

# Pest control in German forests: General patterns of biodiversity and possible impacts of Btk, diflubenzuron and lambda-Cyhalothrin on non-target arthropods, birds and bats – a literature review

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#### Abstract

This review focuses on direct and indirect impacts of three insecticides (*Bacillus thuringiensis kurstaki*, diflubenzuron, lambda-Cyhalothrin) on arthropods, bats and birds. General patterns of ecology, diversity, distribution and aspects of nature conservation of these three non-target animal taxa in Germany were examined, as well as their specific exposure and possible direct and indirect effects of the insecticides after application. We conclude, that a) the knowledge of direct and indirect effects of the above mentioned insecticides is still very scarce, b) there is an urgent need for more in detail studies in field in general, especially on indirect effects on vertebrates (including amphibians and reptilians), and for further ecotoxicological laboratory studies especially on sublethal effects on vertebrates.

Keywords

Bacillus thuringiensis; diflubenzuron; lambda-Cyhalothrin; pest control

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# 1. Introduction

According to the last German wide tree census BM3 approximately 11.4 million ha (= 32%) of the total area of Germany is covered with forests and woodland. A rough estimation accounts up to 90 billion single trees of more than 50 tree species. The four most common tree genera are spruce (*Picea* spec., 25%), pine (*Pinus* spec., 22%), beech (*Fagus* spec., 15%) and oak (*Quercus* spec., 10%).

A wide range of pest insects is present throughout German forests. They are quite well studied and subject of numerous textbooks (overview in Möller, 2007) and research studies. Because environmental conditions may vary substantially over time in forests, the abundance of a pest species may also fluctuate strongly. The dynamic of some pest species populations is cyclic in a predictable manner, but usually outbreaks by defoliators will also occur at irregular, species-specific intervals (Ziesche, 2015). During mass outbreaks forests can suffer substantially from their impacts.

During the last 10 years chemical control agents on insect pests in forests have been used in Germany mainly in oak and pine stands (e. g. AFZ, 2009, 2010, 2011, 2012, 2013, 2014). Thus, the emphasis of this article is on these two types

of forests and their specific defoliators. Both oaks (*Quercus robur*, *Q. petraea*) and pines (*Pinus sylvestris*) are distributed all over Germany (BMEL, 2015). Pines are weak competitors and naturally distributare weak competitors and naturally distributed at sites with extreme environmental conditions. Based on natural conditions and highly supported by forest management resulting in a strong manmade shift towards pine dominated forest units the Northeastern Lowland is the main distribution area of pines in Germany (MIL BB and LU M-V, 2015). For example, in Brandenburg 74% of all forest stands are coeval pine monocultures, or at least pine dominated. In other federal states the situation is different. In Bavaria pine forests represent approximately 18% of the woodland. The share of pine of the natural vegetation of Bavaria was estimated to be around 1% (Walentowski et al., 2002).

Thus, mass outbreaks of pine pests are a common phenomenon in North Eastern Germany. In the last few years namely Brandenburg and Saxony-Anhalt, have been especially concerned. Particularly large areas of monoculture stands of *Pinus sylvestris* suffer from the impact of several insects (Möller, 2007; Schwerdtfeger, 1957). The gradations of pine pests are quite well documented (e. g. Wulf and Berendes, 1995) and regional risk management maps are published or are available as digital information systems.

Larger damages and losses of single trees are caused mainly by leaf-eating moths and sawflies, mainly *Dendrolimus pini* (Lepidoptera: Lasiocampidae), *Lymantria monacha*, *L. dispar* (Lepidoptera: Erebidae), *Panolis flammea* (Lepidoptera: Noctuidae), *Bupalus piniaria* (Lepidoptera: Geometridae) and some Diprionidae species (Hymenoptera: Diprion spec., *Neodiprion sertifer*, *Gilprinia* spec.) (Möller, 2007). Under changing environmental conditions, effects like a shift from univoltine to bivoltine populations and shortening of latency phases mass outbreaks are predicted to occur more often (Gräber et al., 2012).

Oaks are also quite well represented all over Germany (BMEL, 2015), but their distribution is very scattered. High shares of oaks can be found especially in Palatinate Forest, Spessart and other warmish areas of Germany (BMEL, 2015). In oak forests the total impact is often a sum of the damages caused by some pest species and other concomitant herbivore species. This phenomenon is mainly occurring in spring and is thus often called oak spring feeding community ("Eichenfrüh-jahrsfraßgesellschaft"). However also single species, like *Thaumetopoea processionea* (Lepidoptera: Notodontidae) (Sobczyk, 2014) or *Lymantria dispar* (Lepidoptera: Erebidae), can have a heavy impact (Wulf and Berendes, 1995).

Due to the high risks for natural environments the application of insecticides by aircraft is generally prohibited (EU, 2009), but can be approved in exceptional cases under the scope of integrated pest control ("bestandesbedrohende Schäden", prediction of skeletonising > 90% in pine stands, prediction of repeatedly skeletonizing in oak forests).

Currently there are three different insecticides in use for application by aircraft. They differ in terms of legal approval, and the impact on different groups of target and non-target organisms (Table 1).

As the application of pesticides by aircraft is restricted to tree canopies, focal species of this review are non-target species inhabiting the canopies.

Canopy research is a quite young ecological discipline. Sutton (2001) summarized the history of canopy research in general. A state of the knowledge on European canopy arthropods was presented by Floren and Schmidl (2008a). According to the authors the knowledge of arthropods living in European forest canopies has strongly increased since the 1990s. Long-term systematic research has been done mainly in oldgrowth forests in Germany (especially in Bavaria), as well as in other countries (Poland, Slovenia, Romania). But still the knowledge of structure and functionality of canopy communities is very scarce, especially concerning arthropods of temperate regions (Basset, 2001; Floren and Schmidl, 2008a,b; Linsenmair et al., 2001; Sutton, 2001). Without any doubt the canopies have to be regarded as general hotspots of biodiversity in temperate regions (Floren and Schmidl, 2008b).

This review is the result of an extensive literature study of approximately 2500 papers on this topics, documented in a literature database (Endnote) and was supported by the Umweltbundesamt (FKZ: 371 467 4060).

## 2. Disturbance regimes

#### 2.1 Disturbance caused by defoliation

Forest defoliation is one of the main impacts caused by leafeating animal pests. Severe impacts occur on the level of stands and single trees depending on the degree of defoliation and the type of forest (Baker, 1941; Campbell and Sloan, 1977; Heichel and Turner, 1976; Kozlowski, 1969; Twery, 1990). The effects are variable to a high degree.

First of all, hydrocarbon production of trees will be reduced. This results in reduced growth rates, increased vulnerability to pests, and even in the death of stressed trees (Heichel and Turner, 1976; Kozlowski, 1969). In multilayered mixedspecies forest stands defoliation starts usually on understorey trees (Twery, 1990), continuing on overstorey trees.

The majority of forest stands suffers only minor or moderate levels of tree mortality (Gansner and Herrick, 1984). Severe damage is rare (Twery, 1990) and mostly a consequence of heavy mass outbreaks that cover larger areas. In these rare cases stands may experience 80-100% mortality of trees (Campbell and Sloan, 1977; Gansner and Herrick, 1984; Schwerdtfeger, 1957; Wenk and Möller, 2013). These stands are mainly man-made and often far away from the natural tree composition. Resilience is expected to be much greater in multilayered mixed-species forests than in singleaged monocultures (O'Hara and Ramage, 2013; Schuler et al., 2017).

Defoliation can drastically alter environmental variables, like light regimes, temperature and humidity, affecting understory and ground floor vegetation. As a consequence of altered solar radiation the growth of grass species may be promoted. This facilitation of dominant grasses (like *Calamagrostis* spec.) can have longtime negative effects such as the limitation of natural forest regeneration for years.

Further indirect impacts of a mass outbreak are the alteration of above- and belowground fluxes and storages of plant nutrients, especially the C, N and P balance in forests (Grüning et al., 2019), and effects on water resources (Twery, 1990) and ground water recharge.

During the years of outbreak of *Lymantria dispar* Gale et al. (2001) found a significant reduction of birds associated with closed-canopy forests and a temporary increase of openland bird species. Besides this, no effects have been found concerning habitat preference, foraging guild or nesting substrate. of bird communities. The effects of defoliation on birds have been short-term and spatially variable.

