

# Don't kill your allies

The impact of chemical and biological locust and grasshopper control on birds



Wim C. Mullié

## Propositions

1. Political motives and lack of preparedness, and not scientific research, are still the main drivers for the choice for chemical rather than biological control of acridids in Africa.  
(This thesis)
2. Natural bird predation enhances the action of the entomopathogenic fungus *Metarhizium acridum*.  
(This thesis)
3. Data loggers with radar detection on marine birds are a powerful tool to detect illegal fishing on high seas.
4. Greening of the Sahel will never be achieved by the "Great Green Wall" from Senegal to Somalia as it does not provide direct monetary benefits to subsistence farmers and herders.
5. The assumption that citizens through public hearings can fundamentally change Environmental and Social Impact Assessments for offshore oil and gas projects is naive.
6. Monofilament gillnets are a threat to the population survival of the recently described West-African Harbour Porpoise *Phocoena phocoena meridionalis* on the continental shelves of Senegal and Mauritania.
7. Covid-19 lockdown and -restrictions increase creativity: this thesis proves it.

Propositions accompanying the PhD thesis:

**Don't kill your allies**

**The impact of chemical and biological locust- and grasshopper control on birds**

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Wageningen, 24 February 2021

# **Don't kill your allies**

The impact of chemical and biological locust  
and grasshopper control on birds

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# **Don't kill your allies**

The impact of chemical and biological locust  
and grasshopper control on birds

Wim C. Mullié

Thesis

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For my children and grandchildren

Sarah - Amani & Elija

Videau - Kimany

Aïcha

Adrian (Didi)

*"We must work with nature, not against it"*

Jacquie McGlade, Executive Director, European Environment Agency, 2006

*"It always seems impossible, until it's done"*

Nelson Mandela





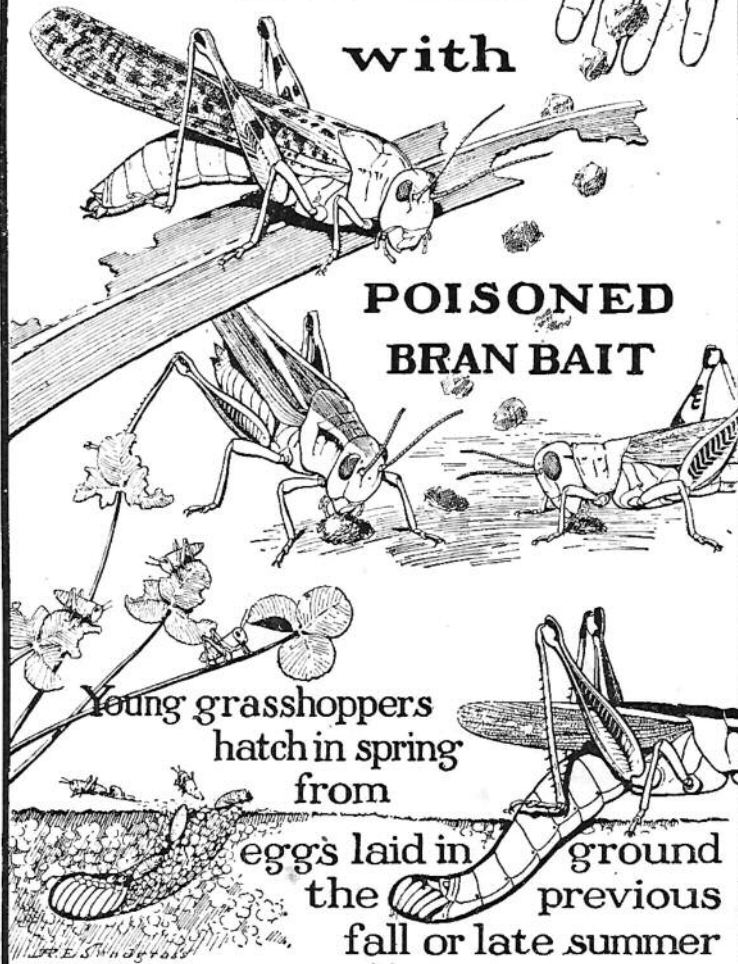
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# DESTROY GRASSHOPPERS

with

POISONED  
BRAN BAIT



## Chapter 1

# General introduction

FROM BIRDS AS NATURAL ALLIES TO BIRDS AS VICTIMS

Wim C. Mullié

## Introduction

Desert Locust *Schistocerca gregaria* upsurges and plagues can destroy harvests and pastures over vast areas and occasionally plunge entire societies into famine. The 2004–2006 Desert Locust upsurge was claimed to have destroyed 80–100% of crops in affected areas (Brader *et al.* 2006), inflicting a major impact on society and livelihoods through: (i) food shortages; (ii) strong price movements in the markets; (iii) loss of grazing areas; (iv) selling of animals at very low prices to meet the subsistence needs of the households and to buy feed for the remaining animals; (v) early transhumance of the herds; (vi) increased tension between transhumance pastoralists and local farmers over resources; and (vii) extensive migration to urban areas (Brader *et al.* 2006). In 2018 a new threat to livelihoods caused by Desert Locusts developed in the Middle East, spreading in 2019 and 2020 to the Greater Horn of Africa in the centre of the locusts' geographical distribution and India and Pakistan in the east of its range. Kenya was particularly hard hit, facing the heaviest outbreaks in 70 years (FAO 2020, World Bank 2020). Overwhelmed by the unprecedented scale of the outbreak and the imminent threat to crops, governments and populations reacted by applying insecticides, such as carbosulfan and fipronil in Kenya and diazinon in Ethiopia (data Food and Agriculture Organization of the United Nations, FAO). Neither of these compounds were recommended for blanket treatments (fipronil) or use in locust control at all, whereas diazinon is also extremely toxic for birds (HD<sub>50</sub> 50% of 0.59 mg kg<sup>-1</sup>; Mineau *et al.* 2001). All uses of carbosulfan were banned in April 2015 in eight West-African countries on the basis of high toxicity for birds and human health concerns (UNEP-FAO-CRC 2016). The use of these and other compounds resulted in widespread reported but unquantified bird and other non-target mortality in the early months of the outbreak. Video footage shown on NTV Kenya in April 2020 (<https://www.youtube.com/watch?v=jnMPXsTnDac>) showed dead and debilitated Superb Starling *Lamprolornis superbus*, Fischer's Starling *Spreo fischeri*, and Wattled Starling *Creatophora cinerea* reportedly affected by a spray of carbosulfan. Wattled Starlings are considered by some as typical "locust birds" (Fry *et al.* 2000, Meinertzhagen 1959) and may have been gorge-feeding on the poisoned locusts. Affected countries in East Africa repeated this typical instinctive reaction that prevailed since the massive introduction of synthetic insecticides after the Second World War and which usually is caused by a lack of preparedness (FAO 2020, Roussi 2020).

Although locusts and grasshoppers, also known as acridids, occasionally develop into plagues in all parts of the world, in this thesis I will mainly focus on the Western Sahel. The Sahel is the transitional semi-arid zone in northern West Africa, which is bordering the Sahara Desert in the North and the Sudan Savannah in the south, or approximately between the 200 and 600 mm isohyets (Le Houérou 1989).

### Locusts

Locusts as far as they are relevant for the scope of this thesis, belong to the orthopteran family Acrididae. Most information in this paragraph is based on Steedman (1990) and

Symmons and Cressman (2001). Locusts are usually large insects, having the capacity of changing behaviour and physiology when they occur in large numbers in dense concentrations. From being solitary, occurring in low densities, they become gregarious, change colour and various physical and physiological characteristics related to reproduction. Winged adults and immatures form swarms and migrate over long distances (sometimes >100 km per day). After mature adults lay their eggs in damp sandy soil, wingless larval stages, called nymphs or hoppers, emerge and form dense bands. A hopper band is a cohesive mass of insects that persists as long as meteorological conditions and predator pressure allow and which moves as a group in one direction. In this aspect locusts differ from grasshoppers, which generally do not form hopper bands or swarms. Several locust species occur in the Sahel, such as the Desert Locust *Schistocerca gregaria*, the African Migratory Locust *Locusta migratoria migratorioides* and the Tree Locust *Anacridium melanorhodon*. Desert Locusts usually occur in arid and semi-arid areas whereas the African Migratory Locust in the Sahel is usually found under more humid conditions such as those in the Lake Chad Basin and in the Inner Delta of the Niger in Mali (Mestre 1988).

Desert Locusts have clear seasonal patterns in their occurrence in the Sahel. The lowest frequencies of occurrence are recorded from January till June, whereas the highest frequencies occur from July till December (Pedgley 1981). This is caused by the circular migration pattern of Desert Locust swarms that originate from the gregarisation areas in Mauritania, Algeria, Mali, Niger, Chad and Sudan. Gregarisation is the process by which initially solitary locusts develop gregarious behaviour (FAO 1980), when weather conditions are simultaneously favourable over large areas. Desert Locusts develop into gregarious populations which invade the Sahel during the rainy season (April to September). When conditions continue to be favourable, they may move into agricultural lands where they can inflict serious damage.

During recessions or remissions, Desert Locusts occur in very low densities in their favourite arid habitats. Recessions are periods without widespread or heavy infestations by swarms, whereas remissions are periods of deep recession marked by the complete absence of gregarious populations (FAO 1980). Their densities become so low (<<25 individuals per hectare) and their behaviour and colouration so cryptic that vertebrate predators are no longer a serious threat to their survival. It has been postulated that high levels of predation by birds will result in strong selective pressures for the evolution of crypsis and other anti-predator defences in acridids, such as micro-habitat selection which facilitate crypsis or active escape (Schulz 1981).

During upsurges, resulting from successful breeding over four or more generations by an initially small population (Symmons & Cressman 2001), Desert Locusts start migrating at the end of the rainy season. In the Western Sahel they migrate to the Maghreb and in the Red Sea Region to the Arabian Peninsula, Ethiopia and Eritrea, but sometimes they reach as far as India and Pakistan or Kenya and Tanzania, where they continue reproducing. In the Western Region they return to the Sahel in late June where they peak at latitudes between 17 and 20 °N from October till December. No clear pattern exists in the frequency of outbreaks of Desert Locusts, data of which have been recorded ever since

1860. Upsurges start with successive seasonal rains, in one or more Desert Locust regions, which are more widespread as well as being more frequent, heavier, and longer lasting than normal (Van Huis *et al.* 2007).

Since 1980, upsurges or plagues in either one or more of the areas covered by the FAO Commission for Controlling the Desert Locust in the Western Region (CLCPRO), in the Central Region (CRC) and in South-West Asia (SWAC) were recorded in 1986–89, 1990, 1992–94, 1994–98, 2003–05 and 2018–present (December 2020) (FAO-ECLO Desert Locust Bulletins, Brader *et al.* 2006, US Congress OTA 1990, Magor *et al.* 2007). During a plague there are widespread and heavy locust infestations, the majority of which occur as bands or swarms (Symmons and Cressman 2001). Densities that have been recorded during upsurges are from 20 to 150 million individuals  $\text{km}^{-2}$  for adults. Maximum densities found for 1<sup>st</sup> instar nymphal stages are 30 000  $\text{m}^{-2}$  and for 5<sup>th</sup> instars 1 000  $\text{m}^{-2}$ . However, average densities over larger surface areas are much lower, probably 50–100  $\text{m}^{-2}$  for late instar bands (Symmons & Cressman 2001). These latter densities are comparable with grasshopper nymph densities that can be encountered under favourable conditions in the Sahel.

### Grasshoppers

Most Sahelian grasshopper species also belong to the Acrididae, although there are several other families, most notably the Pyrgomorphidae. At least the Senegalese Grasshopper *Oedaleus senegalensis* shows incipient gregarious behaviour and forms hopper bands. The Senegalese Grasshopper is considered to be the most important grasshopper pest in the Sahelian zone of West Africa (Cheke 1990). What has been described as “swarms” of *O. senegalensis* in the acridological literature more likely have been clusters of high numbers of individuals during important outbreaks (Maiga *et al.* 2008). In general, grasshoppers do not display coordinated gregarious behaviour like locusts, nor do they undergo massive diurnal movements (Maiga *et al.* 2008). The Senegalese Grasshopper is a species with three generations per year and extensive North-South movements, triggered by rainfall.

