scopus (www.scopus.com), Summon (www.unipa.it/amministrazione/area1/ssp04/set11/summon/) and Web of Science (https://apps.webofknowledge.com)] was conducted, with a combination of the following keywords: "Lepidoptera," "butterfly," "butterflies," "non-target," "Lycaenidae," "Nymphalidae," "Hesperiidae," "Papilionidae," "Danaidae," which were combined with "insecticides," "pesticides," and "plant protection product." Searches were conducted in English and Italian on literature from between 1970 and (15 Jan) 2016.

Screening of Search Results

Duplicates were removed manually and abstracts were screened by two screeners against the target research questions. Exclusion criteria described below were developed and selected. Disagreements between the two screeners were resolved by a third screener when necessary. Cross-checking was performed on the excluded articles. Full-text review was independently conducted by three reviewers and reasons for exclusion were annotated and tracked (e.g., "review paper with no original data"). The primary reasons for excluding papers were: (i) articles completely un-related to search questions (biological, ecological, etc.); (ii) general knowledge papers; and (iii) papers that did not follow the basic criteria of scientific research (e.g., replication, minimum in laboratory and/or field standards). Articles clearly meeting the inclusion criteria were obtained for full-text review unless unavailable. These included articles related to the search inquiry, providing that scientific laboratory experiments or field studies had a minimum number of replicas and a negative control. Articles that could not be assessed for relevance based on the title and abstract screening were also subjected to full-text review. Articles were not considered further when their title and abstract clearly indicated that the study did not meet the inclusion criteria.

Data Analyses

Considering the heterogeneity of the data, observations of the effect of treatment compared to the control were extracted for all investigations, and for both laboratory and field studies. The mean of the effect and confidence intervals were used to calculate effect size lr as follows: $lr = \ln(MH/MC)$, where MC is the mean effect, considering the natural mortality recorded, on the control group, and MH the mean effect on the exposed group.

RESULTS AND DISCUSSION

Search outputs for generic keywords such as "Lepidoptera," "butterfly," and "non-target" in three databases were very extensive, and a total of 192,268 studies were found. Keywords such as "Lepidoptera" or "butterfly" include harmful phytofagous species, so a second search focusing on the most prevalent families of non-target butterflies was conducted. A total of 2097 scientific articles were recorded and then read. After deleting duplicates, we selected 7 articles (**Table 1**) that were useful to answering the proposed research questions.

The review shows that 4 studies were laboratory experiments and 3 were field studies. Overall, 6 different insecticides were tested, and the most common were naled and permethrin. The first is an organophosphate insecticide initially registered for use against adult mosquitoes, but which is also used in agriculture, especially in the United States on cotton crops and alfalfa. The second is a synthetic pyrethroid which, acting as a neurotoxin, affects the nervous system of the organisms and is used in agriculture on cotton, maize and wheat crops. The other insecticides considered in the experiments were dichlorvos, resmethrin, malathion, and imidacloprid. The latter is the second most used PPP in the world, though since 2013 it has been banned in Europe on crops that attract bees because it is considered highly toxic for them (Goulson, 2013). In addition, the U.S. Environmental Protection Agency classifies naled, dichlorvos, and permethrin as highly toxic to aquatic organisms and honeybees, based on acute toxicity data (Hoang et al., 2011).

The Nymphalidae and Lycaenidae have been studied more than Hesperiidae and Papilionidae. The effects of insecticides have been assessed on a total of 20 species, 5 belonging to the Hesperiidae family, 5 to Lycaenidae, 8 to Nymphalidae, and 2 to Papilionidae. The most studied species were *Vanessa cardui*, *Danaus plexippus,* and *Heliconius charitonius*, belonging to Nymphalidae, and *Eumaeus atala*, belonging to Lycaenidae. In addition, 11 species were studied in the field, 1 in the laboratory

TABLE 1 | Studies reviewed in detail in which experiments were carried out to evaluate the effects of insecticides (In) (D, dichlorvos; I, imidacloprid; M, malathion; N, naled; P, permethrin; R, resmethrin) on non-target butterflies.

