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Original article

Indirect effects of bioinsecticides on the nontarget fauna: The Camargue experiment calls for future research

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ABSTRACT

Following its high selectivity and low toxicity to nontarget organisms, *Bacillus thuringiensis var. israelensis* (*Bti*) has become the most commonly used microbial agent to control mosquitoes worldwide. Considered non-toxic to mammals, birds, fish, plants and most aquatic organisms, *Bti* direct effects on the nontarget fauna are largely limited to non-biting midges (Chironomidae). Studies addressing the indirect effects of *Bti* through food web perturbations are scanty and showed no significant results. Mosquito-control in southern France was implemented in 1965 using various insecticides over 400 km of coast. In spite of a high mosquito nuisance, the Camargue wetlands were excluded from this control programme to preserve biodiversity. The expanding use of *Bti* has prompted the implementation of an experimental mosquito control in 2006 involving 2500 of the 25,000 ha of larval biotopes of the Camargue, accompanied by impact studies on the nontarget fauna. Using birds from natural and human-inhabited areas as model species, we assessed trophic perturbations caused by three years of *Bti* applications. The preliminary results of this 5-yr programme revealed significant effects of *Bti* spraying on abundance of reed-dwelling invertebrates serving as food to passerines, as well as on the diet and breeding success of house martins nesting in rural estates and small towns. Very few studies (if any) have provided such compelling evidence of an insecticide affecting vertebrate populations, putting into question the environmental-friendly character of *Bti*, at least in some areas. The significance of these results are discussed within a wider context and completed with an analysis of the current *Bti* bibliography to highlight and orient priorities for future research on this topic.

1. Introduction

Since the discovery of its selective acute toxicity against mosquitoes and black flies in 1977 by Goldberg & Margalit, *Bacillus thuringiensis var. israelensis* (*Bti*) has become the most commonly used microbial agent to control these pest species worldwide (Lacey, 2007; Rowe et al., 2008). *Bti* is considered as non toxic to mammals, birds, plants and most aquatic organisms (Boisvert and Boisvert, 2000; Lacey and Merritt, 2004). Effects on the nontarget fauna are largely limited to other Nematocera (Diptera sub-order including mosquitoes) such as chironomids under laboratory or field conditions (Boisvert and Boisvert, 2000; Lacey and Merritt, 2004).

Mosquito control in southern France has a long history. According to a large-scale environmental planning 50 years ago, some 300 km of the French Mediterranean coast were designated for tourism development, 60 km enclosing the Camargue wetlands for nature preservation and another 50 km for industrial development. A public body, the Entente Départementale pour la Démoustication (Inter-departmental Alliance for mosquito-control or EID), was created in 1965 to control the nuisance caused by mosquitoes, considered as incompatible with economical development, over the whole area except the Camargue.

From the 42 species of mosquitoes occurring in southern France, over half are biting humans. However, mosquito-control operations are targeting mainly three species (*Ochlerotatus caspius, Oc. detritus* and *Aedes vexans*, hereafter referred to *Aedes* mosquitoes), which have the characteristic of laying their eggs on moist soil instead of water. These eggs are quiescent and resist desiccation for long periods until rainfall or water management triggers their development through flooding of small depressions or water-level rising in flooded areas, resulting in a synchronous emergence of adult (biting) forms and a high nuisance. The control of these mosquitoes relies on the frequent monitoring of their potential larval biotopes identified by indicator plant species and reported on 1:5000 scale maps by EID technicians (Franquet et al., 2002). Ground or aerial

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spraying occurs only when mosquito larvae appear in the monitored water bodies, resulting in treatments that are targeted in time and space, which would not be possible with the control of adult forms that rapidly disperse in the environment after emergence. These larvicide treatments, however, were until recently often complemented with adult spraying, using mainly fenitrothion, the main insecticide used by EID until 1990. Temephos (Abate), another organophosphate, was the main larvicide used until recently, but has been replaced by *Bti* in 2007, after it became the only insecticide homologated by the European Commission for controlling mosquito larvae.