Grasshoppers have various different life history traits allowing them to cope with the harsh Sahelian environment. Species can have continuous reproduction (CR), lay diapausing eggs (DE) at the end of the rainy season or survive the dry season as diapausing adults (DA) (Mestre 1988). Furthermore, the species with continuous reproduction can have two to four generations per year, DE species one to three and DA species one or two. After maturation, the majority of grasshopper species which survived the dry season as immature adults (DA) lay eggs after the first significant rains (c. 20 mm) and die. Eggs laid at the end of the previous rainy season hatch and in June till September the population structure is dominated by nymphs. Towards the end of the rainy season the survivors become adults themselves and their numbers peak between September and November when the major part of the population consists of species that will lay eggs and die. Only populations with continuous reproduction and those that will survive as non-reproducing individuals will do so until the next rainy season (Lecoq 1978, Mestre 1988).

There is no good correlation between apparent upsurges in the different countries, which may rather be the result of inconsistencies in scouting, reporting and archiving activities, partially due to insecurity in the field, lack of fuel or decentralization, rather than of real differences in infestations. For grasshopper species surviving as immature adults such information is not available, but it can be safely assumed that such inter-annual fluctuations are the rule (Lecoq 1978). Because of the surface area annually infested with grasshoppers in the Sahel (Fig. 117 in Mullié 2009), annual chemical grasshopper control has the potential for a higher overall environmental impact than the control of locusts during occasional upsurges.

In the years before 1965, when Desert Locust outbreaks were very frequent and received most attention, grasshoppers were considered to be a local problem only (M. Lecoq, *pers. comm.*). It was not until the rainy season of 1974 when widespread crop damage occurred, two years after the major drought of 1972/73, and grasshoppers started to be seen as a regional pest affecting crop production and food security in the Sahel (Launois 1978). The most important species, both as a pest of agriculture and as a prey for numerous birds, is undoubtedly the Senegalese Grasshopper. Between 1974 and 1989 outbreaks in the central Sahel of the Senegalese Grasshopper, and of several other grasshopper species with comparable ecological requirements and life history traits, occurred in 1974, 1975, 1977, 1978, 1980, 1985, 1988 and 1989 (Cheke 1990, Maiga *et al.* 2008).

### **Bird predation seen as important in acridid control until c. 1945**

Predation of locusts and grasshoppers by vertebrates, and birds in particular, has attracted attention since time immemorial. Nevo (1996) cites numerous historic sources which report predators of locusts and grasshoppers in the eastern Mediterranean basin. An example is Eusebius of Caesarea (c. 260–340 AD, Bishop of Caesarea) who stated that the Egyptians honour the ibis for its destruction of snakes, locusts and caterpillars. This was either the Sacred Ibis *Threskiornis aethiopicus*, which was bred by the millions as an offering to the God Thoth, hence "sacred" (Houlihan and Goodman 1986) or the Hermit Ibis *Geronticus eremita* which was commonly depicted in ancient Egyptian art and more of a dryland species feeding on acridids (Bauer & Glutz von Blotzheim 1966, Goodman and Meininger 1989). Pliny (23–79 AD) explained that predators arrived in response to prayers offered to Jupiter by the people in the region of Mt. Cadmus (Jebel el Akra in today's Turkey), when locusts were attacking their fields. According to his information, migratory birds destroyed the invading locusts (Nevo 1996). In the early 20<sup>th</sup> century the Committee of Control of the South African Central Locust Bureau considered bird predation as an important control mechanism against Red Locusts *Nomadacris septemfasciata* and Brown Locusts *Locustana pardalina*. In their annual reports special emphasis was given to this phenomenon (Lounsbury 1909). The word "Locust Bird" or "Sprinkhaanvoël" in Afrikaans (Van Ee 1995) was commonly used for predators which were considered to be important in destroying locusts, such as White Storks *Ciconia ciconia*, Black-winged Pratincoles *Glareola nordmanni* (Lounsbury 1909) and Wattled Starlings (Meinertzhagen



1959). Elsewhere in Africa (Moreau & Sclater 1938, Hudleston 1958, Schüz 1955) and India (Husain & Bhalla 1931, Singh & Dhamdhare 1986), birds were also seen as important natural enemies of locusts and grasshoppers. In North America from c. 1885 to 1940 the Bureau of Biological Survey of the U.S. Department of Agriculture collected numerous data on the diet of birds by analysing stomach contents (Kirk *et al.* 1996). Data were lumped from birds collected all over the United States and differentiated into "good", "bad" and "neutral". Apart from the methodological problems inherent to stomach analysis, the differentiation was heavily criticized, summarized in Evenden (1995). Despite its important contribution to our knowledge about the food of birds in general, the Biological Survey did little to advance our understanding of the role of birds as natural predators (Kirk *et al.* 1996).

Indian Mynahs *Acridotheres tristis*, acridivorous birds from the Indian subcontinent, were introduced to Mauritius in 1762 to control Red Locusts. They were reported to be successful (Long 1981) and they also suppressed smaller outbreaks in 1933 and 1962–64 according to Coppel and Mertins (1977). However, Mynahs introduced in the second half of the 19th century to Madagascar for the same reason, failed to do so (Franc 2007). Rosy Starlings *Pastor roseus* were mentioned as the major predator of the locusts which used to occur in the steppes of the Cis-Caucasus in Russia, i.e. Migratory Locusts *Locusta migratoria* (Belik and Mihalevich 1994) and probably Moroccan Locusts *Dociostaurus maroccanus*. After the frequency of locust upsurges slumped, Rosy Starling numbers dropped sharply, only to increase again during outbreaks of Italian Locusts *Calliptamus italicus* (Belik and Mihalevich 1994).

Control of acridids before the Second World War was dominated by various mechanical methods and in particular the widespread use of arsenicals, such as white arsenic ( $\text{As}_2\text{O}_3$ ), sodium arsenite and Paris Green (copper(II) acetate triarsenite or copper(II) acetoarsenite), the first "green pesticide", by colour only (Casida 2012). These compounds were applied either as a spray or as poisoned bran bait or dust (Lounsbury 1909, Saulnier 1916, Davis 1919, Uvarov 1928). Arsenicals have meanwhile been shown to be teratogenic in mammals, and in Mallards *Anas platyrhynchos* they reduced the growth rate of ducklings and reduce both liver and egg weights (ATSDR 2007). Little field information is known on avian side-effects following the use of arsenicals for acridid control. Lounsbury (1909) mentions some livestock fatalities, but observed that White Storks gorge-feeding on poisoned locusts, after applying recommended dose rates of sodium arsenite, appeared not to be affected and farmers collected large quantities of these treated locusts to feed to poultry and Ostriches *Struthio camelus* (Lounsbury 1909). Whitehead (1934) force-fed chickens with  $\text{As}_2\text{O}_3$  poisoned bran and found  $119 \text{ mg kg}^{-1}$  was a "slightly toxic dose". The same author noticed that poisoned bran strongly repelled domestic fowl, but that consumption of poisoned grasshoppers "may be somewhat injurious" to nestling birds (Whitehead 1934). However, Lilly (1940) did not find negative effects of grasshoppers poisoned with  $\text{As}_2\text{O}_3$  on young pheasants.



### Changing views on avian predation after widespread introduction of chemical locust control

Elliott (1962) mentioned more than one hundred bird species of 34 families as locust predators in Eastern Africa. Husain & Bhalla (1931) did the same for the Indian subcontinent and came up with 35 species, while more than 200 bird species were found to prey on grasshoppers in the USA (Metcalf 1980). An updated survey of published accounts and other relevant sources conducted by Mullié (2009; Table 21) revealed that at least 537 bird species of 61 families are currently known to prey upon locusts and grasshoppers in Africa. Several bird families in Africa, such as the Coraciidae and Laniidae, appear to be specialised acridivores. A wide range of acridid species is recorded as food for Ciconiidae, Glareolidae and Corvidae. Individual bird species which can be associated with a diverse acridid diet are e.g. Black Kites *Milvus migrans* and Pied Crows *Corvus alba*, but also White Storks and Abdim's Storks *Ciconia abdimii*, Lesser Kestrels *Falco naumanni* and Montagu's Harriers *Circus pygargus*. Based on extensive field work in Niger, Petersen *et al.* (2008) and Falk *et al.* (2006) found that pre-migratory movements of Abdim's Storks are in synchrony with the seasonal movements of Senegalese Grasshoppers.

Most published accounts on predation of acridids in Africa since the Second World War are of birds present during major outbreaks of locusts. It is therefore not surprising that several authors concluded that the efficacy of (avian) predators in dealing with swarms during upsurges or plagues was rather limited (Dean 1964, Stower & Greathead 1969). The magnitude of such locust swarms (adults) or of hopper bands (nymphal stages), however, was of an extremely massive nature, as visually described by Meinertzhagen (1959): "*The hoppers were at Middelburg (Cape) and so numerous that trains were held up owing to squashed bodies greasing the lines so that engine wheels would not bite the rails...*".

Given the unpredictable nature of locust outbreaks, grasshoppers, in particular the species surviving the dry season as diapausing adults, are a much more reliable food source for (migratory) birds wintering in the Sahel than migratory locusts. However, it was not until recently that this concept found its advocates (Mullié *et al.* 1995, Jensen *et al.* 2006). The importance of *Ornithacris cavroisi* as a major prey species during the dry season in the Sahel has meanwhile been described for Swallow-tailed Kites *Chelictinia riocourii* (Mullié *et al.* 1992, Pilard 2007, Mullié 2009), White Storks (Mullié *et al.* 1995), Fox Kestrels *Falco alopex* (Brouwer & Mullié 2000), Abdim's Storks (Falk *et al.* 2006), Montagu's Harriers (Trierweiler & Koks 2009, Mullié 2009) and Lesser Kestrels (Pilard *et al.* 2008, Mullié 2009).

The widespread use of chemical pesticides for locust and grasshopper control since the Second World War overshadowed the attention for "economic ornithology" (Kirk *et al.* 1996). Illustrative is the ratio of publications by USDA scientists on chemical vs. biological pest control which went from 1:1 in 1915 to 20:1 in 1946 (Sailer 1972). Pesticide applications induced acute and visible mortality of the pest, which immediately satisfied the desire to reduce it. However, nontarget organisms including birds were also heavily affected. At first, predominantly organochlorine pesticides were used: dinitro-ortho-cresol (DNOC) and hexachlorocyclohexane (HCH), initially as a mixture of  $\alpha$ -,  $\beta$ - and  $\gamma$ -HCH, later only the  $\gamma$ -isomer (lindane), and from c. 1955 onwards aldrin and dieldrin, cyclodiene

organochlorine insecticides. The latter two compounds are also highly toxic to man and livestock (IPCS INCHEM 1989) and were responsible, together with DDE (dichlorodiphenyldichloroethylene), a breakdown product of DDT (dichlorodiphenyltrichloroethane), for severe impacts on wild birds worldwide (Newton 2013). National crop protection services, locust control centres and international organizations rapidly lost interest in natural factors contributing to population regulation of Acrididae. Chemical locust control in Africa since then has been claimed to be responsible for strong avian population declines in Africa and Europe (African Swallow-tailed Kites: Thiollay 2006a,b,c, White Storks: Dallinga & Schoenmakers 1984, 1989). However, the evidence was rather anecdotal as very few field studies on side-effects of locust control were done, contrary to the number of field studies of side-effects of Tsetse Fly *Glossina* spp. control (Peveling and Nagel 2001) including those where dieldrin was used.

The use of dieldrin in locust control has sparked considerable debate before its use was eventually discontinued. Advocates of its continued use for vegetation baiting at ultra-low dosages to target Red Locust hoppers (Gunn 1975) stated that only dieldrin was capable of preventing locust outbreaks from reaching plague dimensions. The United States Environmental Protection Agency (US-EPA) had banned dieldrin and aldrin in 1974 (Federal Register 39 FR 37265) which prevented the United States Agency for International Development (USAID) from providing it or to assist campaigns using it throughout the 1986-88 Desert Locust plague. In the UK it was also banned around the mid-1970s (Newton 2013), but it took another 15 years before FAO eventually commissioned a desk study of the environmental fate and potential hazard of dieldrin in tropical and hot arid climates. This review highlighted a lack of residue data and concluded that both quantitative information on ecological side-effects in arid zones and human exposure through domestic herbivores was missing and badly needed (van der Valk 1988).

Due to international legislation restricting or banning the use of most organochlorine locust control insecticides, mostly to protect humans, by the end of the 1980s most of these compounds for blanket treatments had been replaced by organophosphate and carbamate pesticides, and to a lesser extent by pyrethroids. A new family of Benzoylureas, such as diflubenzuron and triflumuron, replaced dieldrin for barrier treatments. In the 1990s a phenylpyrazole, fipronil, initially introduced by industry as the silver bullet in locust control, quickly fell out of favour for blanket treatments after it was discovered that it had long lasting and severe effects on social insects such as ants and termites (Danfa *et al.* 2002, Mullié *et al.* 2003) and honey bees *Apis mellifera* (Pisa *et al.* 2015) and on vertebrates (Gibbons *et al.* 2015). Fipronil also caused severe food chain perturbations by depriving specialized vertebrate predators of their prey (Peveling *et al.* 2003).