Study	Experiment	In	Exposure modality	Endpoint	Family	Species	Life stage
Hoang et al., 2011	Laboratory	P N D	Direct applications on thorax and wings	LD_{50} (μg/g), Mortality	Lycaenidae Nymphalidae Nymphalidae Nymphalidae Nymphalidae	*Eumaeus atala* *Heliconius charitonius* *Junonia coenia* *Vanessa cardui* *Anartia jatrophae*	Larva Adult
Zhong et al., 2010	Field	N	Aerial application	Mortality	Lycaenidae	*Cyclargus thomasi bethune bakeri*	Larva
Oberhauser et al., 2009	Field	R	Aerial application	Mortality	Nymphalidae	*Danaus plexippus*	Larva
Bargar, 2012	Field	N	Spray application	Mortality Risk assessment	Papilionidae Papilionidae Nymphalidae Nymphalidae Nymphalidae Nymphalidae Nymphalidae Nymphalidae Nymphalidae Lycaenidae Lycaenidae Lycaenidae Lycaenidae Hesperiidae Hesperiidae Hesperiidae Hesperiidae Hesperiidae	*Papilio polyxenes* *Papilio troilus* *Vanessa cardui* *Junonia coenia* *Agraulis vanilla* *Anartia jatrophae* *Heliconius charitonius* *Phyciodes phaon* *Hermeuptychia sosybius* *Leptotes cassius* *Strymon istapa* *Eumaeus atala* *Calycopis cecrops* *Urbanus proteus* *Erynnis* spp. *Thorybes* spp. *Pyrgus* sp. *Pyrgus oileus*	Adult
Oberhauser et al., 2006	Laboratory	P	Oral administration	Mortality survival, feeding interruption, female oviposition choice	Nymphalidae	*Danaus plexippus*	Larva Adult
Krischik et al., 2015	Laboratory	I	Oral administration	Survival, feeding interruption, fecundity, hatching	Nymphalidae Nymphalidae	*Danaus plexippus* *Vanessa cardui*	Larva Adult
Salvato, 2001	Laboratory	N M P	Direct applications on thorax	LD_{50} (μg/g)	Hesperiidae Hesperiidae Lycaenidae Nymphalidae Nymphalidae	*Urbanus proteus* *Pyrgus oileus* *Eumaeus atala* *Agraulis vanilla* *Heliconius charitonius*	Larva Adult

and 8 in both. Regarding the vital stage, experiments were carried out at the larval stage in 6 of 7 studies, the adult stage in 5 of 7 studies, and at both adult and larval stages in 4 of 7 studies. Moreover, the experiments included 10 species at the adult stage, 1 species at the larval stage and 9 at both. The reported data in Hoang et al. (2011), for example, showed that the fifth larval stage was slightly more sensitive to the tested insecticides compared to the adult stage.

A real meta-analysis was impossible to carry out because the reported data in the selected studies, albeit valid, often differed among themselves. Whilst some studies were conducted in the laboratory, others were in the field, with different exposure modalities. For example, some experiments used oral insecticide administration, whilst others employed direct contact on the thorax or wings. In addition, the endpoints examined often differed among studies because some experiments studied the lethal dosage and others the percentage of mortality or feeding behavior.

However, all insecticides had a negative effect on all species for both stages. **Figure 1** reports the effect size (*lr*) of the six insecticides on larvae and adults. Insecticides have more negative effects on larvae than on adults, except for some specific species. The lowest effects was observed for larvae of *V. cardui* exposed to naled and for *Junonia coenia* exposed to dichlorvos; the most dramatic effect was observed for *E. atala* and *H. charitonius* exposed to permethrin.