The Rhone Delta or Camargue in southern France is a mosaic of natural and human-modified ecosystems including agricultural fields (mostly rice), halophilous and freshwater marshes, reed beds, temporary ponds, lagoons, and salt pans spread over 145,000 ha. The high proportion of temporary and semi-permanent wetlands translates into a high nuisance of *Aedes* mosquitoes, which has long been the subject of social and political debates. Use of *Bti* appeared as a promising solution for reducing mosquito nuisance without impacting biodiversity. A first small-scale experiment showed no significant short-term impact on benthic chironomids, should *Bti* spraying be carried out within 4 days after mosquito larvae appearance at a maximum dosage of 3 L/ha. Based on these recommendations, the Parc Naturel Régional de Camargue, created in 1970 to promote sustainable development of the territory, launched an experimental control programme encompassing 2500 of the 25,000 ha of potential larval biotopes in collaboration with EID. This 5-yr programme was accompanied by impact studies on the non-target fauna by applying the precautionary principle. Since August 2006, *Bti* spraying (aqueous solution of VectoBac®12AS at 2.5 L/ha) is systematically carried out whenever *Aedes* mosquito larvae appear in natural or semi-natural water bodies that are regularly monitored by EID technicians. Frequency of *Bti* spraying depends upon meteorological conditions and water management. For instance, 29, 46, and 41 aerial treatments representing a cumulative total of 3831, 5093 and 5282 ha were carried out in 2007, 2008 and 2009, respectively. Passerine birds from natural and human-inhabited areas were selected as model species to assess the effect of potential trophic perturbations caused by *Bti* spraying. The results of these ongoing and partially published studies are briefly presented and interpreted relative to the available literature on *Bti* effects to orient and stimulate future research.

2. Methods

2.1. Bti effects on reed invertebrates

A previous study carried out in reed (*Phragmites australis*) marshes of southern France (Poulin et al., 2002) revealed a positive correlation between the number of passerine birds sampled with mist nests and a food availability index corresponding to the number of sweep-netted invertebrates weighed by their proportion in the birds' diet (Poulin and Lefebvre, 1997). This food index was inversely correlated with the duration of ground dryness in the preceding year (Poulin et al., 2002), so that food availability can be predicted from a regression model involving the length of the marsh hydroperiod. The *Bti* study protocol consists of sampling invertebrates with 500 sweep-net strokes in the reed vegetation within treated and control reed marshes and to monitor monthly their water levels. Invertebrate sampling is carried out once per year during the main passerine breeding season (mid May-early June) in midday when the reeds are dry and the wind low. The aim is to evaluate whether the difference between the observed and expected food availability index based on hydrology varies significantly between control ($n = 10$) and treated ($n = 2$ in 2007–2008, 3 in 2009) areas. The number of treated sites will be increased to five for the remaining two years of this study.

2.2. Bti effects on house martins

The second model species is the house martin *Delichon urbicum*. This migratory aerial insectivore breeds colonially in inhabited areas and feeds upon various arthropod species including Nematocera that are caught on the wing typically within 450 m from the nesting site (Bryant, 1973; Bryant and Turner, 1982). The *Bti* study protocol consists of estimating colony size (1271 active nests counted since 2006), food provisioning rates of chicks (from 9051 feeding flights observed), chick diet (380 faeces collected, totalling 14,857 identified prey since 2006) and breeding success (68 nests visited on average 26 times in 2009) at colonies located within control ($n = 3$) and treated areas ($n = 3$). Further methodological details are provided in Poulin et al. (2010).

3. Results

3.1. Bti effects on reed invertebrates

The food availability index for reed passerines was significantly lower at treated sites during all three years of the study. Overall, its mean value was +22 at control sites and −338 at treated sites relative to the predicted value based on hydrology when the mean food index issued from the reference study is calibrated at 0 (Fig. 1). This significant difference between control and treated sites (Nested-ANOVA, $F_{1,19} = 36.2$; $P = 0.00001$) would correspond to a 47% decrease in passerine numbers based on the relationship between bird abundance and food availability (Poulin et al., 2002).

3.2. Bti effects on house martins

Colony size varied across sites (range: 21–133 active nests), with a decreasing temporal trend at treated sites (Fig. 2), but annual variations were mostly related to bird survival during the non-breeding season. Food provisioning rates were slightly lower at treated sites (Fig. 2), but were mostly influenced by energetic demands of chicks (chick age). Chick diet showed systematic