Defoliation can also improve habitats for many wildlife species and contribute to increased diversity of forests (Twery, 1990). Natural enemies have a strong impact on pest species (Bathon, 1993), but usually with a delay of one to a few years. Thus their impact is usually strongest in the regression phase of outbreaks.

The impact of natural enemies depend mainly on the specific environmental conditions of a site, the specific host species, and population density effects of both pest species and antagonist species from previous years. For example there is a long-time research on the significant impacts of natural enemies on *Lymantria dispar* in Serbia (Glavendekić, 2005).

# 2.2 Disturbance caused by application of pesticides

The evaluation of direct effects of insecticides on non-target organisms has two central aspects: The first one is to "(...) provide evidence of exposure to the contaminant, and the second is to provide evidence of harm (...)". Harm can be measured on population dynamics "through increased mortality and decreased reproductive success" (O'Shea and Johnson, 2009).

First of all, any application is expected to harm non-target animals for sure, as none of the mentioned insecticides is selective on species level (see Table 1). All insecticides differ in their active ingredient, the exposition pathway and mode of action, their taxonomic and live stage specific selectivity. The direct impact on non-target animals is expected to be lower with Dipel ES (selective for some lepidopteran families) and highest with lambda-Cyhalothrin (all arthropods).

Non-target organisms could also be affected on sublethal level. The application of insecticides can cause stress. Insecticides can devitalize specimens and make them more susceptible to diseases while hibernation or migration, and can have long-term impacts on learning, behaviour and fecundity.

Name	Active substance	Exposition pathway	Selectivity (Taxonomic)	Selectivity (live stages)	End of Approval in Germany
Dipel ES	Bacillus thuringiensis kurstaki (Btk)	Feeding of leaves	Lepidoptera only	Larvae only	31.12.2021
Dimilin	diflubenzuron	Feeding of leaves	Several arthropod orders	Live stages before ecdysis (moulting inhibitor), also on eggs	has ended 31.12.2014
Karate Forst Flüssig	<i>lambda-</i> Cyhalothrin	Contact	All arthopods	All live stages	has ended 31.12.2018

**Table 1.** Insecticides in use for application by aircraft at forests.

# 3. Arthropod diversity of canopies of pine stands and oak dominated woodland

#### 3.1 General patterns of diversity

Total species numbers of insects and arachnids of Germany and the federal states are well known. The total number of insect species sum up to more than 33.471 species from 29 orders (Dathe et al., 2001; Gaedike and Heinicke, 1999; Klausnitzer, 2005, 2001, 2003; Köhler and Klausnitzer, 1998; Schumann, 1999). Of arachnids there are approximately 1.000 species living in Germany (Blick et al., 2004; Platen et al., 1995). Species numbers of other arthropods (Chilopoda, Diplopoda, Crustacea) will be omitted here, as they are usually not typical for canopies.

Actually Germany is inhabited by only very few endemic animals species. Likely, no endemic can be harmed by application of pesticides to canopies of oaks and pine, as their populations are very locally distributed and they frequently live under very special habitat conditions (caves or boulder heaps, in the soil etc.) (Reifenstein, 2008).

There is a very strong gradient in species numbers from North to South. Highest species numbers of insects and arachnids occur in Bavaria and Baden-Württemberg. This pattern can partly be explained by area, warmness, and the exclusiveness of Sub-Alpine and Alpine habitats in these federal states (Klausnitzer, 2005).

Southwood (1961) published a list of phytophagous species associated with different tree species of Great Britain. This list was revised later (Kennedy and Southwood, 1984). Brandle and Brandl (2001) updated this list for Germany. In Germany 699 phytophagous insect and mite species can be found on oak, while 335 phytophagous insects and mites feed on pine (Brandle and Brandl, 2001). The highest richness in terms of phytophagous insects and mites can be found on willow, but oaks have only a few species less. Also pines are still rich in phytophagous species, they ranking on the seventh place.

On oaks in Germany Lepidoptera have the highest number of phytophagous species (305 species, 48%), second species rich order is Coleoptera (208 species, 30%). On pines Coleoptera have the highest number of phytophagous species (160 species, 48%), second species rich order is Lepidoptera (67 species, 20%) (Brandle and Brandl, 2001).

Taking into account all other life history strategies and guilds of arthropods, oaks have the overall highest species number. Oaks offer a wide range of different microhabitats, depending on their age, dimension, exposition and other biotic and abiotic variables. Even within one single old oak tree, very different habitats can be found. Not only living tree parts form habitats; wounds, rot holes and cavities, tree humus, larger dead branches and associated fungi offer additional microhabitats (Siitonen, 2012; Speight, 1989; Stokland et al., 2012). Each different microhabitat harbor different assemblies of species (Stokland et al., 2012), thus especially old oaks can indeed be described as "arboreal megalopolises" (Speight, 1989).

Pines are not only less rich in phytophagous insects, they are also less preferred by woodpeckers and other hole drumming and hammering bird species. Therefore also the number of species living in tree holes and cavities is much lower compared to oak. Old solitary pines on sites with extreme environmental conditions are very rare. These solitary trees can offer a wide variety of special microhabitats. Both pines and oaks are most rich in monophagous beetles, 43, respectively 31 species feed exclusively on them (Köhler, 2000).

Several arthropod species are protected by European Habitats Directive (Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora). Many of them are associated with oak, or live in oak forests. On the other hand, only one exceptionally rare beetle could likely be found in pine, but is extinct in Germany since more than hundred years (Table 2).

Highest numbers of arthropods occur in spring and early summer (Goßner, 2004). This pattern can be found in both oak forests as well as in coniferous stands.

Since the striking findings of biodiversity in canopies in tropical areas (Erwin, 1982, 1983, 1988; Stork et al., 1997), intense research has also begun in other regions. Since the 1990s extensively research was made, especially in natural forest reserves of temperate and boreal areas. Many recent

**Table 2.** Insect species protected by European Habitats Directive and their association to oak and pine forests. German legal issue and Red list Germany is only shown for these species.

Species name	Council Directive 92/43/EEC Annex II	Council Directive 92/43/EEC Annex IV	German legal issue and Red list Germany	Oak forests	Pine forests
Bolbelasmus unicornis (Schrank, 1789)	X	X	§, RL 1	X	
Cerambyx cerdo Linnaeus, 1758	X	X	§, RL 1	X	
<i>Cucujus cinnabarinus</i> (Scopoli, 1763)	X	Х	§, RL 1	X	
Eriogaster catax (Linnaeus, 1758)	X	X	§, RL D	X	
Euphydryas maturna (Linnaeus, 1758)	X	Х	§, RL 1	X	
<i>Limoniscus violaceus</i> (P.W.J. Müller, 1821)	X		§, RL 1	X	
Lucanus cervus (Linnaeus, 1758)	X		§, RL 2	X	
Osmoderma eremita (Scopoli, 1763)	X	X	§, RL 2	X	
Buprestis splendens Fabricius,1775	X	X	§, RL 1		X

studies have shown that a rich and specialized canopy fauna exists (Ammer and Schubert, 1999; Bail and Schmidl, 2008; Basset, 1985; Brandle and Brandl, 2001; Bussler and Schmidl, 2009; Dunk and Schmidl, 2008; Floren and Schmidl, 1999; Moran and Southwood, 1982; Schmidl et al., 2004; Schmidl and Bussler, 2008; Southwood et al., 1982, 2004, 2005).

Many canopy arthropod studies have been conducted in oak canopies or oak dominated forests, for example: Orbatid mites (Acari, Sobek et al. (2008)), Diptera (Dunk and Schmidl, 2008), Hymenoptera: Formicidae (Dolek et al., 2009), Auchenorrhyncha (Nickel, 2008), Heteroptera: (Goßner, 2008), Lepidoptera (Hacker and Müller, 2008), xylobiontic beetles (Bail and Schmidl, 2008; Bussler and Schmidl, 2009; Schmidl and Bussler, 2008) and phytophagous beetles (Coleoptera, Floren and Sprick (2007); Sprick and Floren (2008), or arthropods in general (Gruppe et al., 2008).