CONCLUSION

This review shows that the use of insecticides reported in **Table 1** cause negative effects on the most common butterfly families, such as reduced survival rate, feeding interruption, and alteration of oviposition behavior. However, despite the billions of dollars spent on insecticides and the known importance of Lepidoptera, it has been impossible to determine which species is the most sensitive or which insecticide is the most toxic toward the studied species, given the small number of published studies, different methodological approaches and different endpoints examined. Even though it was not possible to perform an exhaustive meta-analysis given the heterogeneity of data and methodological approaches, it is clear, from this review, that the different species have different susceptibility to different insecticides. However, as this review manuscript aims to assist in the formulation of policies, by offering sound scientific evidence on the effects of PPPs on non-target organisms (in this case, butterflies) that are recognized worldwide as good indicators, it is important to mention the lack of data encountered on the concept of sub-lethal effects. This highlights the need for further research

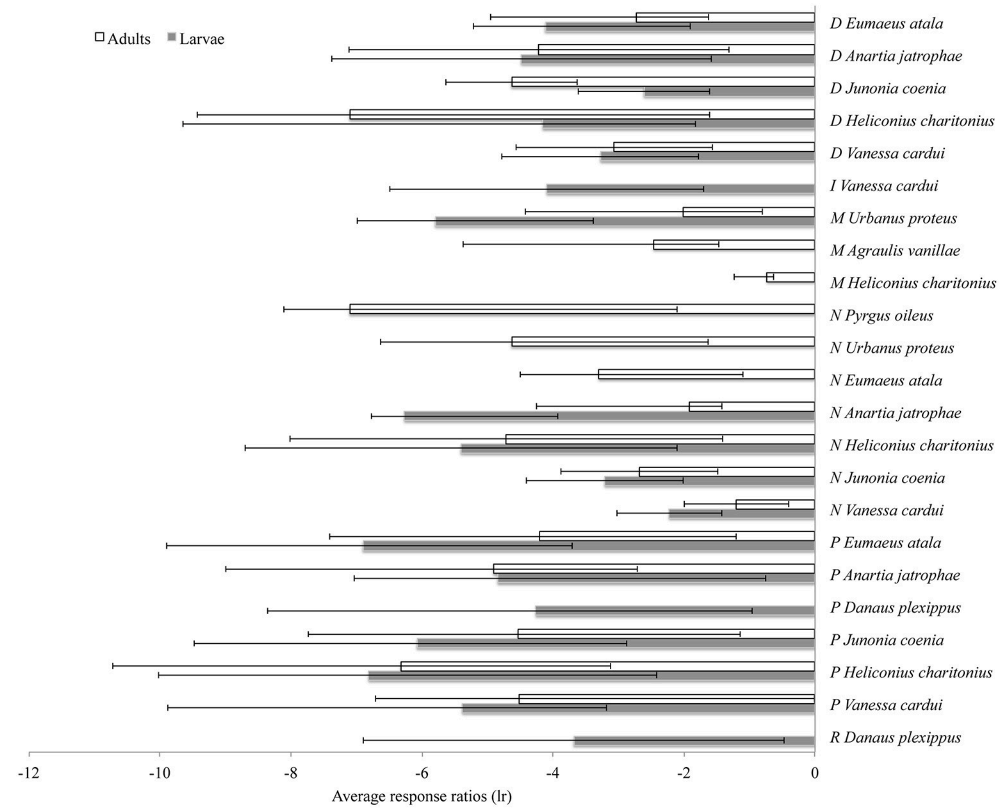

FIGURE 1 | The effect of insecticides (D, dichlorvos; I, imidacloprid; M, malathion; N, naled; P, permethrin; R, resmethrin) on larvae and adults of diurnal Lepidoptera, specimens are grouped in alphabetic order by family and then by species. Data are presented as average response ratios (*lr*) of treated-to-control group. The extremities of the bars indicates the minimum and maximum effects.

on this topic, including the knowledge of the sub-lethal effects of PPPs on NTOs, in accordance with the opinion expressed in 2015 by EFSA's researchers (EFSA, 2015) who made recommendations for further toxicity studies on PPPs, using Lepidoptera larvae as representatives of herbivorous species of non-target arthropods.