In comparison to organochlorines, the newer organophosphates and carbamates, both cholinesterase inhibitors, were non-persistent and relatively short-lived. Their use was intended for blanket treatments over vast areas and FAO had not yet set in place a system to compare efficacy and toxicity to nontargets. Many products were widely used and highly toxic to vertebrates including man and birds, such as diazinon, dichlorvos and cyanophos (Bennett and Symons 1972, McCuaig 1979). Other, extremely toxic, com-

pounds were used occasionally such as carbofuran, monocrotophos, dicrotophos and methyl-parathion. There is much reason to assume that chemical control has caused -at least locally- (mass) mortality of birds throughout the Desert Locust distribution area. This is based on the experiences in Canada (Mineau 1993), the USA (McEwen 1981) and South America, where mass mortality of 20 000 Swainson's Hawks *Buteo swainsoni* following control of acridids with monocrotophos was observed (Goldstein *et al.* 1999). African field studies were notably absent, but Keith and Bruggers (1998) suggest that raptor mortality due to locust control in Africa has been minimal.

It had also become increasingly clear that human health had been compromised in many ways related to chemical locust control activities. These include stockpiles of abandoned organochlorines, the widespread re-use of empty containers for domestic purposes, the inappropriate storage of pesticides, the lack of adequate personal protective equipment, and inefficient or defective and non-calibrated spraying equipment. It would take until the 2003–05 Desert Locust upsurge before these issues were adequately addressed and training was provided to the so-called Quality and Environment Survey of Treatments (QUEST) teams (FAO 2005, 2006; Mullié 2006).

Whereas the protection of birds as acridid predators in the first half of the 20th century was actively promoted to complement the locust mortality induced by arsenicals (Lounsbury 1909), eighty years later birds were often the victims of the indiscriminate use of insufficiently regulated locust control insecticides with high avian toxicity. By 1989 this had culminated into three complementary initiatives:

(1) A multi-compartment pilot study was to be executed in Senegal to quantitatively assess environmental side-effects of two commonly used insecticides frequently used in locust control: fenitrothion and chlorpyrifos. A promising insect growth regulator (IGR), diflubenzuron, for use against hopper bands as a replacement for dieldrin was equally studied (Everts 1990). The pilot study initiated the Locustox Project (Everts *et al.* 1997–2002) which would feed new field-derived and laboratory-verified ecotoxicological information into the Pesticide Referee Group, see hereafter, assessments of locust insecticides (PRG 1991–2014).

(2) The establishment of an independent group of scientists, the Pesticide Referee Group (PRG), whose role was to advise FAO on efficacy trials and the choice of a set of insecticides having the least environmental and human health impact (PRG 1989).

(3) A consortium, called LUBILOSA, was formed by the International Institute for Tropical Agriculture (IITA), CABI and Département de Formation en Protection des Végétaux (DFPV, Niamey) to develop a mycoinsecticide which was selective for acridids (Prior *et al.* 1992). This culminated in the development for ulterior commercialisation and registration of the mycopesticide Green Muscle® (GM) which contained viable conidia of the fungus *Metarhizium acridum* (Lomer *et al.* 2001). The Sahelian Pesticide Registration Committee (CSP) eventually registered GM in 2001 for use in nine Sahelian countries.

These three initiatives would fill major gaps in our knowledge and abilities to deal with acridid outbreaks efficiently and with less impact on human health and environmental integrity. The role of birds as acridid predators which was no longer considered as important needed reassessment. Bird mortality and morbidity due to the indiscriminate use of organophosphorus and carbamate insecticides had further reduced their impact as predators which called for a mechanism to screen and recommend chemical insecticides based on their efficacy and lower human health and environmental side-effects. Perhaps most importantly, more biological alternatives for chemical control were badly needed and had to be developed.

This thesis aims to assess to what extent application of conidia of the entomopathogenic fungus *Metarhizium acridum* can be used as a sustainable alternative for chemical control of adults and nymphal stages of locusts and grasshoppers with a special emphasis on the role of birds as locust and grasshopper predators.

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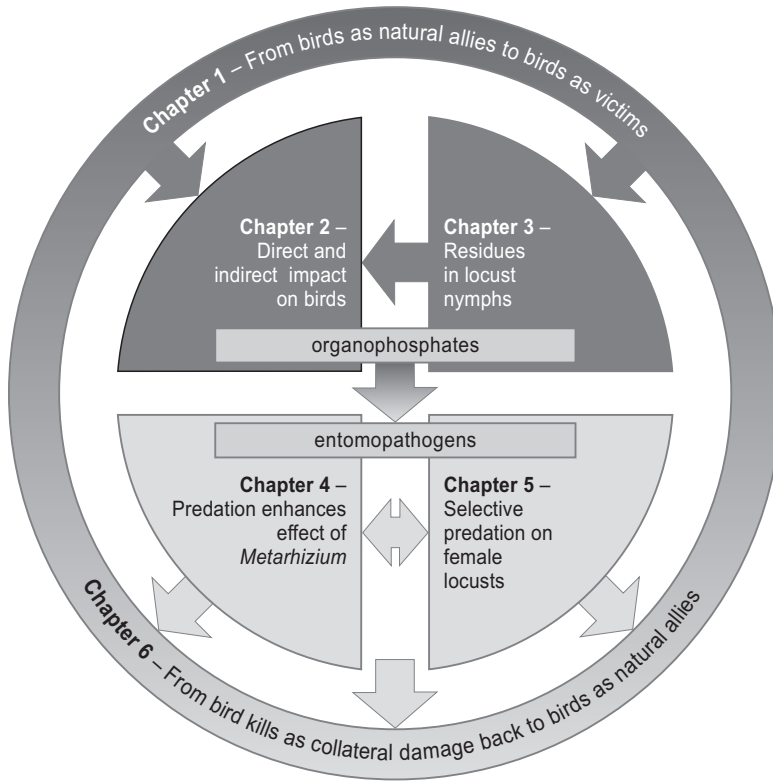
## Thesis outline

This thesis first assesses the impact of aerial fenitrothion and chlorpyrifos applications for locust and grasshopper control on birds (mortality, sub-lethal effects, indirect effects, residues in birds' food to be used in avian risk-assessment, in particular for gorge-feeding birds). Next, the impact of Green Muscle® on grasshopper and locust populations will be studied, as well as on the survival of avian predators and on the interaction of the entomopathogenic fungus effect and bird predation. Thus from birds as victims back to birds as allies, as is visualized in Fig. 1.1.

The field studies were performed in Africa (Senegal and Niger) and Australia (New South Wales and Victoria) and treatments targeted Desert Locusts, Australian Plague Locusts *Chortoicetes terminifera* and Senegalese Grasshoppers.

Four research questions will be addressed in this thesis:

1. What are the consequences of different application rates of the organophosphorous insecticides fenitrothion and chlorpyrifos for locust and grasshopper control on birds in the short and medium term.
2. What is the potential exposure of avian predators via consumption of locust nymphs sprayed with organophosphates, and how can the toxicological risk of this indirect exposure during gorge feeding be addressed in regulatory avian risk-assessments.
3. What are the consequences of application of the entomopathogen *Metarhizium acridum* for locust and grasshopper control on avian predators in the short and medium term.
4. How do chemical- and bio-pesticide applications compare with respect to effects on avian predators and what does this mean for locust and grasshopper control in the shorter and longer term?



**Figure 1.1.** The direct and indirect negative impact on birds of acridid control with organophosphorous insecticides (Chapters 2 and 3) will be compared to enhanced and selective predation by birds on acridids controlled by entomopathogenic fungi (Chapters 4 and 5). This is visualized as the transition from "*birds as natural allies to birds as victims*" (Chapter 1) to "*bird kills as collateral damage back to birds as natural allies*" (Chapter 6). This thesis therefore intends to provide some of the answers on how natural avian predation of locusts and grasshoppers can fit in an environmentally sound approach of acridid control and management.

**Chapter 1** introduces the background about insecticides used for locust and grasshopper control and its environmental side-effects including the impact on birds over the past century. It describes the successive steps that eventually lead to a strong civil society driven demand for (1) field research to better understand the impact of chemical locust and grasshopper control in semi-arid environments, (2) to make a scientifically sound choice of insecticides recommended for use based on efficacy field trials and a risk assessment of nontarget impact and (3) the development of efficient but more environmentally friendly methods of control. This chapter further defines the aim and research questions and presents the outline of the thesis.

**Chapter 2** describes the study of the impact on birds of aerial treatments with fenitrothion and chlorpyrifos under recommended and double dosages for locust control. This was part of a large pilot to study the environmental effects of chemical locust and grasshopper control. The objective was to determine the range of effects on birds that will most likely result from large scale chemical pesticide treatments.

In **Chapter 3** the results are presented of the chemical residues found in the different physiological states of locust nymphs following aerial exposure as operational treatment with fenitrothion of Australian Plague Locusts. Particular attention was paid to the risks for gorge-feeding birds, which currently is not yet taken into account in regulatory frameworks.

**Chapter 4** describes the result of biopesticide *Metarhizium acridum* application in two realistic dose rates compared to a control. The study was performed in a formerly deforested sylvo-pastoral reserve for the efficacy of the biopesticide and the impact on birds from September till June the following year. Grasshopper densities were determined from transect and quadrat counts on the plots and bird numbers were counted on the same transects by the Distance Sampling technique. The relation between the long term effects on grasshoppers and locusts and predation by birds are discussed.

**Chapter 5.** Avian predation on a population of Desert Locusts in northern Niger aerially sprayed operationally with *M. acridum* was studied. Populations of adult locusts and birds and vegetation greenness were assessed simultaneously along two transects. The food of Common Kestrels was studied through pellets and prey remains.

**Chapter 6** discusses in general the results, answers to the research questions posed and places the application of biopesticides with high specificity in a broader context of respecting and profiting of natural allies in reducing pressure of acridid pests. The inherent differences of chemical and biological treatments have been compared in relation to their impact on birds and bird predation and their ecological and economic consequences. Recommendations for habitat restoration and grazing regulation to reduce acridid breeding conditions and increase bird numbers are given.

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# The effects of aerially applied fenitrothion and chlorpyrifos on birds in the savannah of northern Senegal

Wim C. Mullié & James O. Keith

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## Abstract

1. Studies of the effects of applications of locust insecticides on birds were conducted in the savannah of northern Senegal in 1989. The insecticides studied were fenitrothion at 485 and 825 g ha<sup>-1</sup> and chlorpyrifos at 270 and 387 g ha<sup>-1</sup>.
2. Total bird numbers decreased on all treated plots. Decreases in three of the most abundant species were significant on the fenitrothion plots. Some of the decrease was due to bird mortality, but apparently most of it represented movements of birds in reaction to a reduction in their arthropod food.
3. The reduction in grasshoppers was four times greater on the fenitrothion plots than on the chlorpyrifos plots, and this difference was reflected by a decrease in the insect foods eaten by birds after the treatments.
4. Buffalo weavers and singing bush-lark reproduction was monitored on plots. Weavers deserted most colonies in all plots except the Control plot, while large numbers of debilitated fledgling larks were found during plot searches.
5. Effects on weaver desertion probably were indirect through food reduction, but debilitation in lark fledglings was due to direct poisoning. Fledglings showed severe reduction in brain cholinesterase levels; inhibition was comparable to that in adult birds found dead or debilitated immediately after treatments.
6. Brain cholinesterase levels in live birds collected 1 week post-treatment were slightly reduced, but levels were normal after 3 weeks.
7. Reduced reproductive success was the most serious impact identified, as it had the greatest potential for long-term effects on bird populations.

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## Introduction

An eruption of the desert locust (*Schistocerca gregaria* (Forskål)) occurred through the African Sahel between 1986 and 1989. Affected African countries responded by initiating surveillance and control programmes with the assistance of international donors. Between June 1988 and June 1989 alone, 12 million ha were treated with insecticides (Everts 1990). In addition, major outbreaks of the Senegalese grasshopper (*Oedaleus senegalensis* (Krauss)) occurred in 1985, 1986 and 1989. This species is at present considered to be the most destructive grasshopper pest in the Sahelian zone of West Africa (Cheke 1990). During 1986, insecticides were sprayed from aircraft and by ground applications on 3.4 million ha of grasshopper-infested zones between Senegal and Chad (FAO 1987), and in 1987 on 1.3 million ha in the same general region (US Congress 1990). A variety of insecticides was used in these programmes, few of which had been evaluated for possible effects on Sahelian environments.