These studies have found usually a very species rich fauna in canopies with high abundances. But overall variability is high, even in-between single trees in a stand. Usually strong differences between canopy and near-ground communities can be found and even within lower and higher layers of the canopy (review in Gruppe et al. (2008)). With increasing age of oaks the number and complexity of microhabitats increase and the variability and availability even at a single tree varies to a great extent (Floren and Schmidl, 2008b; Speight, 1989).

Arthropods of pine canopies in general have been studied by Engel (1941), Höregott (1990), Ozanne et al. (1997, 2000), Thunes et al. (2004, 2003), Basset (1985), Borkowski (1986), Schmidl et al. (2004), and Floren and Schmidl (2008b). Other research was restricted to several insect orders or families: Diptera: Syrphidae (Bańkowska, 1994), Hymenoptera: Diprionidae (Simandl, 1993), Heteroptera (Cmoluchowa and Lechowski, 1993), Auchenorrhyncha (Nickel, 2008), Neuropteroidea (Czechowska, 1994), Coleoptera: Cantharidae (Chobotow, 1993), Chrysomelidae (Sprick and Floren, 2007; Wąsowska, 1994), Curculionidae (Cholewicka-Wisniewska, 1994a,b), saproxylic Coleoptera families (Gutowski et al., 2006), Aphidae (Kolodziejak, 1994), Araneae (Simon, 1995; Sterzynska and Slepowronski, 1994).

Often succivore Insects (Heteroptera, Homoptera) are the most dominant insect group in canopies (Nickel, 2008; Schmidl et al., 2004), followed by Coleoptera and Hymenoptera. These dominant groups are mostly phytophagous insects and have a share of approximately 80% of the total arthropod community. The other belongs mainly to the Lepidoptera, Araneae and Diptera.

For most tree species Auchenorrhyncha are the phytophagous animal group with highest shares of species numbers in canopy (Nickel, 2008). So approximately 12% of phytophagous Auchenorrhyncha species live on oaks. Here only few species are monophagous, most are polyphag (Nickel, 2008). Only few live in canopies of pines, the shares are much less, compared with oaks. Also on *Quercus robur* the share of monophagous weevil species is high (Sprick and Floren, 2008).

The number of canopy species is significantly increasing

with increasing age of pine (Engel, 1941; Thunes et al., 2003). The species number, abundances and number of Red List xylobiontic beetles was highest in pine stands with higher shares of canopy dead wood and dead wood diameter (Schmidl et al., 2004). A close-to-nature pine forest the percentage of xylobiontic Red List beetles was 19% of all beetles, while in a managed pine stand no Red List beetles have been found. But in another younger pine forest also xylobiontic Red List beetles have been found in canopies (Schmidl et al., 2004). Strong differences can be found concerning the species composition of canopies and other tree strata.

Additionally, there exists a plenty of unpublished research, mainly academic theses or documentations of the impact of insecticide applications (mainly lambda-Cyhalothrin) at pine stands done by authorities or universities (e. g. Hugger, 2005; Meußling, 2000). Most of these studies cover only very short time periods, or determination was not done to species level.

Floren and Schmidl (2003) did a rough estimation on the number of arthropods living in German forest canopies. They estimates 1532244 x 106 specimens in total, based on extensive own fogging datasets. Arthropod studies in canopies have revealed many rare and endangered species. Also species new to science have been found (Floren and Schmidl, 1999, 2003; Thunes et al., 2008, 2004, 2003).

Still many questions remain unanswered. Often research has only been descriptive or suffering from several statistical problems or simply hard to compare with other studies. The studies report a quite high temporal and spatial variation of diversity.

The high importance of forest edges was shown several times (Foggo et al., 2001). Köhler (1996, 2000) has studied several old primary and managed forest stands. The highest numbers of rare and endangered saproxylic, as well as thermophilic beetles were mostly found with old grown oak trees at the forest edges.

After fogging with pyrethroids empty tree crowns of *Quercus robur* have been recolonized fast, especially by Diptera, Coleoptera, Psocoptera and Plecoptera. (Floren and Schmidl, 2003) concludes that the recolonization is very stochastic and not predictable and environmental conditions may have a great importance on this process.

Movement patters are highly variable for different phytophagous insect groups. In canopies adult Ensifera and Heteroptera have a higher degree of mobility than their specific larvae, caterpillars (Lepidoptera), aphids and cicada (larvae & adults) (Asshoff et al., 2008).

#### 3.2 Effects of insecticides

#### 3.2.1 General

Adverse effects of insecticides in general on non-target arthropods are more or less well documented for some insecticides (Easton and Goulson, 2013; Gill et al., 2012; Hallmann et al., 2014; Henry et al., 2012; Roessink et al., 2013; Whitehorn et al., 2012). Lepidoptera-specific insecticides can also have neutral or even beneficial effects on the level of communities

or rare species (Manderino et al., 2014).

Information about ecotoxicological side effects of the three insecticides (see Table 1) on non-target arthropods is scarce, and at least usually only available for a few model organisms and laboratory conditions. For *Bacillus thuringiensis* var. *kurstaki* and diflubenzuron it is documented in Forster et al. (1993). The first one is usually ranked as the one with the lowest threat on bees, natural antagonists (parasitoids, predators) and soil organisms (Forster et al., 1993).

*Chrysoperla carnea* (Neuroptera: Chrysopidae). *Chrysoperla carnea* is a general predator and quite well studied, as at least more than 150 formulated pesticides have been tested on their larvae and pupae, and more than 100 pesticides on the adults (Bozsik, 2009). Bozsik (2010) have shown, that the impact of lambda-Cyhalothrin (Karate 5 EC, in terms of LC50 and LT5) was significantly more detrimental for two other tested close related *Chrysoperla* spec. than for *Chrysoperla carnea*. All the tested species play a considerable role in controlling insect pests. The susceptibility was "moderately harmful" (92% mortality, *Chrysoperla perla*) to "harmful" (98,8% mortality, *Chrysoperla formosa*), while it was "harmless" for *Chrysoperla carnea* (39,4% mortality, Bozsik 2010).

Especially for diflubenzuron a higher toxic potential was found in several ecological studies (Addison, 1993; Geels and Rutjens, 1992; Wimmer et al., 1993) in contrast to laboratory studies (Forster et al., 1993). Examples of impacts of different insecticides in oak forests and pine stands are shown in Table 3.

The persistence of diflubenzuron is high and can last at least for two vegetation periodes (Skatulla and Kellner, 1989). During the annual leave fall an additional contamination of litter was found for *Pinus sylvestris* (Mutanen et al., 1988). But in contrast a significant influence of diflubenzuron was not found on oak leaves after annual leave fall (Emmerich, 1995). Also Wimmer et al. (1993) found tree species specific persistence of diflubenzuron in North America (3 different oak species).

#### 3.2.2 Exposure

Taking into account the selectivity, residual time and the food plants, habitats, and seasonal appearance of the specific species seems to be very high, especially for non-target Lepidoptera (100% of macrolepidopterean species, North America: Kolodny-Hirsch et al. (1990), overview for Germany: Brunk et al. in prep.). Usually highest abundances and species numbers of arthropods occur in spring and early summer (Araneae: Bräsicke (2009), Coleoptera & Heteroptera: Goßner (2004). This coincides with the occurrence of larvae of many of the relevant pest species.

#### 3.2.3 Direct effects

All three insecticides focus on target species belonging to the arthropods, the effects are different, but high in general, especially for non-target Lepidoptera (Table 3).

Many studies have shown the high impact of the three insecticides on target species, adverse direct effects were found also in many studies, so Table 3 gives only a overview over some of the more important studies. Not all studies did show negative impacts, in some cases they are mixed with effects of concurrence or by the alteration of the environments.

#### 3.2.4 Indirect effects

Also indirect effects are likely for zoophagous arthropods, parasitoids and other arthropods, but this is not very well studied. The application of diflubenzuron significantly reduced the numbers of workers of a wasp species, possibly because of the reduction of caterpillar prey in the year of the application (Barrows et al., 1994).

# 4. Avian diversity in forests and impact of pesticides

#### 4.1 Diversity of birds in Germany

Germany inhabits 305 breeding bird species, among them 260 are regularly breeding (Gedeon et al., 2014; Südbeck et al., 2009). There is a strong gradient in numbers of breeding bird species per grid from East towards the West, and from North towards the south, with highest numbers per grid in East Germany (Gedeon et al., 2014). Due to high proportions of endangered species, there is a good knowledge base of distribution especially inside of nature conservation areas and for species protected by Habitats Directive (Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora).