AUTHOR CONTRIBUTIONS

Conception and design of study: BM. Acquisition of data: RM, GS, and LR. Analysis and/or interpretation of data and drafting the manuscript: BM and RM. Revising the manuscript critically for important intellectual content and editing assistance: BM, LR, and RM.

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REFERENCES

AAVV (2015). *Global Crop Protection Chemicals (Pesticides) Market – Growth, Trends and Forecasts* (2016–2021). Hyderabad: Mordor Intelligence.

Aktar, M. D. W., and Sengupta, D., Chowdhury, A. (2009). Review article–Impact of pesticides use in agriculture: their benefits and hazards. *Interdisc.Toxicol.* 2, 1–12. doi: 10.2478/v10102-009-0001-7

Bargar, T. A. (2012). Risk assessment for adult butterflies exposed to the mosquito control pesticide naled. *Environ. Toxicol. Chem.* 31, 885–891. doi: 10.1002/etc.1757

Bonebrake, T. C., Ponisio, L. C., Boggs, C. L., and Ehrlich, P. R. (2010). More than just indicators: a review of tropical butterfly ecology and conservation. *Biol. Conserv.* 143, 1831–1841. doi: 10.1016/j.biocon.2010.04.044

Boriani, L., Burgio, G., Marini, M., and Genghini, M. (2005). Faunistic study on butterflies collected in Northern Italy rural landscape. *Bull. Insectol.* 58, 49–56.

Collins, N. M., and Morris, M. G. (1985). *Threatened Swallowtail Butterflies of the World The IUCN Red Data Book*. Cambridge: IUCN.

EEA (2013). *The European Grassland Butterfly Indicator: 1990–2011.* EEA Technical Report 11/2013. Publications Office of the European Union.

EFSA (2010). Guidance of EFSA. Application of systematic review methodology to food and feed safety assessments to support decision making. *EFSA J.* 8:1637. doi: 10.2903/j.efsa.2010.1637

EFSA (2015). PPR Panel (EFSA Panel on Plant Protection Products and their Residues). Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods. *EFSA J.* 13:3996. doi: 10.2903/j.efsa.2015.3996

Fiedler, K. (1996). Host-plant relationships of lycaenid butterflies: large-scale patterns, interactions with plant chemistry, and mutualism with ants. *Entomol. Exper. Appl.* 80, 259–267. doi: 10.1111/j.1570-7458.1996.tb00931.x

Fiedler, K. (1998). Diet breadth and host plant diversity of tropical - vs. temperate - zone herbivores: South-East Asian and West Palaearctic butterflies as a case study. *Ecol. Entomol.* 23, 285–297. doi: 10.1046/j.1365-2311.1998.00132.x

Fileccia, V., Santorsola, S., Arpaia, S., and Manachini, B. (2015). Seasonal patterns in butterfly abundance and species diversity in five characteristic habitats in sites of community importance in sicily (Italy). *Bull. Insectol.* 68, 91–102.

Goulson, D. (2013). Review: an overview of the environmental risks posed by neonicotinoid insecticides. *J. Appl. Ecol.* 50, 977–987. doi: 10.1111/1365-2664.12111

Griffis, K. L., Mann, S. S., and Wagner, M. R. (2001). "The suitability of butterflies as indicators of ecosystem condition: a comparison of butterfly diversity across stand treatments in northern Arizona," in *5th Biennial Conference of Research on the Colorado Plateau, Conference Proceedings*, eds C. van Riper, K. A. Thomas, and M. A. Stuart (Report Series USGSFRESC/COPL/2001/24), 125–135.

Gullan, P. J., and Cranston, P. S. (2008). *Lineamenti di Entomologia*. Bologna: Zanichelli.