When applied to wetland habitats, chlorpyrifos has consistently caused mortality and other effects in aquatic invertebrates and fishes. In contrast, when used for insect control in terrestrial habitats, chlorpyrifos has not had severe effects on resident animals (Odenkirchen & Eisler 1988).

Fenitrothion applications to forests in Canada at 300 g ha<sup>-1</sup> and higher have usually had acute effects on passerine birds (Busby *et al.* 1983), while at lower rates sublethal effects are observed (Millikin & Smith 1990). Reductions in bird abundance were found at application rates of 140–280 g ha<sup>-1</sup>, especially in canopy feeders (Pearce & Peakall 1977). In addition, almost all breeding attempts (84%) at 13 nests of white-throated sparrows (*Zonotrichia albicollis* Gmelin) were disrupted by a 420 g ha<sup>-1</sup> spray and reproductive success in the sprayed area was only one-third of that in the control area (Busby, White & Pearce 1990). However, at an application rate of 300 g ha<sup>-1</sup> in Scotland, Spray, Crick & Hart (1987) did not find effects on the size of the breeding bird populations, on bird counts immediately before and after spraying, or on reproduction in coal tits (*Parus ater* L) although this may have been caused by a difference in exposure or by out-of-area feeding by the birds due to the relatively small plot size in the latter study. Of greater pertinence to locust control programmes are the findings of mortality and decreases in bird abundance following applications of 210–420 g ha<sup>-1</sup> of fenitrothion to rangelands in the western United States for grasshopper control (McEwen 1982). Evidence was provided by Mineau & Peakall (1987) and by D.B. Peakall (*personal communication*) that heavily exposed birds seek sheltered locations and are less likely to be found. Therefore, they suggested that data collected thus far showed a strong collection bias, and that in fact they were only the 'tip of the iceberg'.

The information on birds we present was part of a larger FAO study to evaluate the effects of experimental applications of fenitrothion and chlorpyrifos on aquatic and terrestrial habitats in northern Senegal (Everts 1990). An experimental approach was chosen because it was impossible to predict when and where the insecticides would be applied for operational control of locusts. Thus, treatments were not made to areas containing locust

swarms or bands; however, relatively high populations of grasshoppers (predominantly *Oedaleus senegalensis*) were present on studied areas.

This pilot study was conducted primarily to observe the kinds of gross effects that took place and differences in the intensity of effects associated with the different chemicals and application rates. Fenitrothion and chlorpyrifos are both widely used in locust control and the amounts applied may exceed recommended rates. Occasionally mass mortality among birds has been reported (e.g. Mullié *et al.* 1991). Treatments were not replicated. For this pilot study, it was decided to use the research capabilities to differentiate among the variables of chemicals and rates. In the future, long-term work will be needed to examine experimentally the more important effects identified in this pilot study.

## Study area

### Plots and treatments

The study was conducted in a 400 km<sup>2</sup> area 15 km south of the Senegal River near Richard Toll, Senegal, between June and October 1989 (Fig. 2.1). Five 2 × 3-km study plots, each separated by at least 2 km, were used to evaluate the effects of four individual insecticide treatments and one control. These consisted of fenitrothion in study plot 1F at 485 g ha<sup>-1</sup> (recommended rate for desert locust control is 500 g ha<sup>-1</sup>); fenitrothion in study plot 2F at 825 g ha<sup>-1</sup>, an untreated control (Control); chlorpyrifos in study plot 1C at 270 g ha<sup>-1</sup> (recommended rate for desert locust control is 240 g ha<sup>-1</sup>); and chlorpyrifos in study plot 2C at 387 g ha<sup>-1</sup>. Insecticides were applied aerially to plots in ULV formulations between 5 and 12 September using an Islander aircraft fitted with electric pumps, two AU4000 Micronairs and short twisted blades (Courshee 1990).

### Rainfall

Total rainfall for the rainy season (June–October) ranged from 183.5 to 313.9 mm (average 235.5 mm,  $n = 16$ ) in the sugar cane plantation of the Compagnie Sucrière Sénégalaise, immediately north of our study area.

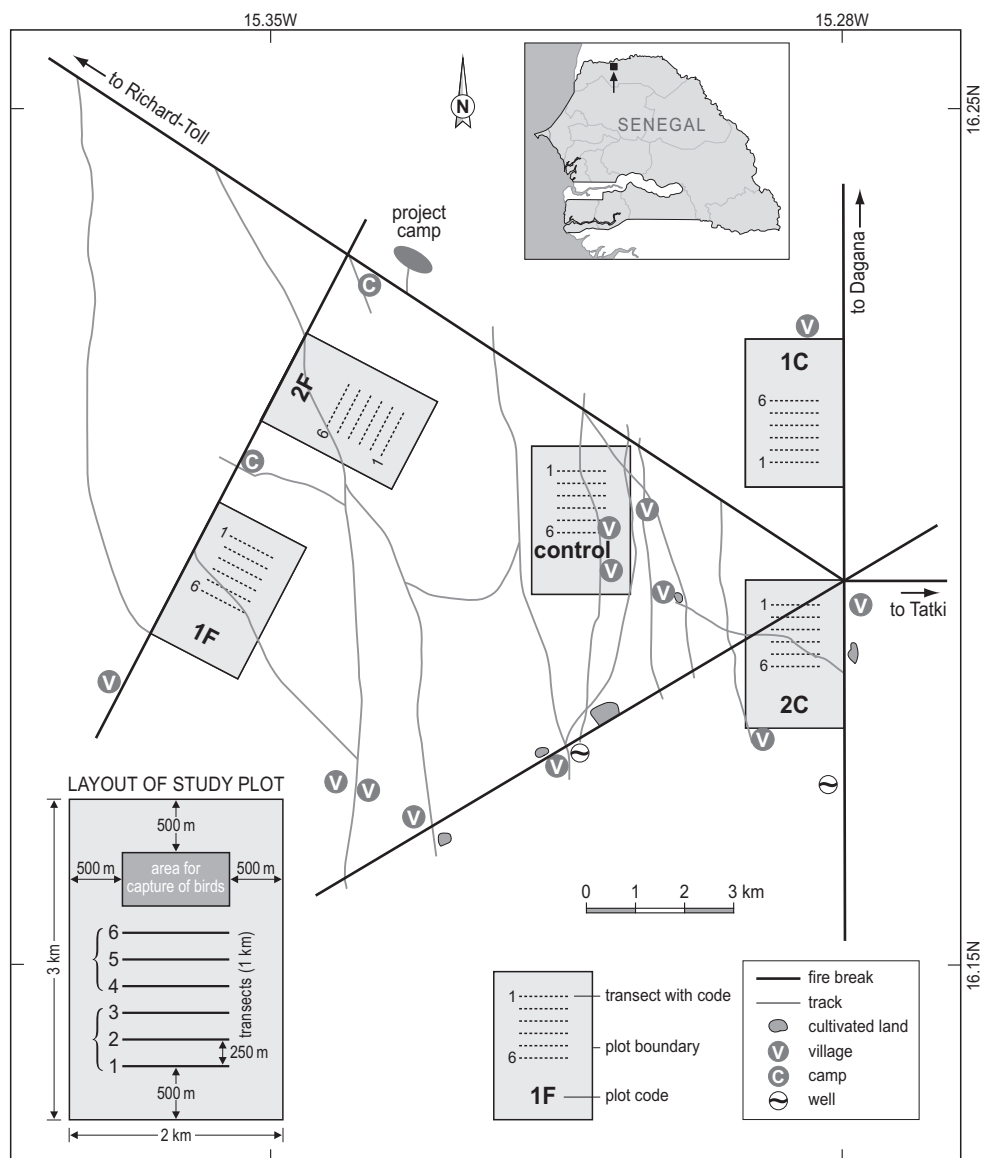
Long-term rainfall in the region has averaged  $319 \pm 39$  mm (Morel & Morel 1980). Thus, 1989 had less than average precipitation.

### Vegetation

The semi-arid thornbush savannah of the northern Sahel, which has also been termed Mimosaceae thorn scrub (Le Houérou 1989), is characterized by short trees, bushes and annual grasses, such as *Aristida* spp., *Cenchrus biflorus* Roxb., and *Schoenefeldia gracilis* Kunth. and common dominant herbs such as *Zornia glochidiata* Reichb. and *Cassia* spp..

Counts on 4–10 ha along a representative transect (see Methods) within each of our five study plots indicated *Boscia senegalensis* (Pers.) Lam. ex Poir., *Balanites aegyptiaca* (L.) Del., and *Acacia* spp. (e.g. *A. senegal* (L.) Wild., *A. tortilis* Hayne, and *A. seyal* Del.) made up 97% of the trees present. Total tree density ranged from 51 to 84 trees per ha; tree





**Figure 2.1.** Study area in northern Senegal and layout of experimental plots.

density and species richness was highest in plot 1F. Of trees counted, 57% were shorter than 2.5 m in height. Baobabs (*Adansonia digitata* L.) were widely, but thinly, distributed and occurred on all study plots (estimated density, 5–10 trees per 1000 ha). Large herds of cattle, goats, and sheep grazed the study area.

There were numerous shallow depressions scattered throughout the study area, ranging from 0.25 to 1 ha or more in size. Water and sediments tended to drain into



depressions after rain storms. The higher soil moisture in depressions supported a greater diversity and biomass of vegetation and usually twice the number of trees as in the surrounding savannah (Bille & Poupon 1972). After rains began, annual and perennial herbs in depressions responded immediately and grew tall and dense, in striking contrast to the shorter grasses in the savannah.

## Methods

### Bird counts in transects

To help assess treatment effects on bird numbers, six 1-km long transects, 250 m apart, were established in each of the five study plots (Fig. 2.1). Along each transect a white marker was placed every 100m. Bird counts were generally made weekly and in the same sequence between 24 July and 7 October (Table 2.1).

Transect counts on a single plot, each lasting 50 min, were conducted by two observers between 07.00 and 10.00 h. One observer visited transects 1–3, the other transects 4–6 (Fig. 2.1). Birds heard or seen within 50 m of the transects were tallied. A constant pace was maintained by covering each 100 m of the transect in 5 min. Our counts did not always measure actual bird densities but they provided a relative index of abundance. Secretive and nocturnal birds were seldom recorded, and counts did not evaluate treatment effects on these species.

There was some variation in the observers' knowledge of and ability to identify birds. However, analysis of counts of common birds that were ultimately used to evaluate treatment effects showed no difference ( $P > 0.20$ ) between observers.

### Bird counts on depressions

Three to five of the depressions in each plot were selected for monitoring bird abundance. One to four 15-min counts were obtained in each depression both before and after treatments. An observer sat or walked around and through depressions while noting the birds present. Such counts were taken between 10.00 and 13.00 h, after transect counts.

**Table 2.1.** Timetable of transect counts.

	Start of week on										
	July		August				September			October	
	23	30	6	13	20	27	3	10	17	24	1
<b>Count</b>	1	2	3	4	5	6 <sup>a</sup>	7 <sup>b</sup>	8 <sup>c</sup>	9	10	11
Treatments											

<sup>a</sup> Three transects per plot.

<sup>b</sup> Only three transects on control plot, none on other plots.

<sup>c</sup> Three transects on Plot 1C, six on other plots.

### Evaluation of breeding performance

Colonies of synchronously breeding buffalo weavers (*Bubalornis albirostris* (Vieillot)) were present throughout the study area, primarily in large baobab and acacia trees. The breeding stage of colonies was noted during bird counts before and after insecticide treatments.

Before treatments, the breeding biology of the singing bush-lark (*Mirafrja javanica* (Horsfield)), was studied. Details are presented elsewhere (Mulli  & Keith 1991).

### Searches for dead and debilitated birds

#### SEARCHES

To measure any direct mortality from treatments, special carcass searches were organized. Twelve young men were recruited from nearby camps and villages. Two searches were conducted on each of the four treated plots: one at 24 h and another at 48 h after treatments. Two searches were made on the control plot during the same period on 2 consecutive days. In each search, the 12 men spread out 20 m apart over a distance of 250 m and walked abreast for 2.0 km for a period of 2–3 h, depending on the density of vegetation. Searchers covered about 8.3% of the area within each plot and searched 1.3–1.9 ha h<sup>-1</sup> person<sup>-1</sup>. In addition to data on dead birds found, a value for search efficiency and for carcass disappearance rate was needed to calculate the proportion of the population killed (Fite *et al.* 1988).