European bird species diversity has been reported to be widely declining (Butchart et al., 2004; Newton, 2004; Siri-wardena et al., 1998), but effects are varying on different spatial scales (van Turnhout et al., 2007), and differ for birds characteristic for different landscapes (farmland, wetlands, woodland) and with specific life history traits.

In Germany there is still a decline in abundance of breeding birds. Woodland species' populations have not had negative population trends for a long time (regional example: Böhning-Gaese and Bauer (1996), German Monitoring 1989-2003: Flade and Schwarz (2004)), but since some years, also the numbers of woodland species are significantly declining (German Monitoring 1991-2010: Flade and Schwarz (2010)). Assumed cases for this effect are intensification in forest management, as well as effects of changing fructification of trees (Flade, 2012; Flade and Schwarz, 2010; Flade et al., 2012).

In Netherlands both rare and very abundant bird species have recently (1973-2000) decreased on average, and especially in species rich regions. But overall, some scale dependent positive trends have been found as well in the last 30 years, as more bird species increased in abundance and distribution than decreased. A positive change in abundance and distribution was found especially for breeding woodland bird species (van Turnhout et al., 2007). The total woodland area of Netherlands expanded by 29%, existing forests maturated (many have been established in beginning of last century) and forest management changed (shift towards higher shares of deciduous trees, increase of dead timber). In France an overall decline of bird species was found between 1989 and 2001. Especially common breeding birds had declined, and forest specialists as well (Julliard et al., 2004). There are multiple reasons for the declines, such as intensification of land use, shifts in management practices (e.g. tree species), climate change, and the use of pesticides.

#### 4.2 Ecology, patterns of variability

Woodland bird communities are very varied (Flade, 1994; James and Wamer, 1982; Whitmore, 1969). Very important are stand conditions, especially structural variables and stand age. Usually the diversity of breeding birds increases strongly with increasing structural variability. Birds can be very good indicators for some special environmental conditions, as well as for naturalness, intensity of forest management practice and tree species diversity (Utschick, 2006).

Communities of breeding birds in oak dominated forests are well studied in Germany (Flade (1994): 79 study sites). The bird communities are very varied, depending on oak species, type of forest and environmental variables. There is a large number of typical breeding species (17 species). Most of the typical birds breed in tree cavities and have preferences for thick barks.

Bird communities of pine stands are very well studied in terms of typical breeding species (Flade (1994): 297 study sites in total, 101 study sites for monodominance pine forests, 24 for pine thickets). Typical pine forests have extremely low numbers of breeding bird species and individuals. The higher the share of deciduous trees in a specific stand, the higher the numbers of breeding birds. There are only four typical species for pine forests namely Crested Tit (Parus cristatus), Coal Tit (Parus ater), Mistle Thrush (Turdus viscivorus) and Woodlark (Lullula arborea). Typical communities of pine thickets are Dunnock (Prunella modularis), Nightjar (Caprimulgus europaeus) and Crested Tit. Crested Tit and Coal Tit are well adapted to forage in canopys and breed in tree cavities, while Woodlark and Nightjar forage on surface ground (Flade, 1994). There is a well predictable succession of bird communities with typical species for each age of a pine stand (Dierschke, 1973).

# 4.3 Effects of insecticides

#### 4.3.1 Exposure

Direct exposure of birds during application of pesticides is assumed to be high, as the main daily activity time of birds (especially insectivorous species) is concurrent with the often forenoon application flights above forests. Seasonal activity is highly correlated with times of high activity by arthropods, with highest arthropod numbers in spring and early summer (see chapter 3.2.2) and during the breeding season. Many forest bird species build open cup nests, and here also a direct contamination of nestlings is possible.

**Table 3.** Examples of impacts of different insecticides in oak forests and pine stands (+ positive effect, ? – unknown effects, o – no effect, - negative effect, - - high negative effects).

Active substance	Group of non-target arthropod studied	Type of forest	Effects	Type of Effects	Sources
Bacillus thuringiensis kurstaki (Btk)	Forest canopy Lepidoptera	Oak forests (North America)		negative impact on abundances and diversity of several systematic or functional groups especially macrolepidoptera	Butler et al. (1997)
Bacillus thuringiensis kurstaki (Btk)	Forest Lepidoptera	Oak forests (North America)	0	"no effects of disturbance history on local ( $\alpha$ ) moth diversity or diversity of moths among sites ( $\beta$ -diversity). The $\alpha$ - or $\beta$ -diversities of moths classified by their dietary overlap with gypsy moths (overlapping, partially overlapping, not overlapping) were also not affected by disturbance history."	Manderino et al. (2014)
Bacillus thuringiensis kurstaki (Btk)	All arthropds on taxon level: order and family	Pine stands	0	in year of application and 2 years later no impacts found	Möller (2007); Möller and Majunke (1996, 1997)
Bacillus thuringiensis kurstaki (Btk)	Soil arthropods (epigäic Collembola)	Pine stands	(+)	significant higher abundances of epigeic Collembola, but not studied on species level	Jäkel (1998)
Bacillus thuringiensis kurstaki (Btk)	Ground floor ants	Oak and Pine stands	0	no impacts on <i>Formica polyctena</i> , <i>Leptothorax nylanderi</i> and ground floor wood ants in laboratory and field studies	Kneitz (1966); Lange (1966); Lischke (1993)
Diflubenzuron	All arthropods	Oak forests		a significant negative on species diversity and number of specimens of arthropds one and two months after application	Schönfeld (2007)
Diflubenzuron	Forest canopy Lepidoptera	Oak forests (North America)		significant negative impacts on abundances and diversity of several systematic or functional groups especially macrolepidoptera	e.g. Butler et al. (1997); Butler and Kondo (1993); Martinat et al. (1988); Sample (1991)
Diflubenzuron	Forest canopy Lepidoptera	Oak forests (North America)	0	low or no influence on few Lepidoptera, due to different biology strategies ("mature larvae "in ground vegetation layer)	Butler et al. (1997)
Diflubenzuron	Heteroptera	Pine stands, Oak forests, laboratory	0	low impacts on Heteroptera	Skatulla (1975)
Diflubenzuron	Heteroptera	Laboratory		high mortality of larval stages and impacts on fertility	Ahmad (1992); Clercq et al. (1995)
Diflubenzuron	Coleoptera: Carabidae	Oak forests	-	negative impacts on abundances of ground beetles	Klenner (1990)
Diflubenzuron	Herbivorous Coleoptera	Oak forests (North America)		significant negative impacts on abundances of herbivorous Coleoptera on foliage	Butler et al. (1997)

Continued on next page

**Table 3.** Examples of impacts of different insecticides in oak forests and pine stands (+ positive effect, ? – unknown effects, o – no effect, - negative effect, - - high negative effects) (continued).

Active substance	Group of non-target arthropod studied	Type of forest	Effects	Type of Effects	Sources
Diflubenzuron	Diptera	Pine stands		High significant reduction of Diptera, especially Sciaridae	Jäkel (1998)
Diflubenzuron	Diptera	Pine stands		Significant negative impacts on abundances	Möller and Majunke (1997)
Diflubenzuron	Orthoptera	Oak forests (North America)		Significant negative impact on abundances	Martinat et al. (1993)
Diflubenzuron	Araneida	Oak forests (North America)		Significant negative impact on abundances	Martinat et al. (1993)
Diflubenzuron	Araneae	Oak forests (North America)	0	No significant effects	Butler et al. (1997)
Diflubenzuron	Hymenoptera: Formicidae	Oak forests (North America)	0	No significant effects	Butler et al. (1997)
Diflubenzuron	Ground floor ants (Hymenoptera: Formicidae)	Oak and Pine stands		Significant higher mortality on Leptothorax nylanderi workers in laboratory studies, no pupation of larvae, possible negative effect in field study	Lischke (1993)
Diflubenzuron	Myriapoda (Diplopoda, Chilopoda)	Oak forests (North America)	0	No significant effects	Butler et al. (1997)
Diflubenzuron	All arthropds on taxon level: order and family	Pine stands	0	In year of application and 2 years later no impacts found	Möller (2007); Möller and Majunke (1996, 1997)
Diflubenzuron	Several arthropods	Oak forests (North America)	/0	Lepidoptera (reduced abundance and species richness at treated sites), especially larvae biomass. No effects on Coleoptera, Diptera or Hymenoptera	Sample et al. (1993a,b)
Diflubenzuron	Soil arthropods (epigäic Collembola)	Pine stands	(+)	Significant higher abundances of epigäic Collembola in the first 6 weeks after application, but not studied on species level	Lischke (1993)
lambda- Cyhalothrin	All arthropods	Pine stands	-	Mostly Syrphidae & Coleoptera (each shares approx. 1/3th)	Jakobitz (2003)
lambda- Cyhalothrin	All arthropds on taxon level: order and family	Pine stands	0	In year of application and 2 years later no impacts found	Möller (2007); Möller and Majunke (1996, 1997)
lambda- Cyhalothrin	Hymenoptera. Platygastroidea	Pine stands	-	Short time reduction of densities of parasitoids, 3 months later densities same again as on untreated site	Möller (2007)
lambda- Cyhalothrin	Coleoptera: Staphylinidae	Pine stands	0	Lowest numbers on untreated site with heavy defoliation	Möller (2007)