Hahn, M., Schotthöfer, A., Schmitz, J., Franke, L. A., and Brühl, C. A. (2015). The effects of agrochemicals on Lepidoptera, with a focus on moths and their pollination service in field margin habitats. *Agric. Ecosys. Environ.* 207, 153–162. doi: 10.1016/j.agee.2015.04.002

Hoang, T. C., Pryor, R. L., Rand, G. M., and Frakes, R. A. (2011). Use of butterflies as nontarget insect test species and the acute toxicity and hazard of mosquito control insecticides. *Environ. Toxicol. Chem.* 30, 997–1005. doi: 10.1002/etc.462

Krischik, V., Rogers, M., Gupta, G., and Varshney, A. (2015). Soil-applied imidacloprid translocates to ornamental flowers and reduces survival of adult *Coleomegillamaculata*, *Harmoniaaxyridis*, and *Hippodamia convergens* lady beetles, and larval *Danaus plexippus* and *Vanessa cardui* butterflies. *PLoS ONE* 10:e0119133. doi: 10.1371/journal.pone.0119133

Nicholls, C. I., and Altieri, M. A. (2013). Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review. *Agron. Sustain. Dev.* 33, 257–274. doi: 10.1007/s13593-012-0092-y

Oberhauser, K. S., Brinda, S. J., Weaver, S., Moon, R. D., Manweiler, S. A., and Read, N. (2006). Growth and survival of monarch butterflies (Lepidoptera: Danaidae) after exposure to permethrin barrier treatments. *Environ. Entomol.* 35, 1626–1634. doi: 10.1093/ee/35.6.1626

Oberhauser, K. S., Manweiler, S. A., Lelich, R., Blank, M., Batalden, R. V., and De Anda, A. (2009). Impacts of ultra-low volume resmethrin applications on non-target insects. *J. Am. Mosq. Control Assoc.* 25, 83–93. doi: 10.2987/08-5788.1

Pierce, N. E., Braby, M. F., Heath, A., Lohman, D. J., Mathew, J., Rand, D. B., et al. (2002). The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annu. Rev. Entomol.* 47, 733–771. doi: 10.1146/annurev.ento.47.091201.145257

Popp, J., Pető, K., and Nagy, J. (2012). Pesticide productivity and food security. A review. *Agron. Sustain. Dev.* 33, 243–255. doi: 10.1007/s13593-012-0105-x

Salvato, M. H. (2001). Influence of mosquito control chemicals on butterflies (Nymphalidae, Lycaenidae, Hesperiidae) of the Lower Florida Keys. *J. Lepidopt. Soc.* 55, 8–14.

Sciarra, D., Foderà, I., and Ceriani, N. (2015). *Stop Pesticidi: Analisi dei Residui di Pesticidi Negli Alimenti e Buone Pratiche Agricole–Dossier Legambiente*. Ufficio Stampa; Legambiente.

Wang, K., Hao, J., and Zhao, H. (2013). Characterization of complete mitochondrial genome of the skipper butterfly, *Celaenorrhinus maculosus* (*Lepidoptera*: *Hesperiidae*). *Mitochondrial DNA* 26, 690–691. doi: 10.3109/19401736.2013.840610

Zakharov, E. V., Caterino, M. S., and Sperling, F. A. H. (2004). Molecular phylogeny, historical biogeography and divergence time estimatesfor swallowtail butterflies of the genus *Papilio* (*Lepidoptera*: *Papilionidae*). *Syst. Biol.* 53, 193–215. doi: 10.1080/10635150490423403

Zhong, H., Hribar, L. J., Daniels, J. C., Feken, M. A., Brock, C., and Trager, M. D. (2010). Aerial ultra-low-volume application of naled: impact on non target imperiled butterfly larvae (*Cyclargus thomasi bethunebakeri*) and efficacy against adult mosquitoes (*Aedes taeniorhynchus*). *Environ. Entomol.* 39, 1961–1972. doi: 10.1603/EN10089

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