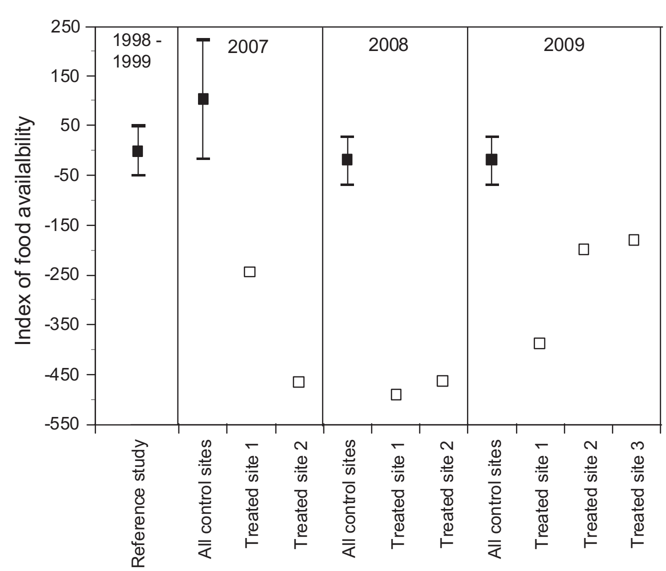

Fig. 1. Observed and predicted food availability index based on hydrology for control sites (mean ± 95% CI, $n = 10$) and each treated site sampled in 2007, 2008, and 2009 when the mean food index from the reference study is calibrated at 0.

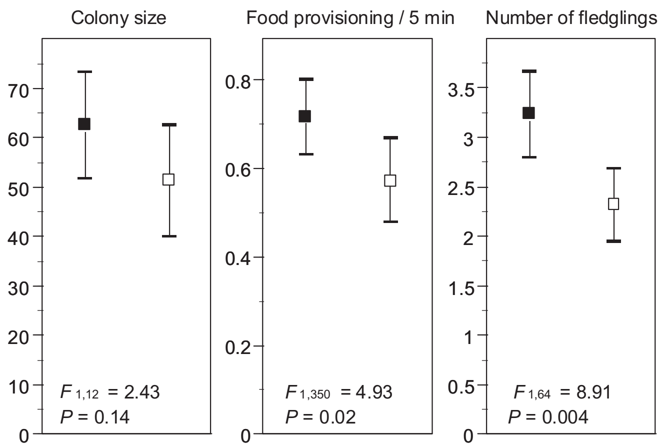

Fig. 2. Colony size, chick feeding rates and overall breeding success of house martin colonies located in control (filled square) and treated (open square) sites from 2006 to 2009 (mean ± 95% CI, treatment effect from Nested-ANOVA).

differences between treated and control sites after the start of *Bti* spraying in both prey composition (Nested-ANOVA, $F_{9,311} = 14.28$, $P < 0.00001$) and size ($F_{6,314} = 17.20$, $P < 0.00001$). Intake of Nematocera (it was not possible to distinguish mosquitoes from midges in the faecal samples), as well as spiders and dragonflies was higher at control sites (Fig. 3). Because dragonflies and spiders are favourite prey of swallows' nestlings (Foelix, 1996; McCarty and Winkler, 1999), and major predators of Nematocera (Foelix, 1996; Corbet, 1999), these results suggest indirect effects of *Bti* treatments through the food web. The lower intake of Nematocera at treated sites was mostly compensated by a higher intake of flying ants (Fig. 3), considered as a substitute prey of low quality for young nestlings (Poulin et al., 2010). Prey size also differed between treated and control sites, with energetically profitable large prey (<7.5 mm) taken more frequently at control sites and small prey (≥2.5 mm) taken more frequently at treated sites. Clutch size, as well as fledging success, were significantly smaller at treated sites relative to control, resulting in an overall breeding success of respectively 2.3 ± 0.2 and 3.2 ± 0.2 young fledged (Fig. 2). The reduced productivity in treated areas was attributed to chick starvation, with breeding success being positively correlated with intake of Nematocera and their predators at the nest level (Poulin et al., 2010).

4. Discussion

Studies on the direct effects of *Bacillus thuringiensis israelensis* on nontarget invertebrates are relatively common, with chironomids being the taxa most commonly affected (Boisvert and Boisvert, 2000; Lacey and Merritt, 2004; Lundstrom et al., 2010). Indirect effects of *Bti* through food web perturbations have, however, received much less attention. Some 311 references on *Bti* relative to its use in the environment published over the 1974–2010 period were compiled by Maffei (1997) and supplemented with Maffei (pers. comm.) and the author's current database. Although not exhaustive, this bibliographic search is used to evaluate the proportion of *Bti* research related to various topics and its trend overtime (Fig. 4). Of these 311 studies or reports, 184 (59%) were related to *Bti* efficacy, 100 (32%) addressed at least partially the direct effects of *Bti* on the nontarget fauna, 14 (4%) dealt with *Bti* persistence in the environment, mostly from the perspective of risks for mosquito-resistance, and only 13 (4%) addressed the indirect effects of *Bti*, potentially through food web perturbation. Although *Bti* is increasingly used worldwide, field investigations addressing the indirect effects of *Bti* have not increased in recent years (Fig. 4). This could be at least partially due to the lack of significant results from pioneer and subsequent studies.