#### 4.3.2 Direct effects

"Poisoning of insectivorous birds by diflubenzuron, after spraying in orchards as recommended, is highly improbable". This conclusion based on daily intake rates by nestling of *Parus major* and *Passer montanus*, on maximum whole body loadings of diflubenzuron prey insects, and observations of normal growth and afterwards subsequent breeding of nestlings in the same orchards (Muzzarelli and Marks, 1986; Muzzarelli, 1986).

We have not found any literature on direct negative effects caused by the application on lambda-Cyhalothrin on birds. On sublethal level, negative impacts, especially on nestlings, are imaginable, especially by feeding contaminated prey.

#### 4.3.3 Indirect effects

According to several authors temperate deciduous birds are generally food limited, especially during the breeding season (Cramp and Perrins, 1994; Holmes and Schultz, 1988; Martin, 1987; Simons and Martin, 1990). Birds lay their clutches when food is most abundant (Perrins, 1965; van Noordwijk et al., 1995). Food supplementation can increase the number of nestlings, the feeding rates and the number of breeding times per year and enhances the body mass and size of nestlings (Martin, 1987; Nagy and Smith, 1997; Simons and Martin, 1990). During a mass outbreak of an insect pest species the food supply is superabundant.

Larvae of Lepidoptera are preferred by insectivorous birds for feeding the nestlings, as they combine a high amount of fat with a low amount of chitin (Redford and Dorea, 1984). They are well digestible and contain a higher amount of energy compared to other insects. Especially in forests Lepidoptera larvae are the major dietary component for insectivorous birds (Cooper et al., 1990; Holmes and Schultz, 1988; Robinson and Holmes, 1982; Sample et al., 1993b).

Birds have specific foraging traits so shifts in the availability of prey will influence several community patterns, as habitat selection and bird community structure (Robinson and Holmes, 1982). In arable landscapes several studies have demonstrated statistically significant effects of pesticides on food abundance and foraging behavior of farmland bird species, on the availability of prey specimens on nestling condition, nestling growth rate, brood size and nestling survival, and for Grey Partridge (Perdix perdix) even an effect of breeding performance on population change (Boatman et al., 2004; Brickle et al., 2000; Brickle and Harper, 2002; Evans, 2001; Green, 1984; Hill, 1985; Morris et al., 2001; Newton, 2004; Potts, 1980, 1986; Potts and Aebischer, 1991, 1995; Rands, 1985, 1986; Sotherton and Robertson, 1990) (overview in Boatman et al., 2004). Recently, declines in 15 insectivorous birds have been found to be associated with neonicotinoid concentrations in surface water (imidiclorid in agricultural landscapes in Netherland). In this case bird numbers decreased on average by 3,5% /year in areas with more than 20 ng of imidacloprid / liter. A shortage of food, the consumption of contaminated prey insects, or both have been proposed as possible explanations. Also feeding on contaminated seeds

could not be excluded as explanation for negative effects on seed-consuming birds (Hallmann et al., 2014).

The impact of the use of *Bacillus thuringiensis israelensis* (Bti) for controlling mosquitos' on breeding house martins (*Delichon urbanicum*) has been researched in France (Poulin, 2012; Poulin et al., 2010). They found that the intake of Diptera (Nematocera), as well as their predators (Arachnida & Odonata) decreased significantly at treatment sites. At treated sites, two effects occurred: a significantly higher rate of very small prey was consumed, but total foraging rates were lower. Clutch size and nestling survival were significantly lower at treatment sites. "Breeding success was positively correlated with the intake of Nematocera and their predators at the nest level" (Poulin et al., 2010).

Little is known about the impact of insecticide-reduced prey availability on forest birds. Bell and Whitmore (1997) found significant negative impacts on the bird communities after the application of *Bacillus thuringiensis* and diflubenzuron, while the skeletonizing of forest by *Lymantria dispar* had no negative impacts, and also increased the density of a few bird species (due to increased vegetation structures). Only minimal effects on reproduction of hooded warbler (*Wilsonia citrina*) have been found. But nesting success was significantly higher in untreated sites and significant differences in feeding rates has been found only for small clutches (Nagy and Smith, 1997).

One of the better known studies from Germany concerns on the impact of a diflubenzuron application in an oak-dominated forest (Schönfeld, 2007, 2009). A few days after the application the birds fed higher proportions of other arthropods than Lepidoptera larvae, but not a significantly lower total number of prey specimens. Breeding success of first clutch was only somewhat lower on application site than control site, but birds moved away from this site for a second breeding attempt. However all-in-all the application had a significant negative impact on the number of insect-feeding bird species and individuals.

In laboratory studies 2000 mg/kg body weight of diflubenzuron was "insufficient to kill 50%" of *Anas platyrhynchos*, but caused anorexia the day after treatment (Hudson et al., 1984). Also daily dietary was "insufficient to kill 50%" of *Anas platyrhynchos* in 8 days (Farlow, 1976). In some other laboratory studies on domestic *Gallus* no adverse effects has been found (Eisler, 1992).

In a study diflubenzuron was applied on an oak forest to control *Lymantria monacha* (70,75 g/ha). The maximum residue recorded in 8 different insectivorous canopy foraging birds was 0,21 mg/kg whole body FW. In six ground or low foraging insectivorous birds a similar maximum value was found (0,2 mg/kg whole body FW, Muzzarelli and Marks (1986)).

In another study *Abies* and *Pseudotsuga menziesii*-forest were sprayed aerially (140 and 280 mg/ha) with diflubenzuron. No significantly changes in species diversity, survival, morbidity, brain cholinesterase activity, behavior was found at

either dose (Richmond et al., 1979).

A decreasing number of prey specimens is exponentially correlated with increasing searching time per prey specimen (Parus major: Naef-Daenzer and Keller (1999)). An insecticide-induced reduction of food would likely prolong the foraging time, whereas the energy consumption have an overall negative impact on the individual fitness and life-history perspective, including reproductive success (Martin, 1987; Reed, 2000; Schönfeld, 2007). Post-fledging mortality is high, when post-natal growth rate, body size and body weight are low at fledging (Alatalo and Lundberg, 1986; Davies, 1986; Magrath, 1991; Owen and Black, 1989; Schmutz, 1993; Smith et al., 1989). Additionally, the recruitment rate into the next year breeding population has been found to be lower (Gebhardt-Henrich and van Noordwijk, 1991; Tinbergen and Boerlijst, 1990) and adult birds had a lower fertility (Kolodny-Hirsch et al., 1990).

Sample et al. (1993b) found a strong dietary shift in songbirds at diflubenzuron treated sites. They studied the gut contents of nine songbirds and found significantly reduced total biomass of gut contents for two bird species. Significant multivariate effects have been found for 4 species, the canonical variable was significant negatively correlated with biomass of Lepidoptera larvae and positive with biomasses of other Arthropods (Araneae, Hemiptera, Homoptera, Coleoptera, adult Lepidoptera, Diptera).