#### SEARCH EFFICIENCY

On two occasions 36 and 61 dead birds were placed respectively in an area to be searched in order to establish search efficiency. The search team was not informed of this prior to counts. Each labeled specimen was placed haphazardly in a habitat where it probably could have died. After the searches, remaining marked carcasses were not removed but were left to help evaluate subsequent searches. Separate efficiency coefficients were calculated for small birds (weight <30 g) and larger birds.

#### CARCASS DISAPPEARANCE RATE

Dead birds were also used to determine the disappearance rate of carcasses due to scavenger activity. In plot 1C 14 birds were placed at five locations and in plot 2F 33 birds were placed at seven locations along a transect. They were checked after 24 and 48 h. The disappearance rate was used to calculate the proportion of carcasses remaining ( $R = 1 - \text{disappearance rate}$ ).

### Bird collections for food habits and cholinesterase analyses

#### COLLECTIONS

Singing bush-larks, buffalo weavers, and golden sparrows (*Passer luteus* (Lichtenstein)) were initially chosen for monitoring changes in ChE levels and food habits following treatments. These species were abundant, widely distributed, and ranged in food habits from insectivores to granivores. Golden sparrows decreased rapidly in numbers after rains started and they were deleted from collections. Abyssinian rollers (*Coracias abyssinica*

(Hermann)), hoopoes (*Upupa epops* L.) and woodchat shrikes (*Lanius senator* L.) proved susceptible to treatments: they were also collected. Birds were taken with mist nets, a 4.5-mm air rifle, and a 16-gauge shotgun. For each species an attempt was made to collect 10 individuals in each plot in the first and again in the third week after treatments. Birds from untreated areas were taken for controls. These untreated areas were at least 2 km from the edge of any treated plot. Birds found dead or debilitated during searches were also saved for analyses.

#### SPECIMEN HANDLING

Usually fresh specimens were dissected within 15 min of death. Weights were taken using spring scales with a precision of 0.3%. During dissection, birds were sexed and aged and relevant information was noted. Developmental stage ('age') of fledgling larks was assessed by taking wing lengths and tail lengths and adding these measurements. The gizzard and crop, if present were removed, and the contents or the complete gizzard was stored in ethanol (96%). Empty gizzards were discarded. Brains were removed and placed in 15-ml cryogenic vials, and subsequently stored in liquid nitrogen until they could be processed in the laboratory.

#### FOOD HABITS ANALYSIS

Gizzard contents were identified to order using a 7 × 40 × binocular microscope. Remains of some taxa, such as those of *Oedaleus senegalensis*, could be identified to the species level. The presence of grit was also noted. Bird species to be sampled could not always be obtained on all plots. Using numbers of food items as a basis for determining food habits tends to bias results in favour of the small, numerous items.

#### CHOLINESTERASE ANALYSIS

Brains were transferred to storage in a laboratory freezer after fieldwork was completed. They were held in the freezer for a maximum of 14 weeks. ChE activity was determined by the colorimetric method of Ellman *et al.* (1961) as modified by Hill & Fleming (1982). Analyses were conducted at the University Cheikh Anta Diop, Dakar, Senegal (Ciss & Niane 1990). Results are expressed as  $\mu$ moles of acetylthiocholine iodide hydrolysed per minute per gram (wet weight) of brain tissue ( $\mu$ moles min<sup>-1</sup> g<sup>-1</sup>).

#### Statistical analyses

The experimental design of this study did not include replications of treatments and, therefore, it did not fulfill theoretical requirements to permit general inferences from the results. However, statistical analyses were conducted with the understanding that any differences detected could be due either to the effects of the chemicals, to inherent differences among plots, or to both factors.

Analyses of bird count data were made using a two-factor, repeated measures ANOVA with unbalanced data. Analyses were conducted for birds grouped by systematic relationships and life-history traits using a three-factor, repeated measures ANOVA with unbalanced

data. Data were unbalanced because several counts on some plots were conducted by only one of the two observers.

Differences among-plots and within-plots among weeks, in the proportion of grasshopper remains in gizzards of selected species, were tested separately in single classification ANOVAS. The same analysis was conducted to test differences among and within plots in weights of captured specimens and in development of captured fledgling larks. Means were separated with Duncan's Multiple Range test.

## Results

### Bird counts on transects

131 species of birds were documented on the study plots between June and October (Keith & Mullié 1990). Afrotropical species (both residents and intra-African migrants) dominated the avifauna in June and July, but Palaearctic migrants increased in August, September and October. Some species of local breeding birds were presumably augmented by migrants. It was sometimes unclear whether increases observed over time were the result of local movements or of an influx of Palaearctic migrants. Observations on the control plot and other untreated areas showed that normal increases and decreases in certain species occurred unrelated to insecticide treatments.

Counts on study plots were conducted during (July–September) and after the rainy season (October). Some birds had reproduced during the dry season (Abyssinian rollers), while others initiated breeding with the beginning of the rains (singing bush-larks, buffalo weavers, blue-eared glossy starlings (*Lamprocolius chalybaeus* (Hempr. & Ehrenb.)), cricket warblers (*Prinia clamans* (Temm.)), and fantailed warblers (*Cisticola juncidis* (Rafin.)).

Many of the species seen on study plots were not used in the assessment of treatment effects. Some species were wide-ranging and not known to be exposed to insecticides. All rare and incidental birds and all palaearctic migrants, most of which arrived after treatments, were not considered in evaluating treatment effects. Golden sparrows were so

**Table 2.2.** Percentage change in bird numbers between periods on study plots.

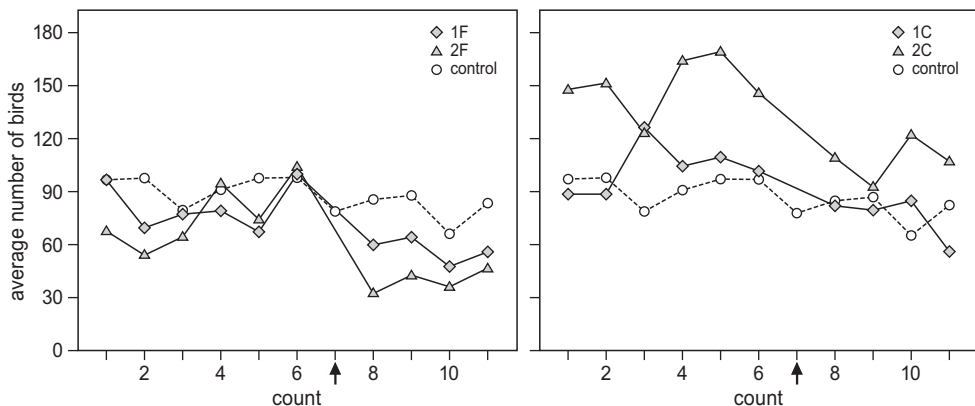
Data and means compared	Plot				
	1C	2C	1F	2F	Control
<b>Sum of 71 species</b>					
Pre-treatment vs. post-treatment	-26	-28	-30	-46	-8
Count 6 vs. post-treatment	-26	-26	-42	-61	-14
<b>Sum of 21 species</b>					
Pre-treatment vs. post-treatment	-26	-28	-32	-51	-13
Count 6 vs. post-treatment	-24	-26	-46	-63	-16

numerous that changes in their abundance were capable of masking effects on the less abundant species and data for this species were considered separately.

The removal of the above birds left 71 species, 21 of which were common, for consideration in assessing insecticide applications. Means and standard errors for transect counts of these 21 species and of golden sparrows on each plot are given in Keith & Mullié (1990) and are available upon request.

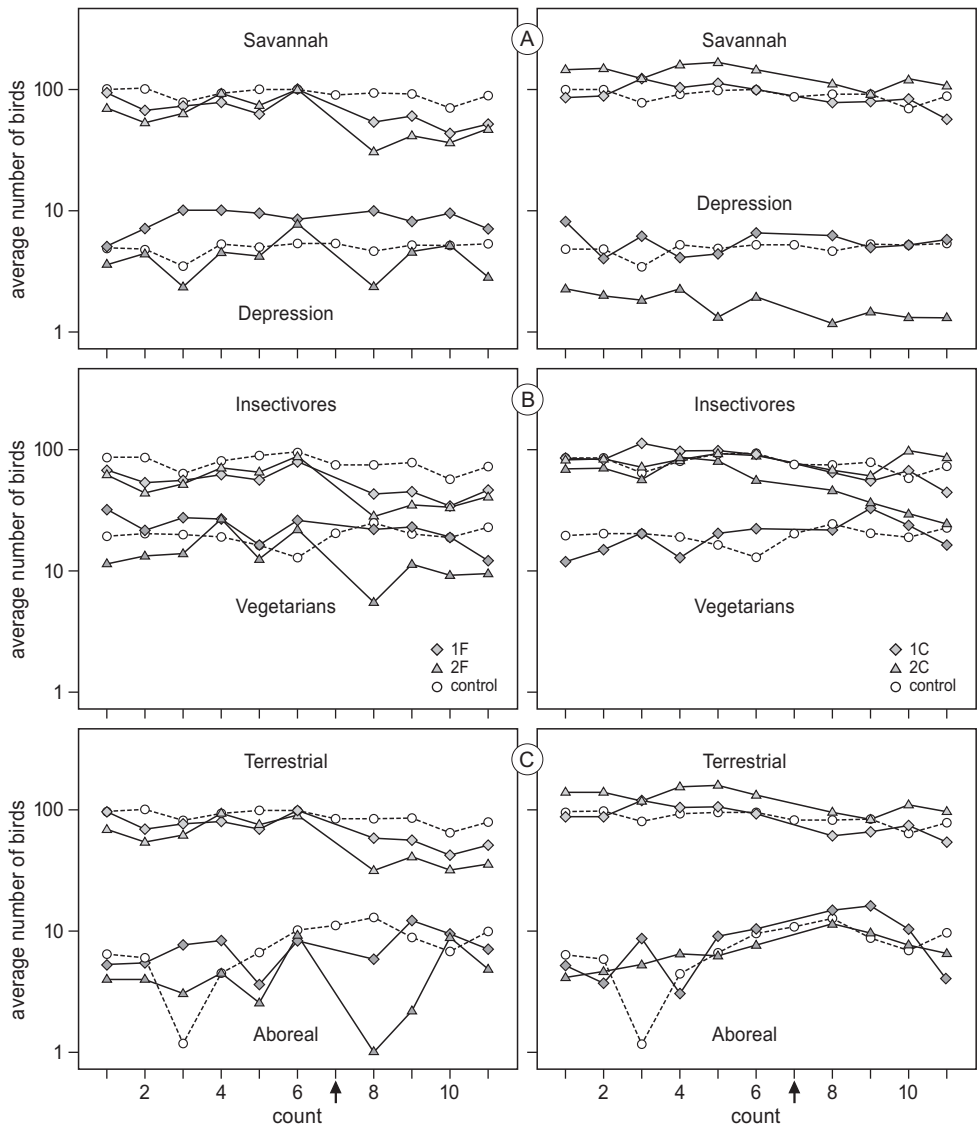
To determine whether life-history characteristics of the 71 species made them more or less sensitive to treatments, analyses were conducted based on systematic relationships (passerine, non-passerine), macrohabitat preferences (depression, savannah proper), feeding strategies (terrestrial, arboreal), and diet (insectivore, omnivore, vegetarian) (Keith & Mullié 1990).

The total number of birds (sum of 71 species) and the total of the most common species (sum of 21 species) decreased after treatments ( $P < 0.01$ ). The percentage decrease in bird numbers was greatest on plot 2F and was greater on all treated plots than on the control plot (Table 2.2). In general, a greater decrease in bird numbers on plot 2F was indicated by all assessments ranging from the sum of 71 species, the sum of 21 species (Fig. 2.2), and the life-history traits of birds (Fig. 2.3A,B,C) to many of the individual species, such as the singing bush-lark.



**Figure 2.2.** Average number per transect of 21 species seen during each count on experimental plots. An arrow indicates the moment of treatment.

Abyssinian rollers increased during the study, but by the third week after treatment (week 10), roller numbers on all treated plots were 36-40% lower than on the control plot ( $P = 0.01$ ). Blue-naped mousebirds (*Urocolius macrourus* (L.)) also tended to increase during the first 8 weeks of the study, especially on plots 1C and 2C, which by the eighth week had more than twice the numbers as the control plot ( $P = 0.01$ ). In contrast, on plots 1F and 2F, mousebirds numbers had dropped to zero 1 week after treatments. Singing bush-larks were the second most abundant birds on plots (after golden sparrows). They were likely to be a good indicator species for treatment effects in the savannah because



**Figure 2.3.** Average number per transect of (A) savannah and depression birds, (B) insectivores and vegetarians, and (C) terrestrial and arboreal feeding birds seen during each count on experimental plots. An arrow indicates the moment of treatment.

they were abundant, widely distributed, sedentary, and nested in the grasslands. After treatments, lark numbers decreased on all plots as young fledged and birds presumably left the area. However, decreases were greatest and occurred more rapidly on plots 1F (3.2 transect<sup>-1</sup>), 2F (8.0 transect<sup>-1</sup>) and 2C (10.0 transect<sup>-1</sup>), all of which on the first or second week after treatment had significantly ( $P < 0.01$ ) fewer larks than the control plot (20.8 transect<sup>-1</sup>).