Most of the studies on indirect *Bti* effects found no significant difference in abundance of nontarget taxa, based on the monitoring of treated sites before and after spraying or the comparison of treated and control sites (Purcell, 1981; DeJong and Rusterholz, 1989; Merritt et al., 1989, 1991; Wipfli and Merritt, 1994; Hanowski et al., 1997a, 1997b; Hershey et al., 1998; Balcer et al., 1999; Niemi et al., 1999; Vinnersten et al., 2009). One of the few

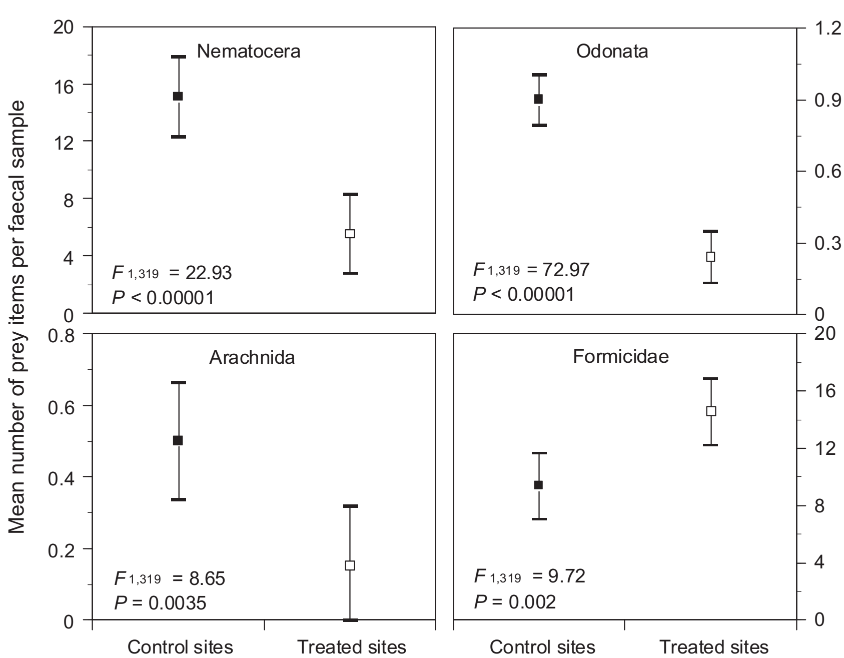

Fig. 3. Prey taxa of which the intake (mean ± 95% CI) differs significantly between house martin colonies located in control (filled square) and treated (open square) sites (treatment effect from Nested-ANOVA).

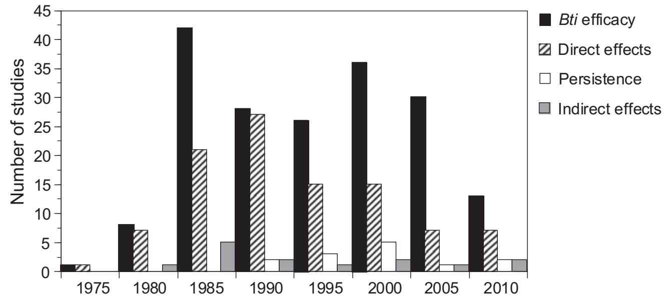

Fig. 4. Number of articles/reports on *Bti* use in the environment dealing with control efficacy, persistence, direct and indirect effects on the nontarget fauna from 311 studies published between 1974 and 2010.