Male red-eye Vireo (*Vireo olivaceus*) spent on diflubenzuron treated sites more time foraging and covered larger searching areas (DeReede, 1982). Similar effects of an insecticide-induced reduction of food and dietary sifts has been found in other studies as well (Cooper et al., 1990; DeReede, 1982; Stribling and Smith, 1987) (Table 4).

We can conclude that pesticides most likely have no direct effects on avian populations. However they have a high impact on the amount of available prey specimens and their nutrient value, and can increase the time spent for foraging.

# 5. Chiropteran diversity in forests and impacts of pesticides

#### 5.1 Diversity of bats in Germany

Germany inhabit 25 bat species (Meinig et al., 2009). There is a gradient in species numbers from North towards the south, with highest numbers in the South (Meschede et al., 2002). Due to high proportions of endangered species, there is a good knowledge base of distribution especially inside of nature conservation areas and for species protected by Habitats Directive (Council Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora).

#### 5.2 Ecology and patterns of variability

Bats can be found in all types of forest and woodland (summarized in Meschede et al. (2002)). Patterns of distribution and species diversity are variable to a high degree. Even within a stand, compositions of bat communities and their spatial distribution are uneven. There is also a very high daily, seasonal and annual fluctuation due to various factors, especially based on specific site conditions, as well as on individual mobility and foraging strategies.

The specific species composition depends mainly on the spatial complexity of a forest, as well as the accessibility of insects as diet and the availability of water (Almeida and Ditchfield, 2014). Tree species composition was also found to be important (Johansson et al., 2013). Deciduous forests have usually higher importance for bat specimens and yield higher species numbers than coniferous forests (Meschede et al., 2002). Possible reasons are the usually higher numbers of prey specimens, as well as availability of different types of long-lasting quarters (Meschede et al., 2002). Bats frequently change their roosting sites, many of them every night.

In managed landscapes old growth structures e.g. dead wood and old forest have the highest importance for biodiversity of bats, as they are correlated with higher densities and species numbers (Johansson et al., 2013). Also very specific structures, tree retention, specific habitats for sensitive species (e.g. understory or edge zones) can be woodland key habitats for bats (Johansson et al., 2013).

Usually oak forests have higher numbers of bat species, but also large pine forests can be rich in terms of species numbers. But in the latter only low densities were found, because of the usually very unfavourable situation of quarters (Meschede et al., 2002; Schmidt, 1998).

During flight bats face exceptionally high energy costs (Voigt et al., 2010). These energy was gained directly from rapid combustion from dietary nutrients (foraged prey), and not from endogenous sources (lipids and glycogen, (Voigt et al., 2010)). Each bat feed at night arthropods with a share of 20 - 50% of their own body mass.

There is also a high variation in energy consumption related to different life cycle strategies (Barros et al., 2013). According to (Srivastava and Krishna, 2008), the reproduction is frequently synchronized with food availability in the environment, this pattern is especially significant for bats from temperate regions (Barros et al., 2013; Turbill et al., 2003).

Bats are opportunistic hunters (Barclay, 1985; Fenton, 1982; Neuweiler and Fenton, 1988). They usually feed on different species from very different arthropod orders and families, according to preferred prey body size (Barclay and Brigham, 1991) and also on their specific way of echolocation and the hearing response of prey (Fenton and Fullard, 1981). Prey consist mainly of small Lepidoptera and larvae, but also of Neuropteroidea, Coleoptera, Araneae, Diptera, Coleoptera and other small insects (Neuweiler and Fenton, 1988; Teubner et al., 2008). Sometimes strong preferences for a specific prey have been found, for example when there is a large offer of specimens of a specific prey species, as found during a mass outbreak (Meschede et al., 2002).

All types of woods and forest were used for foraging activities (Meschede et al., 2002). For foraging activities most species have strong preferences for specific structures in a

Table 4. Dire	ct and indirect Effect	s of insecticides or	n forest birds (?	<ul> <li>unknown effect</li> </ul>	ts, o – no effect, ·	<ul> <li>negative effect, -</li> </ul>	- high
nega	tive effects).						

Classes of contaminants	Effect	Type of Effect	Sources
Bacillus thuringiensis kurstaki (Btk)	-	Negative impacts on the bird communities, while the skeletonizing of forest by <i>Lymantria dispar</i> had no negative impact	Bell and Whitmore (1997)
Bacillus thuringiensis kurstaki (Btk)	0	Only minimal effects on reproduction of hooded warbler ( <i>Wilsonia citrina</i> ). Nesting success was significantly higher in untreated sites, significant differences in feeding rates only for small clutches	Nagy and Smith (1997)
Bacillus thuringiensis israelensis (Bti)		Intake of Diptera (Nematocera), as well as their predators (Arachnida & Odonata) decreased significantly at treatment sites. A significantly higher rate of very small prey was consumed, but total foraging rates were lower. Clutch size and nestling survival were significantly lower at treatment sites.	Poulin et al. (2010)
Diflubenzuron	0	Intake of diflubenzuron had no negative impact on birds, based on Martinat et al. (1987)	Eisler (1992)
Diflubenzuron	0	Acute toxicity is extremely low for birds, acute oral LD50 was reported as 3,8 g/kg body mass for a songbird ( <i>Agelaius</i> <i>phoeniceus</i> )	Maas et al. (1981)
Diflubenzuron	-	negative impacts on the bird communities, while the skeletonizing of forest by <i>Lymantria dispar</i> had no negative impact	Bell and Whitmore (1997)
Diflubenzuron	-	Application has had a significantly negative impact on number of insect-feeding bird species and individuals	Schönfeld (2007, 2009)
Diflubenzuron	0	Aximum residue in 8 different insectivorous canopy foraging birds was 0,21 mg/kg whole body FW.	Martinat et al. (1987)
Diflubenzuron	0	Maximum residue in 6 different insectivorous ground or low foraging birds was 0,2 mg/kg whole body FW.	Martinat et al. (1987)
Diflubenzuron	-	Strong dietary shifts. Gut contents of 9 songbird species was analysed. Total biomass of gut contents was significantly reduced for 2 species, but greater for 1 species	Sample et al. (1993b)
Diflubenzuron	0	No significantly changes in species diversity, survival, morbidity, brain cholinesterase activity, behavior was found at either dose (140 and 280 mg/ha)	Richmond et al. (1979)
Diflubenzuron	-	Male <i>Vireo olivaceus</i> spent more time foraging and covered larger areas (3,2 and 2 times), shift in dietary related to decrease of caterpillars at treated plots	DeReede (1982)
Diflubenzuron	0	Reductions in the percentage abundance of Lepidoptera larvae in the diet of birds	Cooper et al. (1990)
Diflubenzuron	0	No directly endangerment birds, after reducement of arthropods as diet, no significant decrease in the overall size or diversity of breeding bird populations.	Stribling and Smith (1987)

landscape, like ecotones and edges alongside roads, trails and open water.

Meschede et al. (2002) classified native bat species according to preferences for foraging activities in forests. Some species are specialized to hunt in the air above or inside canopies (*Barbastella barbastellus*, *Eptesicus nilssoni*, *Nyctalus noctula*, *N. leisleri*, *Vespertilio murinus*), while other hunt mainly below canopy. Some bat species are specialized to pick up the prey from leaves (e.g. *Myotis bechsteini* and *M. nattereri*, *Plecotus auritus*) or from the soil surface (*Myotis myotis*).

According to several authors (e.g. Agosta et al. (2003); Almeida and Ditchfield (2014); Boonman et al. (1998)) the efforts for flying and catching insects are higher in structurally complex patches than in open areas. Also prey detection can be harder. On the other hand structurally complex patches can also provide higher numbers of prey specimens and species. Preferences of understory are different in different bat species, depending on their specific echolocation and hunting behaviour. Other bat species searches for prey by above the trees or close to water surfaces (Boonman et al., 1998).

Seasonally activity time is synchronized with main activity of potential prey and daily activity time is at dawn and in the night. During cool weather, insect activity and foraging success and energy intake declines dramatically (Hickey and Fenton, 2016; Paige, 1995). Torpor is a common strategy to substantially reduce metabolic rates (up to 98% of basal rates (Studier, 1981)) and to manage their energy expenditure and survival in temperate climates during these times (Turbill et al., 2003). Bats have low reproduction rates and "require high rates of adult survival to avoid population declines" (Barclay and Harder, 2003).