Buffalo weavers decreased on treated plots immediately after insecticide applications. Although these changes were not highly significant ( $P = 0.11$ ), they probably were real.

Golden sparrows were apparently not affected by treatments; however, their numbers decreased significantly ( $P = 0.01$ ) on all plots over time. They constituted 80% of total birds seen on the control plot during the first five counts and only about 30% during the last four counts. Their decrease was correlated with breeding elsewhere in the region. Sparrows were increasing during our last counts as grasses dried and a new crop of seeds ripened. At that time flocks consisted largely of recently fledged birds.

In contrast to golden sparrows, woodchat shrikes tended to increase on plots during the study period but, like sparrows, the shrikes were not apparently affected by treatments although two individuals were found debilitated. Cricket warblers increased ( $P < 0.01$ ) during the study and did not appear to be affected by insecticide treatments. Increases were due partly to the appearance of young with adults: the cricket warblers bred during the period of the study.

Other afrotropical species that were widely distributed and bred during the study period did not appear to be affected by insecticide treatments. Numbers of black bush-robins (*Cercotrichas podobe* (Müller)) ( $P = 0.03$ ), grey-backed camaropteras (*Camaroptera brachyura* (Vieillot)) ( $P < 0.01$ ), and fantail warblers ( $P = 0.07$ ) varied among plots and over time, but not in relation to treatments. These species were considered obligate depression species, and the lack of an effect on them suggests the depression habitat was not affected by spraying to the same degree as the savannah habitat (see also Fig. 2.3A).

Effects of fenitrothion were indicated, especially on plot 2F, for most groups of birds, whether separated on the basis of systematic relationships, diet or feeding strategy (Table 2.3). This suggests that these traits did not predispose birds to fenitrothion effects. In contrast to birds on the control plot, those on fenitrothion plots tended to decrease after treatments regardless of their systematic relationships or their life-history traits (except that depression species increased). This situation probably reflects the fact that birds are somewhat opportunistic in their choice of foods. The abundance of grasshoppers on plots (Balança & De Visscher 1990) may have supported high bird populations. Reduction of that food source, therefore, could have caused some birds to leave plots.

Normal variations were observed among plots in the abundance of certain species. These differences were not related to treatments, but undoubtedly reflected the preference of birds for habitat resources on specific plots. Such habitat preferences were not identified, but such variations in the abundance of a species among plots illustrate why replication of plots is necessary in experimentation. Resources affected by insecticide treatments were not uniform among plots, so the possibility of each treatment's effect on birds was not equal. Replication of plots increases the probability that variations in resources and thereby in the kinds and numbers of birds will be equally tested against each treatment.

### **Bird counts in depressions**

A total of 55 species of birds was seen during depression counts, including incidentals and palaearctic migrants. Of these, only 17 species were relatively abundant. There were large

variations in counts, both within individual depressions and among different depressions. Numbers of observations were too few to test for significant changes due to treatments. Still, it was of interest that the data, when compiled, suggested the same detrimental effects of fenitrothion as indicated by transect counts and other observations. Whereas depression counts in the control and chlorpyrifos plots indicated an increase in total birds present after treatments, numbers in fenitrothion plots apparently decreased. These findings do not conflict with the increase in 'obligate depression species' shown in Table 2.3. Counts in depressions reported here included all birds that were seen in depressions and were not limited to only the obligate depression species.

These results again suggested that fenitrothion treatments affected birds to a greater extent than chlorpyrifos treatments. In contrast to transect counts, depression counts on the chlorpyrifos plots and the control plot suggested bird numbers increased. Birds on those plots may have used the verdant depressions more frequently as the savannah habitat dried following the rainy season.

### Evaluation of breeding performance

#### BUFFALO WEAVERS

It was impossible through casual observation to monitor the establishment, progress, and success or desertion of all colonies on study plots. However, records were maintained for a small number of active colonies observed during the first five and last four weekly transect counts on each plot. These records documented the locations of colonies, their desertion,

**Table 2.3.** Percentage change in numbers of birds grouped by systematic relationships and life-history traits on control and fenitrothion plots\*.

Traits	Plot		
	1F	2F	Control
<b>Systematic relationship</b>			
Passerine	-39	-67	-12
Non-passerine	-40	-65	+91
<b>Habitat preference</b>			
Depression	+32	-67	+12
Savannah	-72	-81	+24
<b>Feeding strategy</b>			
Terrestrial	-42	-60	+10
Arboreal	-34	-78	+76
<b>Diet</b>			
Insectivores	-20	-54	+77
Omnivores	-54	-74	-20
Granivores/frugivores	-38	-66	+89

\* Percentage difference between birds seen during Count 6 (last pre-treatment count) and Count 8 (first post-treatment count).



and in many cases their re-establishment. The number of colonies active before and after treatment compared to the total initiated is shown in Table 2.4.

Eleven of 13 colonies on treated plots were deserted at about the time of spraying. None of three colonies on the control plot was deserted. Casual observations in plot 1C indicate that two weaver colonies were deserted the day following treatment. Observations were insufficient to determine if desertion was due to treatments, but results indicated insecticides may have caused changes that occurred. Some evidence suggested that, during applications, areas containing colonies that persisted on plot 2C were not sprayed (I.F. Grant, personal communication; W.C. Mullié, personal observation).

#### SINGING BUSH-LARKS

Breeding performance of bush-larks was studied only before treatment (Mullié & Keith 1991). Therefore, there was no direct evidence of treatment effects on bush-lark breeding performance. However, singing activity of male bush-larks in both plots sprayed with fenitrothion decreased significantly after treatment compared to singing activity in the control plot (Mullié & Keith 1991), while mortality of adults in breeding condition and debility or mortality of immatures strongly suggested that treatment might have affected breeding success.

**Table 2.4.** Occupancy rate of buffalo weaver nesting colonies before and after treatment of plots\*.

Period	Plot				
	1C	2C	1F	2F	Control
Pre-spray (end of August)	3/6	5/7	3/6	2/4	3/6
Post-spray (mid-September)	0/6	2/7	0/6	0/4	3/6
* Data represent number of active colonies/total number of colonies initiated on plot.					

#### Searches for dead and debilitated birds

##### SEARCHES

A few dead or debilitated birds were found in all treated plots (Table 2.5), while none was located in the control plot. The greatest number and variety of birds were found in plot 2F. Button quail (*Turnix sylvatica* (Desfont.)), Abyssinian rollers, hoopoes, and singing bush-larks were most frequently affected on plots. Searchers captured a number of fledglings from the ground, and these also were predominantly singing bush-larks. These were likely to be affected by treatments, based on the ChE analyses.

##### SEARCH EFFICIENCY

Of the 22 larger birds placed in the search area of plot 1C, nine (42%) were found, while only two (14%) of 14 smaller birds were located. These results, and the fact that vegetation was more dense on most other plots, prompted us to intensify the searches by

increasing search time from 1 h to 1.5 h km<sup>-1</sup>. In plot 2F, 33 (56%) of 59 larger birds were recovered. After 24 h, another search was made, and seven more birds were found, giving a total recovery of 68% for larger birds. No individuals of the two smaller birds were recovered. Calculated efficiencies are given in Table 2.6.

**Table 2.5.** Dead (D) and debilitated (d) birds found on plots after treatment\*.

Species	Plot			
	1F	2F	1C	2C
Button quail	–	2(d)	1(d)	–
White-throated bee-eater	–	–	–	1(d)
Abyssinian roller	1(d)	1(d)	–	3(D)
Hoopoe	1(D)	2(d)	–	–
Singing bush-lark	1(D)	1(D)	–	1(D)
Tree pipit	–	1(d)	–	–
Woodchat shrike	–	2(d)	–	–
Cricket warbler	–	1(d)	–	–

\* Fledglings of the long-tailed beautiful sunbird (2), buffalo weaver (1), singing bush-lark (28), pink-headed dove (1) and black-headed shrike (1) were picked up during searches. Results of ChE analysis suggest that at least a number of these birds were debilitated, rather than simply flightless (see text). Two fledgling larks and one fledgling pink-headed dove and no dead or debilitated birds were found on the control plot.

**Table 2.6.** Calculated percentage minimum mortality (p) of the bird populations occurring in savannah habitat due to treatment with insecticides.

Plot	Size of birds	Birds found (N) <sup>d</sup>	Density of birds (ha <sup>-1</sup> ) (D) <sup>a</sup>	Carcasses remaining (R) <sup>a</sup>	Search efficiency (E) <sup>a</sup>	Hectares searched (A) <sup>a</sup>	Population mortality (p(%)) <sup>a</sup>
1F	>30g	2	3.6	1.0	0.68	50	2
	<30g	1	2.7	1.0	0.12 <sup>b</sup>	50	7
2F	>30g	7	2.8	1.0	0.68	50	7
	<30g	3	8.6	1.0	0.12 <sup>c</sup>	50	6
2C	>30g	3	5.8	0.8	0.55 <sup>c</sup>	50	2
	<30g	2	12.6	0.8	0.14	50	3

<sup>a</sup> The proportion of the population that died has been calculated by using the formula  $p = n \text{ 100\%/DREA}$  (after Fite *et al.* 1988).

<sup>b</sup> Search efficiency coefficients used for birds <30 g in plots 1F and 2F are the combined figures for the experiments in plots 2F and 1C

<sup>c</sup> The search efficiency coefficient for birds >30 g in plot 2C has been calculated using the combined results from the only search in plot 1C and from the second search in plot 2F.

<sup>d</sup> The fledglings that were picked up in the searches (Table 2.5) are not included in the calculations. At least a number of these birds were not simply flightless, but also debilitated due to the effect of treatment (see text). Adult birds found debilitated are included in the calculation; based on brain ChE levels it is assumed that they would have died as well.

### CARCASS DISAPPEARANCE RATE

Of 14 birds put out on plot 1C, three (22%) disappeared within 24 h. After 48 h, most carcasses contained fly larvae. In plot 2F, none of 33 birds was missing after 24 and 48-h checks, and there was little evidence of sarcophagic fly and beetle activity. For calculations it was assumed that the proportion of carcasses remaining on plot 2C was the same as on 1C, and on plot 1F the same as on 2F. Therefore, the proportion of carcasses remaining was taken to be 1.0 on plots 1F and 2F, and 0.8 on plot 2C (Table 2.6).

### POPULATION MORTALITY

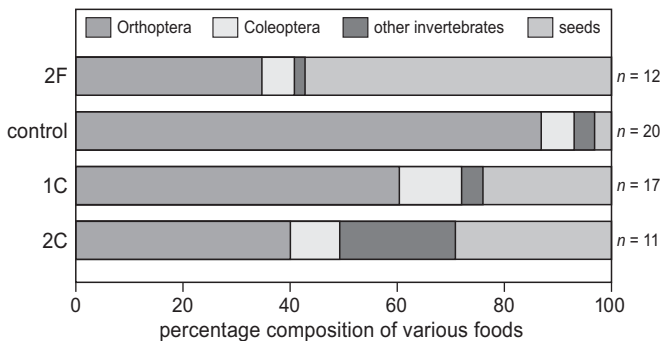
Values for calculation of mortality in and small species populations of savannah birds on plots 1F, 2F, and 2C are given in Table 2.6. In plot 1C, only one debilitated bird was found, and population mortality was not calculated. Calculated population mortality, corrected for search efficiency and carcass disappearance rate, was low on all plots, but perhaps somewhat greater on plot 2F. Mortality (2–3% in chlorpyrifos plots, 2–7% in fenitrothion plots: Table 2.6) was not sufficiently high to account for all decreases observed in bird numbers on transect counts, corrected for the natural decrease in numbers on the control plot (8–10% decrease on chlorpyrifos plots, 30–47% on fenitrothion plots; Table 2.2).