exceptions involves a reduced abundance of water bugs in salt marshes (Purcell, 1981). Merritt et al. (1989) found no detectable effect of *Bti* applications on survival, diet, and functional structure of species assemblage of both invertebrate and fish downstream of a river after a *Bti* treatment in June. Hershey et al. (1998) found a strong reduction in insect density (from 57 to 83%) and biomass (50–83%) in the second and third year of *Bti* treatment when comparing 18 wetlands randomly assigned to an equal number of control and *Bti* treated sites, but no negative effects were observed on breeding birds (Niemi et al., 1999). Likewise, Hanowski et al. (1997a, b) could not detect changes in bird abundance and breeding success two years before and three years after *Bti* treatment in spite of a large reduction in aquatic insects attributed to *Bti* spraying. Similar results were obtained from extensive bird studies involving other varieties of *Bacillus thuringiensis* (Holmes, 1998; Sopuck et al., 2002). The lack of significant *Bti* effects at the vertebrate level is explained conceptually by predation opportunism and prey substitution or practically by the presence of confounding factors such as annual variation in habitat hydrology, weather and nest predation effects, and temporal mismatch between spraying and bird breeding. The Camargue studies, which involved relatively simple protocols, provided significant results in the first year after *Bti* spraying. From a retrospective analysis, I identified four main conditions that are rarely fulfilled in other studies and could explain these positive results. They are outlined below.

4.1. Dependence of the model species on Bti-depressed prey

Predatory species cannot specialize or depend extensively on ephemeral prey, and mosquito production is often highly concentrated within a short period at a specific water body. It is unlikely that significant effects could arise from *Bti* treatment if a wetland is sprayed once or twice over a season. *Bti*-sensitive insect must be abundant over a relatively long period and their control (reduction) must be severe and sustained for translating into impacts on the nontarget fauna. House martins have 35% of their diet made of Nematocera in the Camargue. These Nematocera are produced from neighbouring wetlands which are sprayed within a radius of 8 km from the treated colony sites whenever mosquito larvae are developing. These various surrounding wetlands play a complementary role overtime to provide a regular and high abundance of Nematocera which can be heavily preyed upon by house martins.

4.2. Well-designed controlled or uncontrolled experiment relative to potential treatment effects

The random selection of control/treated sites across a range of wetlands, or the *a posteriori* assignment of sprayed wetlands to treated sites and of unsprayed wetlands to control sites, is inappropriate for faithful assessment of *Bti* effects on the nontarget fauna. Wetlands that do not require mosquito control are unlikely to produce large amount of *Bti*-sensitive insects and would hence share more ecological similarities with treated than control wetlands in terms of community organization. In that respect, the Camargue provided excellent experimental conditions with a globally high density of Nematocera, including at sites that were not *Bti* sprayed.

4.3. Minimisation of confounding factors

Mosquito production is often a highly dynamic phenomena triggered by weather, resulting in potentially strong annual and seasonal variations, with repercussion on plant phenology, insect abundance, bird breeding success, etc. Hence the simultaneous monitoring of treated and control areas over a few years appears as a much better approach than the monitoring of sites before and after treatment, since the latter does not permit the distinction of effects related to year, season and treatment. Adding years and sites will not necessarily reduce the contribution of confounding factors, however. The reed invertebrate study would not have produced significant results without knowledge of ecosystem functioning which lead to the modelling of invertebrate availability relative to the marsh hydrology.

4.4. Adequate sample size relative to statistical power of tests

The lack of significance does not mean “no effect”, and in many studies the large fluctuation among samples (e.g. numbers of insects captured) or their small size (e.g. number of successful breeding attempts) makes the detection of significant differences nearly impossible. House martins were an ideal model species in that respect. In comparison with birds nesting in natural habitats, their nests could be found and monitored relatively easily and suffered little predation, permitting to assess quantitatively the contribution of diet to breeding success. The narrow diet breadth of these aerial foragers compared to vegetation-dwelling species, further limited individual variation and reduced the diversity of alternative prey, resulting in highly significant diet differences between colonies located in control and treated areas.

5. Conclusions

The Camargue experiment suggests that wide scale *Bti* spraying can have deleterious effects on the demography of insectivorous birds through multitrophic interactions. I suspect that these effects are mainly associated with the sustained reduction of chironomids, which in contrast to mosquitoes, are major component of wetland food webs (Ali, 1995; Batzer and Wissinger, 1996), and owing to their benthic habits, are exposed to *Bti* over periods well-extending those of *Bti* efficacy against mosquitoes, especially in standing water bodies (Dupont and Boisvert, 1985; Ohana et al., 1987). Hopefully, the results of the Camargue experiment will contribute to stimulate and orient additional studies on the indirect effects of *Bti* in natural environments.

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