#### 5.3 Effects of insecticides

#### 5.3.1 Exposure

Direct exposure of bats during the application of insecticides is assumed to be low, as activity time of bats is mainly during dawn and in the nighttime while application of insecticides occurs mostly during daytime. But seasonal activity is also highly correlated with times of high activity by arthropods. Highest numbers of arthropods occur usually in spring and early summer (see chapter 3.2.2).

#### 5.3.2 Direct effects

Many negative effects of insecticides on bats have been reported in the past. Especially carbamates and organophosphate insecticides (direct mortality during exposure, and subletal effects on thermoregulation, reproduction and food consumption, as well as they can lose their orientation and can fall to the ground, at least be more easily predated) and organochlorine insecticides (Cyclodines, DDT, DDE: high bat mortality, several sublethal effects, high rate of metabolization) are threats for bats (extensive review in O'Shea and Johnson (2009). Besides of these also other chemical non pesticide contaminants (polychlorinated biphenyls, polyaromatic and aliphatic Hydrocarbons, heavy metals) have negative impacts on bats, but usually to a lesser extent or less well documented (O'Shea and Johnson, 2009).

No data are available on effects of diflubenzuron on mammals in wildlife (Eisler, 1992). Results of studies on laboratory or domestic species are available, and have shown that diflubenzuron is not mutagen, teratogen or carcinogen on mammals (Eisler, 1992).

Application of pyrethroids can likely be related to subletal effects, like impairing of flight ability or having negative effects during hibernation, but their significances still remains unknown to a large extent (see Table 5, for review see O'Shea and Johnson (2009). Also bats were known to have higher

metabolic rates, compared to birds and mammals of similar size (Clark Jr and Shore, 2001).

#### 5.3.3 Indirect effects

Bats face exceptionally high energy costs during flying activities (Voigt et al., 2010). They also depend on high energy resources for hibernation, daily and seasonal migration, reproduction and lactation (Srivastava and Krishna, 2008; Voigt et al., 2010). As energy is mainly gained directly and rapidly from metabolizing foraged prey the sudden reduction of food items seems to be a crucial point for the individual survival and fitness (individual reproductive success).

Application of insecticides leads not only to a strong reduction of the availability of prey species and individuals. If main prey items are less present, bats have to migrate or shift to other prey objects. These can be harder to hunt or contain less proteins and energy equivalents. There is a large variation of protein content between and within different insect orders. Protein content is usually high in insects, but varying to a very large extent between 15 - 81% of dry matter. The protein content of insects also varies strongly depending on species and life stage. Usually instars of Lepidoptera have high protein content ranging from 52 to 80% of dry matter (Bukkens, 1997; Ramos-Elorduy, 1997; Xiaoming et al., 2010).

Prey specimens can also be contaminated. Due to hunting by echolocation no dead specimen will be feed. After application the death of a prey specimen is usually a little bit delayed in time (few minutes to several days), depending on exposition and active substance. For example the impact of diflubenzuron is delayed up the next ecdysis.

Research of residues of other insecticides (fenoxycarb, chlorpyrifos-methyl) from different nocturnal arthropod samples have shown highest peaks directly after application. Only small moths had highest residues on day 1 after application. Afterwards the residue values decreased, in large moths at day 8 values were below quantification level. Residues from foliage-dwelling arthropods has been found to be 20 to 50 times higher than all the other tested groups (Stahlschmidt and Brühl, 2012).

Arthropods harmed by pesticides, are likely more easy be located and hunted. Contaminated arthropods also share the diet of bats. So it is imaginable that contaminated diet may have a time delayed influence on reproduction and milk feeding.

Even without exact knowledge about subletal effects on bats, impacts on fitness seem likely and follow up deaths possible. Hibernation can depress immune functions. Any additional environmental insults could lead to population declines, e.g. due to Pathogenic Fungi while hibernation (Eskew and Todd, 2013; Quarles, 2013; Warnecke et al., 2012). The newly described infection with the Pathogenic Fungi *Pseudogymnoascus destructans* during hibernation is worldwide increasing, but seems to be less crucial for Palaearctic bats than for others (Gargas et al., 2009; Zukal et al., 2014, 2016).

Bats are often reported as being threatened by pesticides (Meschede et al., 2002; Stahlschmidt and Brühl, 2012), but

Classes of contaminants	Effect	Type of effect	Sources
Pyrethroids	?	"There has been little research on the presence or effects of pyrethroids on bats, a void that should be filled in light of the extensive and growing use of these compounds."	O'Shea and Johnson (2009)
Pyrethroids	o ?	<i>Cis</i> –Permethrin and <i>Trans</i> -Permethrin was found in Guano of <i>Tadarida brasiliensis</i> , but not in carcasses and milk samples (North America)	Sandel (1999)
Pyrethroids	0	"Low toxicity in laboratory mammals."	Peterle (1991)
Pyrethroids	-	"Some pyrethroids may persist in the environment and may adversely affect bats, particularly chlorinated forms such as cypermethrin."	Clark Jr and Shore (2001)
Pyrethroids	-	Carcasses of two hibernating bat species ( <i>M. lucifugus</i> , <i>M. septentrionalis</i> ) have been reported to contain permethrin and esfenvalerate, but toxicological significance remains unknown (North America: Missouri)	McFarland (1998); O'Shea and Johnson (2009)
Pyrethroids	-	Laboratory dosing of permethrin to <i>M. lucifugus</i> , showed that field-grade formulations were more toxic than analytical grade permethrin	McFarland (1998); O'Shea and Johnson (2009)
Pyrethroids		Ability to fly of <i>M. lucifugus</i> was impaired by exposures much lower than the lethal doses	McFarland (1998)
Pyrethroids	0	"Experimental studies of <i>Pipistrellus pipistrellus</i> held in captivity of permethrin treated timber woods showed no mortality, and permethrin was not accumulated in tissues or fur (Great Britain)."	McFarland (1998)

Table 5. Effects of pyrethroids on bats (? – unknown effects, o – no effect, - negative effect, - high negative effects).

evidences for direct impacts of Btk, diflubenzuron or lambda-Cyhalothrin have not been found. Recent studies have shown that bats are most likely "potentially more sensitive to reproductive effects of pesticides than other mammals" (Stahlschmidt, 2007; Stahlschmidt and Brühl, 2012).

#### 6. Summary and Conclusion

#### 6.1 Information is scarce

The knowledge on directly or indirectly effects of the above mentioned insecticides (*Bacillus thuringiensis kurstaki*, diflubenzuron, lambda-Cyhalothrin) applied by aircraft in forest canopies is still scarce. Of course the impact of active substances is well studied by laboratory experiments for some specific test species, but these results can hardly be generalized for other species or natural environments. Some studies have shown that the impact of lambda-Cyhalothrin can be significantly more detrimental for closely related species, than for the test species in laboratory itself or that (chapter 3.2.1).

Only very few field studies are available in general. Few more studies have been conducted, but haven't been published mostly. Many of these studies in Germany belong to so called grey literature, like unpublished surveys and reports of authorities (monitoring or efficiency controls) or academic theses (Master, Bachelor, Diploma theses).

Often the reported effects are hard to be interpreted and suffer from several methodological restrictions and short comings. Some will be illustrated here.

Although there is a very good monitoring of pest species by the appropriate authorities there is still an uncertainty of prognosis of calamities to some extent. The planning and approval process of research studies needs preparation time, so several studies suffer from an asynchrony of the research time schedule and specific pest species calamities. Most studies began during or shortly afterwards defoliation or insecticide application. Often the missing knowledge about the prior state of communities inside stands was taken from direct comparison of reference study plots either without defoliation or without insecticide application.

Usually there is no or little detail knowledge about the animal communities of a specific stand site and their dynamics and spatial variation prior insect pest calamities or insecticide application.

The reductions of densities of non-target animals due to

insecticide application by aircraft are often overlapped by natural, especially phenological effects. In spring and early summer months highest densities of arthropods can be found, synchronized usually as well with the breeding seasons of birds (see chapter 4.3.1) and bats (chapter 5.3.1). Most pest species are feeding as larvae during this time and are the target species of application of insecticides (chapter 3.2.2). So normally the application takes place near the natural peak of densities of both pest species and non-target species.