### Food habits analyses

#### SINGING BUSH-LARKS

The gizzard contents of singing bush-larks collected after treatments are shown in Fig. 2.4. In untreated areas, food items consisted predominantly of grasshopper instars, with seeds present in only two of the 17 gizzards containing food (11%). In the sprayed plots, however, 17 of 38 gizzards (45%) had seeds in them. The proportion of Orthoptera in the gizzard remains from the control areas was statistically greater ( $P < 0.05$ ) than in the chlorpyrifos and fenitrothion plots (plots 2C and 2F).

There were no apparent differences in prey selection between adults and fledglings. There was, however, a marked difference in the presence of grit in gizzards between adults



**Figure 2.4.** Percentage composition of various foods in gizzards of singing bush-larks on experimental plots after treatments (based on numbers of food items in gizzards).

and juveniles. In juvenile birds, 16 of 25 gizzards (64%) contained 1–25 small stones (maximum weight 0.25 g per gizzard), while in adult birds only four out of 33 birds (12%) contained grit in the gizzard.

Flightless fledgling singing bush-larks were significantly heavier ( $P < 0.05$ ) in the control (17.2 g,  $n = 3$ ) than in the treated plots (1C (13.4 g,  $n = 8$ ), 2C (12.3 g,  $n = 4$ ), 1F (12.3 g,  $n = 2$ ), 2F (13.4 g,  $n = 14$ )), while among treated plots there were no significant differences in weights. Weight differences could not be attributed to differences in 'age', as tail plus wing length did not differ significantly among plots ( $P > 0.10$ , NS): control (83.3 mm,  $n = 3$ ), 1F (73.0 mm,  $n = 2$ ), 2F (90.2 mm,  $n = 16$ ), 1C (78.7 mm,  $n = 9$ ), and 2C (66.0 mm,  $n = 4$ ). There were no significant differences in adult lark weights between control and treated plots.

#### ABYSSINIAN ROLLERS

Treatment effects were not obvious in Abyssinian roller gizzard contents. Grasshoppers made up 60–95% of all prey remains in birds from all areas. The main species was *Oedaleus senegalensis*, but *Acrida bicolor* (Thunberg) and *Cataloipus cymbiferus* (Krauss) were also identified in the prey remains. Based on the size of the jaws, predominantly adults or late instars were eaten. Four rollers that were found dead or debilitated 24 h after spraying (1 in plot 1F and 3 in plot 2C) had 35, 32, 29 and 51 grasshoppers, respectively, in their gizzards. In contrast, up to 14 grasshoppers were found in each of the nine rollers from untreated areas and up to 19 grasshoppers were found in each of the 39 gizzards collected in treated plots. An immediate shift to feeding on dying grasshoppers and rapid intoxication is likely, therefore, to have been responsible for the observed direct effects on Abyssinian rollers in the study plots. A comparison of the relative proportion of the different prey items in roller gizzards between week 1 and week 3 post-treatment shows a decreasing proportion of grasshoppers present in treated plots and a stable proportion in untreated areas. However, because of large individual variation, decreases were not statistically significant.

Abyssinian roller weights were not significantly different in control and treated plots. There were also no significant differences in weights of rollers between week 1 and week 3 post-treatment.

#### BUFFALO WEAVERS

Treatments had little effect on gizzard contents of buffalo weavers. Orthoptera were an important prey of buffalo weavers, making up 25–90% of the total remains present in individual gizzards and averaging 35–70%. Apparently, buffalo weavers were opportunistic feeders, adapting rapidly to the prey that was locally available. Birds from a colony at the project camp, used as controls, showed a dramatic shift in their diet within 1 week. Therefore, it was more difficult to determine if changes in diet were an effect of treatments. Like Abyssinian rollers, the buffalo weavers had a lower proportion of Orthoptera in their diet in week 3 than in week 1 post-treatment, but changes were significant ( $P < 0.05$ ) only in plot 2C.

Adult female buffalo weaver weights decreased significantly from week 1 to week 3 post-treatment in both plots 2C and 1F. Weavers captured while flying to or from their nests, when feeding young, showed a decrease in weight from 71.0 g ( $n = 10$ ) to 56.3 g ( $n = 10$ ) ( $P < 0.05$ ) in Plot 2C and from 67.8 g ( $n = 9$ ) to 53.0 g ( $n = 2$ ;  $P < 0.05$ ) in Plot 1F. No such changes were observed in untreated areas; insufficient data were available from other plots to examine possible effects.

### Cholinesterase analyses

Due to breakage of tubes in which frozen brains were stored, only 66 of the 216 brains collected were available for study. Sufficient samples of unexposed birds were available for two species: Abyssinian rollers and singing bush-larks. After treatments, singing bush-larks, Abyssinian rollers, and buffalo weavers were the principal species collected. Buffalo weavers decreased on all plots, except plot 2C, and were difficult to collect, especially on plot 2F. Likewise, singing bush-larks decreased on all plots, and adequate collections were not always obtained.

Dead and debilitated birds found on plots immediately after treatments (Table 2.5) had low ChE levels in their brains (Table 2.7). Compared with unexposed birds (Table 2.8),

**Table 2.7.** Brain cholinesterase levels ( $\mu\text{mol min}^{-1} \text{g}^{-1}$ ) in brains of individual dead (D) and debilitated (d) birds and in groups of fledglings found on plots after treatments\*.

Birds	Plot											
	1C			2C			1F			2F		
	(n)	mean	SE	(n)	mean	SE	(n)	mean	SE	(n)	mean	SE
<b>Adults</b>												
Button quail		–			–			–		(1)	5.2(d)	
								–		(1)	6.2(d)	
Abyssinian roller		–		(1)	20.5(D)		(1)	5.1(d)			–	
				(1)	16.5(D)							
Hoopoe		–			–			–		(1)	8.6(d)	
										(1)	7.9(d)	
Singing bush-lark		–		(1)	23.5(D)			–			–	
Woodchat shrike		–			–			–		(1)	11.2(d)	
										(1)	7.7(d)	
<b>Fledglings</b>												
Singing bush-lark												
24h	(4)	20.1	5.8	(2)	16.2	0.9		–		(4)	11.6	2.4
48h	(2)	15.9	5.9	(2)	20.5	0.4	(2)	16.8	0.5	(3)	12.6	3.2
72h		–			–			–		(3)	7.9	0.8
Buffalo weaver												
24h		–		(1)	15.5			–			–	
Pink-headed dove												
24h		–		(1)	18.5			–			–	

\* See Table 2.8 for ChE levels in control birds.

ChE levels were sufficiently inhibited (39–85%) to have caused death and debility of the birds. ChE levels also were low in fledglings found by searchers. In the young larks from plot 2F, ChE ranged from 6.32 to 16.27  $\mu\text{mol min}^{-1} \text{g}^{-1}$ ; lowest levels were in birds found 72h after treatment. ChE levels in brains of fledgling larks from all plots were well below those in adult larks (mean = 40.0  $\mu\text{mol min}^{-1} \text{g}^{-1}$ ) and in one fledgling (29.9  $\mu\text{mol min}^{-1} \text{g}^{-1}$ ) from untreated areas (Table 2.8). This suggested that lark fledglings were impaired by exposure to insecticides, but reliable means for their normal ChE levels were not determined.

ChE inhibition of 50% or more is accepted as severe and is considered diagnostic as the cause of death (Hill & Fleming 1982). Debilitated adult birds and fledgling larks in plots 1F and 2F showed an inhibition greater than 50% compared with controls. ChE inhibition was not as severe in dead birds found in plots 1C and 2C.

Live birds collected from the plots 1 week after treatments often had lower ChE levels than controls, but after 3 weeks ChE levels in birds from treated plots were about the same as those in controls (Table 2.8). ChE inhibition usually was not severe in live birds collected from plots after treatments, but singing bush-larks collected 1 week after treatment of plot 2F showed a 50% inhibition. After 1 week, Abyssinian rollers on plots 1F and 2F had ChE levels in the same range as those in rollers found dead on plot 2C. One red-

**Table 2.8.** Brain cholinesterase levels ( $\mu\text{mol min}^{-1} \text{g}^{-1}$ ) in live unexposed (Control) birds and in birds collected on study plots after treatments.

Species and period post-treatment	Plot											
	1C			2C			1F			2F		
	(n)	mean	SE	(n)	mean	SE	(n)	mean	SE	(n)	mean	SE
Abyssinian roller												
1 week	(1)	30.7		–			(4)	21.3	1.2	(5)	27.1	2.5
3 weeks		–		(1)	46.4		(6)	37.4	5.2	(10)	34.8	3.5
Singing bush-lark (adult)												
1 week	(7)	33.1	3.8	(2)	32.0	7.5	–			(4)	20.8	1.7
3 weeks		–		(4)	41.1	4.5	–			–		
Singing bush-lark (juv.)												
1 week	(2)	18.5	7.5	–			–			(1)	15.2	
Buffalo weaver												
1 week	(8)	30.6	2.7	(10)	32.4	2.1	(7)	22.0	2.2	(1)	14.4	
3 weeks		–		(9)	29.5	2.2	(1)	54.2		–		
Golden sparrow												
3 weeks		–		–			(1)	38.8		–		
Woodchat shrike												
3 weeks		–		(2)	28.6	2.3	–			–		
Red-beaked hornbill												
72 hours		–		–			–			(1)	15.2	
Hoopoe		–		–			–			–		
Pink-headed dove		–		–			–			–		

beaked hornbill (*Tockus erythrorhynchus* (Temm.)) shot 3 days post-treatment on plot 2F, had a low ChE activity ( $16.6 \mu\text{mol min}^{-1} \text{g}^{-1}$ ) compared with a control level of  $27.8 \mu\text{mol min}^{-1} \text{g}^{-1}$  found in this species in Kenya (Bruggers *et al.* 1989). According to Hill (1988), and based on generally low coefficients of variation, the use of such data is allowed as emergency substitute in diagnosis of lethal anticholinesterase poisoning when concurrent controls cannot be obtained.

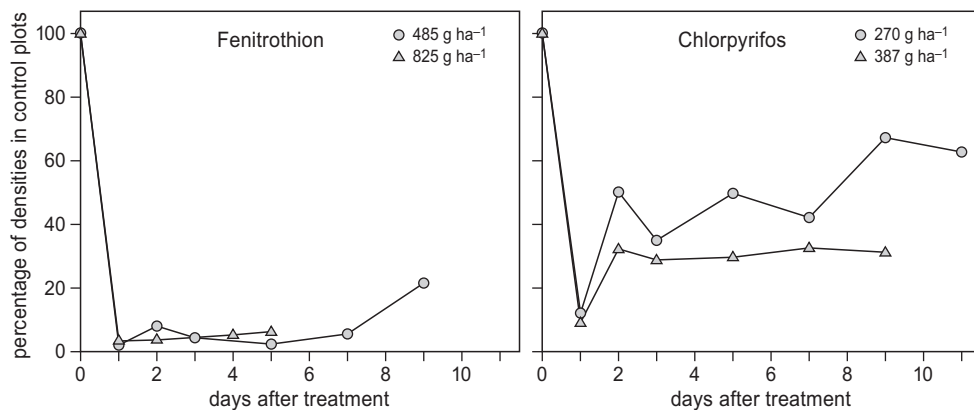
## Discussion

The varied and abundant avifauna on study plots provided an excellent situation for study of insecticide effects on the habits and population abundance of diverse species. Total bird numbers (sum of 71 species) decreased on all plots after treatments. Some of this decrease was due to bird mortality, but most apparently represented movement of birds from plots. Mortality, debility, and decreases in bird numbers were greatest on plots treated with fenitrothion. Decreases in Abyssinian rollers, blue-naped mousebirds, and singing bush-larks were statistically significant, but decreases were also indicated in numbers of hoopoes and buffalo weavers. Fenitrothion treatments caused decreases in these species and, in addition, reduced numbers of birds grouped on the basis of either their systematic relationships or life-history traits.

Insecticides kill insects and other arthropods, reducing the food supply of birds. Decreases in bird numbers observed on study plots probably were largely due to such decreases in food. Results suggested fenitrothion more seriously reduced food availability than chlorpyrifos. For instance, singing bush-larks ate few seeds on the control plot after treatments, and relied primarily on insects. On the chlorpyrifos plots, larks ate about 75% insects and 25% seeds, but on the high dose fenitrothion plot (2F), larks ate more seeds than insects. These findings suggest larks were forced to eat seeds as insect biomass decreased.