Application of insecticides by aircraft in forest canopies will lead to a reduction of densities of arthropods, depending on the selectivity of the used active substance. The success of an application can be measured by the number of falling down specimens of the target pest species, or by measurements of pest species rates before and shortly after application and felling of single test trees.

Usually in late spring and early summer time (May – June) the number of species and their abundances in invertebrate communities are declining due to the specific phenologies of most of the species (e.g. Gruppe et al. (2008)). Therefore the impacts of an application of an insecticide on non-target species can often not clearly be interpreted and distinguished from natural declining effects.

There is still little knowledge about population dynamics of canopy inhabiting arthropods. Most studies are based on small samples or characterizing only a single tree canopy. The overall variation of species numbers and their densities within a specific stand is usually high. Monocultural stands, like many pine stands may have smaller variation inside communities, but inside canopies of mixed forest stands, especially mixed stands with oak, the distribution of arthropods is very uneven and may vary to a very great extent (review and discussion in Gruppe et al. (2008)). Also they point out, that the knowledge about the horizontal distribution is very scarce, citing only Stork et al. (2001) (oak canopies) as single known other reference. They conclude that "we are still far from understanding the communities of different compartments within tree crowns and their interaction". Even the idea of a simple division in a near crown and a near ground compartment in monocultural stands (pine stands) is to "crude" due to their complexity (Gruppe et al., 2008; Horchler and Morawetz, 2008).

Pest species can heavily alter their environments at least by a drastically reduction of leaves and the dropping of excrements. During defoliation and skeletonizing they alter several environmental conditions inside a stand, and can have an impact on surface vegetation and the top soil layer (chapter 2.1). The altering of environmental variables can increase diversity and abundances of animals with different life strategies (predators, species with preferences for less shady environments, species with preferences for dead or decaying wood) and could lead to shifts in ground floor vegetation.

Little is known on the effects of concurrence on non-target species during the dominance of a pest species. Especially in canopies of oak forests this seems to be very complex as many phytophagous species can occur simultaneous or can be part of a several species pest phenomenon ("Eichenfrühjahrsfraßgesellschaft").

#### 6.2 Methodological problems

Beside the often low comparability of stands due to the high spatial and temporal variation of their specific communities, and their different stand histories (including pesticide use or other disturbances in past years), several other methodological problems occur.

Most studies are short term studies. Documentations of down falling dead specimens after the application of lambda-Cyhalothrin were done usually only for the following up next 24 hours. The impact of Btk and diflubenzuron is delayed in time for days or weeks, the measurement of the impact of these insecticides in nature is very sophisticated and usually not monitored.

The impacts on target and non-target arthropods can partly be derived from monitoring of number specimens and species found dead after the application of pesticides. Usually this monitoring lasts only one day. The monitoring should be applied for several days and the results should be published. Down falling dead specimens cannot clearly be attributed to single trees or their canopies and are defined usually based on the area where they were collected.

The determination is often restricted to a simple account of numbers of specimens of a higher level taxon, like family. For interpretation they should always be determined to species level and at least evaluated for functional groups or ecological guilds.

Different sampling and observation methods bear different results. Results from below ground canopy fogging, felling of sample trees, monitoring of down falling dead specimens after application of insecticides from aircraft are nor easily comparable among each other, neither with standard methods like light traps, flight interception traps or other types of eclectors. Only a good combination of them and a same time, same place sampling will offer good results. They should complement each other, and should include several compartments of a stand.

Most studies begin in the year of calamities or skeletonizing of stands, or afterwards pesticide use. Usually there is no knowledge about densities of target or non-target animals prior pesticide use. As especially arthropod communities are spatially and seasonally highly variable, many samples are hard to be interpreted, and short-term effects are needed to be distinguished from long-term effects. Recolonization can only be interpreted if the long-term dynamics of communities are known. Population densities prior the uses of pesticides remain usually unknown to a large extent.

There is a need of meaningful long term studies for stands that are susceptible and exposed, beginning in years of latency and more than one year afterwards skeletonizing of stands. Only few studies are available on the recolonization of forest canopies after pesticide treatments. Also in well studied species groups, like lepidopterans the knowledge on recolonization ability and movement patterns of single species is scarce. A first evaluation of threatening and recolonization abilities for Lepidoptera will be published (Brunk et al., in prep.).

Also the studies of birds and bats are limited by methodological restrictions, especially as they face normally no direct effects after the use of insecticides. They are often opportunistic hunters and their reproduction is frequently synchronized with food availability in the environment. Stands with high availability of food will possibly inhabit more reproducing birds and bats. In these years the size of breeding territories can become smaller and the number of breeding pairs can increase per area for several species (e.g. Brünner et al. (2008)). But there are a number of species with large territories during the period of nest-building and egg-laying, or nestling time (review for several species in Møller (1990)).

All three insecticides will reduce drastically the availability and quantity of food (crucial especially for bats), nestling food and their qualities (chapter 4.3.3) and can have therefore indirect negative effects on birds and bats, and their offsprings. Overall the sudden reduction of arthropod densities and energy rich prey availability seems to be far less crucial for birds than for bats.

But for interpretation of reductions of numbers of breeding bird pairs/ territories per area, or the number of second clutches per year after the application of insecticides there is a need of datasets of the distributional patterns before. Ideally this should be observed for more than 1 year prior the application.

#### 6.3 The impact of pesticides on non-target species

All analysed active substances can have negative effects on non-target species. These effects are direct ones for arthropods, and there is some literature available (and several, mostly unpublished studies) on the impact on pest species and nontarget species after application.

Only few studies are available on the impact of these active substances on vertebrates, especially on bats. Btk and diflubenzuron have been used widely because of an assumed low toxicity to non-target vertebrate species and their low persistence in the environment. But both substances can drastically reduce populations of larvae of Lepidoptera and Symphyta as invertebrate food items. Due to their specific life strategies this reduction of energy-rich food supply may have a higher impact on bats than on birds.

Bats are a group of mammals with high ecological requirements. All bat species of Germany belong to annex II (and mostly as well annex IV) of the European Habitats Directive. Bat species should be considered as a higher ranked subject of protection as before. Many bat species live in oak forests ore uses oak forests as specific habitats.

As presented above oak forests own a particularly high value as habitat for other organisms and for biodiversity in general. They are one of the habitats with highest biodiversity in Germany, and are extremely rich in species that are valuable for nature conservation (red list species, species protected by German or European law (BArtSchVO, European Habitats directive, European Bird Directive). Oak dominated forests and stands should be considered as especially high ranked subject of protection.

#### 6.4 Knowledge gaps and suggestions for further research

Especially studies on indirect effects on vertebrates are scarce. For bats and birds very few studies are available (see above), but almost nothing is known on effects on amphibians and reptilians living inside forests.

There is an urgent need for more intensified research on the decline of bird and bat species in relation to the use of neurotoxins, especially of lambda-Cyhalothrin. Toxicity of the discussed insecticides is assumed to be much lower for bats and birds, than for arthropods. Subletal (or lethal) effects through trophic accumulation of neurotoxins are widely unknown and unstudied. The consumption of contaminated prey for bats and insectivorous bird species as well as the ingestion of contaminated seeds for granivorous bird species are still a possible reason for the decline of many bird species (Goulson, 2013; Hallmann et al., 2014). There is a need in further ecotoxicological studies over longer time scales especially on small and common forest specialists and the inclusion of impacts on "learning, behaviour and fecundity" (Goulson, 2013). Furthermore, long-term monitoring of bird and bat populations in stands with perseverative gradations, not only during and afterwards of mass outbreaks of insect pests is needed. In these stands and areas vertebrates (insectivorous and granivorous birds, bats, reptilia) that were found dead, should be analysed for residues of insecticides or their metabolites.

Facilitation of mixed forest stands: The facilitation of mixed forest stands, with higher shares of broad-leaved trees and higher diversity of structures especially in the larger *Pinus sylvestris* stands in North-East Germany will be an important task for the future (Möller, 2007). Positive Effects of broad-leaved trees and heterogeneity of stands have been found (e.g. Rös et al. (2003), reduced threat of *Panolis flammea* on *Pinus sylvestris* stands). The facilitation effects should be monitored and evaluated for relevance for populations parasitoids, of predators and other natural antagonists of pest species).

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