The two insecticides appeared to differ in their impact on birds. Fenitrothion applications resulted in greater decreases in bird numbers. If decreases were caused by reductions in food availability, it follows that fenitrothion must have reduced arthropod biomass to a greater extent than chlorpyrifos. This appears to have been the case. Singing bush-larks and Abyssinian rollers consumed primarily grasshoppers in the study area before treatments. After insecticide applications, four to five times as many adult grasshoppers remained on chlorpyrifos plots as on fenitrothion plots (Balança & De Visscher 1990; Fig. 2.5). Also, in contrast to the chlorpyrifos plots, grasshopper nymphs were absent on fenitrothion plots after treatment, and grasshopper recolonization began later and progressed at a slower rate. These findings support the idea that a greater decrease in food resources was responsible for a greater movement of birds from fenitrothion plots and thereby a greater decrease in their numbers.

Insect biomass should increase as insects invade or otherwise re-establish populations on plots. Bird abundance would respond to increased food resources and return to normal.



**Figure 2.5.** Reduction in numbers of adult grasshoppers after treatment. Redrawn with permission from data in Balança & De Visscher (1990).

Under such conditions, the effects of treatments should be temporary. Birds are opportunistic in their feeding habits and tend to respond negatively to food decreases and to congregate where food resources are the richest. However, food restrictions can have more serious and long-lasting effects if they occur during the reproductive period and adversely influence nesting success. Observations suggested nesting success of singing bush-larks and buffalo weavers were affected by fenitrothion treatments. Both species were reproducing during spraying, and their numbers decreased rapidly afterwards. This implied that the insecticide terminated the process of reproduction in some buffalo weavers and may have caused some singing bush-larks to move before young were fully fledged. Young larks usually leave the nest well before they can fly (Green 1985; Cramp 1988). However, fledgling larks analysed were debilitated by ChE inhibition, and many probably died on all treated plots. Stromborg *et al.* (1988) dosed nestling European starlings (*Sturnus vulgaris* L.), with dicotophos to examine its influence on post-fledging survival and development. They found effects were rapid (death and reduced ChE levels), but survivors recovered rapidly and adverse effects did not extend into the post-fledging period.

Birds are not equally exposed to insecticides applied to the environment. Their activities and habits at the time of treatments largely determine the intensity of their exposure. ChE measurements suggested that a few adults of species eating grasshoppers ingested sufficient insecticides to cause intoxication and death. Fledglings, and especially those of the singing bush-lark, received high exposure to the insecticides, which appeared to result in an even greater inhibition of ChE than in adults. However, normal ChE levels of young larks deserve further study. It has been demonstrated that nestling starling brain ChE activity was age-dependent and increased linearly over 17 days to 70% of adult levels (Grue, Powell & Gladson 1981). Serum ChE activity in tree sparrows (*Passer montanus* L.) was also age-dependent when measured during 2 weeks after hatching. Levels increased at a much higher rate after birds were c. 11 days old (Thompson 1988). As young larks were in grasslands, they were probably subjected to greater dermal contamination than



birds active in trees and depressions. Dermal absorption and preening were recently identified as major exposure routes leading to ChE inhibition in birds exposed to field application of organophosphates. Contrary to a widely held view, ingestion contributed only 10–20% to the overall anticholinesterase impact on bobwhite (*Colinus virginianus* (L.)) under simulated field conditions (Driver *et al.* 1991). Young birds being fed by adults probably were given contaminated insects, as insect protein is a prerequisite for growth in young of most bird species. ChE levels in fledglings decreased substantially during the first 3 days following insecticide treatments.

In passerines, reduced food intake was observed following sublethal exposure to organophosphorus compounds under laboratory conditions (Grue, Powell & McChesney 1982). The significantly lower weights of flightless fledgling singing bush-larks collected 24 and 48 h post-treatment compared with those collected in the control area may have been an effect of exposure to the insecticides. This is supported by the observation that among the five plots no significant differences in the 'age' of the fledgling larks were found. Delayed growth or loss of weight in nestling songbirds, in the range of 5–25%, in the first 24 h after experimental oral exposure to organophosphates has been reported in various studies (Grue & Shipley 1984; Stromborg *et al.* 1988). If the parent birds are also affected by exposure to organophosphates, an even stronger effect on the development of the nestlings may be expected. Female starlings given an oral dose of dicotophos made significantly fewer sorties to feed their young and they remained away from their nests longer than controls (Grue, Powell & McChesney 1982). ChE levels in some adult singing bush-larks in breeding condition indicated that they were probably affected.

Weights of female buffalo weavers (c. 70 g) tending nests with young decreased 21% in plot 2C between week 1 and week 3 post-treatment. It is unlikely that the observed loss of weight is entirely due to energy requirements for feeding young, and it may have partially resulted from exposure to the pesticide or from severe reduction in food supplies. In temperate regions, loss of weight in the starling -a species of comparable size and prey choice- due to energy requirements for feeding young, is much less than the 21% observed in this pilot study. Female starlings (c. 80 g) attending adjusted broods of 3, 5 and 7 young lost an average of 5.0, 5.5 and 7.1 %, respectively, of their initial weight between 1–5 and 16–20 days after hatching (Westerterp, Gortmaker & Wijngaarden 1982).

ChE measurements in mature birds after insecticide treatments did not indicate serious inhibition at 1 week, and ChE levels in general were near normal after 3 weeks. These findings are consistent with the observation of minimal mortality and debility in adult birds resulting from insecticide applications. Applications of fenitrothion at 300g ha<sup>-1</sup> in forests of northern Scotland resulted in ChE inhibition in four species of songbirds. Inhibition averaged 47% on the day after treatments in one species, and it was still 34% after 1 week and 13% after 3 weeks in another species (Hamilton, Hunter & Ruthven 1981).

Re-analysis of these data by Hart (1990) revealed evidence of significant differences in the effects of fenitrothion between species and between spraying operations under similar conditions and with the same nominal dosage. This could possibly have resulted from

differences in deposition. If birds cannot be recognized individually or if observers are insufficiently familiar with their behaviour, it is virtually impossible, 3 weeks post-treatment, to establish whether or not an individual was already present at the time of spraying. If many new individuals have entered the plot, measured ChE activities may severely underestimate a treatment effect. These effects are less likely to occur when surveyed plots are within a larger sprayed area.

Chlorpyrifos degrades rapidly in birds and residues largely disappear after about 9 h (Odenkirchen & Eisler 1988). In wheat fields treated with 560 and 1000 g ha<sup>-1</sup> of chlorpyrifos, horned larks (*Eremophila alpestris* (L.)) showed a 22% reduction in ChE after 3 days and only 8% after 16 days. No dead larks were found in treated fields (McEwen, DeWeese & Schladweiler 1986). Our results also indicated that ChE inhibition was brief, and mortality in adult birds was low in areas treated with chlorpyrifos.

Residues of 1.0 ppm and higher have been reported from grasshoppers following applications of organophosphate insecticides (Stromborg, McEwen & Lamont 1984). Dead and dying grasshoppers collected the day of spraying with azinphos-methyl at 280 g ha<sup>-1</sup> (ULV) even contained 14 ppm of the insecticide (McEwen, Knittle & Richmond 1972). In consuming their own weight of grasshoppers carrying 1.0 ppm of fenitrothion residues, birds would ingest 1.0 mg kg<sup>-1</sup> of fenitrothion. Zebra finches (*Poephila guttata* (Viellot)) dosed with about 1.0 mg kg<sup>-1</sup> fenitrothion showed 50% ChE inhibition (Holmes & Boag 1990). ChE inhibition increased at higher doses, and some mortality occurred. It follows that singing bush-larks that consumed their weight or more in contaminated grasshoppers could possibly suffer ChE inhibition of 50% or more and die. Fledgling larks collected on treated plots and showing ChE inhibition of 50% or more were apparently debilitated, rather than just flightless, and probably would have died if left in the field.

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## Conclusions

The objective of this study was to determine the kinds of effects on birds most likely to result from aerial applications of fenitrothion and chlorpyrifos for locust and grasshopper control. Chlorpyrifos and fenitrothion treatments resulted in temporary decreases in the abundance of birds, bird foods, and ChE levels in several bird species. Fenitrothion effects appeared somewhat greater than those of chlorpyrifos. It is possible that both insecticides decreased reproductive success on plots either by reducing numbers of birds fledged or by killing fledglings soon after they left the nest. Reproductive effects were apparent and could potentially cause the greatest long-term effects on bird populations. Further study of avian reproductive effects in plots treated with fenitrothion would be of high priority in future programmes of study in Senegal and throughout Africa where insecticides are applied to control locusts and grasshoppers.

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# Insecticide residues in Australian plague locusts (*Chortoicetes terminifera* Walker) after ultralow-volume aerial application of the organophosphorus insecticide fenitrothion

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## Abstract

The need for locust control throughout eastern Australia during spring 2010 provided an opportunity to quantify residues of the organophosphorus insecticide fenitrothion on nymphs of the Australian plague locust, *Chortoicetes terminifera* Walker. Residues were collected across the different physiological states – live, dead, and debilitated (characterized by ease of capture, erratic hopping, and the inability to remain upright) – of locust nymphs observed following exposure to fenitrothion. The time course of residue depletion for 72 h after spraying was quantified, and residue-per-unit dose values in the present study were compared with previous research. Fenitrothion residue-per-unit dose values ranged from 0.2  $\mu\text{g g}^{-1}$  to 31.2  $\mu\text{g g}^{-1}$  (mean  $\pm$  standard error [SE] =  $6.3 \pm 1.3 \mu\text{g g}^{-1}$ ) in live *C. terminifera* nymphs, from 0.5  $\mu\text{g g}^{-1}$  to 25.5  $\mu\text{g g}^{-1}$  ( $7.8 \pm 1.3 \mu\text{g g}^{-1}$ ) in debilitated nymphs, and from 2.3  $\mu\text{g g}^{-1}$  to 39.8  $\mu\text{g g}^{-1}$  ( $16.5 \pm 2.8 \mu\text{g g}^{-1}$ ) in dead nymphs. Residues of the oxidative derivative of fenitrothion, fenitrooxon, were generally below the limit of quantitation for the analysis (0.02  $\mu\text{g g}^{-1}$ ), with 2 exceptions – 1 live and 1 debilitated sample returned residues at the limit of quantitation. The results of the present study suggest that sampling of acridids for risk assessment should include mimicking predatory behavior and be over a longer time course (preferably 3–24 h postspray) than sampling of vegetation (typically 1–2 h postspray) and that current regulatory frameworks may underestimate the risk of pesticides applied for locust or grasshopper control.

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## Introduction

Broad-scale locust and grasshopper control programs worldwide rely primarily on the aerial application of chemical insecticides (Story *et al.* 2005). Many bird species are known to feed on Acrididae, the family of locusts and grasshoppers to which most economically damaging species belong. As such, locust outbreaks provide an important ephemeral food source for birds and are often accompanied by wide and diverse avian assemblages (Story *et al.* 2007, Szabo *et al.* 2003). Locusts provide a rich source of protein (62% dry mass) and lipid (17% dry mass) for predators (Bodenheimer 1951, Ekop *et al.* 2010) and, in regions of inland Australia characterized by low rainfall and variable resource availability, provide valuable nutrients for the many bird species that descend on areas experiencing locust population increases (Szabo *et al.* 2009). Several of these species specialize on locust outbreaks and have been observed feeding on this superabundant food source, often in large flocks (Piersma & Hassell 2010).

Environmental cues that trigger increases in locust populations are also responsible for the physiological mechanisms that stimulate breeding in birds, enabling avian species to manage the natural variation in energy availability and reproduce while conditions are favorable (Fildes *et al.* 2006). Consequently, locust outbreaks and avian reproductive events can co-occur (Szabo *et al.* 2009) and, combined with the ability of avian species to gorge feed, increase the risk to avian populations from pesticide exposure beyond the scope of the standard risk-assessment paradigm. Furthermore, granivorous birds often feed their young with insects, broadening the risk. The field record reflects this with mass intoxications of Swainson's hawks (*Buteo swainsoni* Bonaparte) witnessed a decade ago when landowners in Argentina began spraying grasshoppers with the organophosphorus insecticide monocrotophos (Goldstein *et al.* 1999). Cases of bird mortality have also been reported following desert locust (*Schistocerca gregaria* Forskål) control operations, and experimental research on aerially applied fenitrothion and chlorpyrifos under operational conditions has shown this to be lethal to 2% to 7% of individuals from the species assemblages exposed to pesticides in savannah habitat (Mullié & Keith 1993, Chapter 2 of this thesis).

There is a specific lack of data quantifying residues on locusts and grasshoppers for avian risk assessments. Current recommended default values for residues in insects in the European Union (EFSA 2009) are mainly based on pitfall-trapped insects in a variety of horticultural crops and therefore may not fulfill the requirements for avian risk assessments of locust and grasshopper control given the specific nature of these spray programs (e.g., the use of ultralow-volume formulations in arid and semiarid ecosystems with irregular vegetation structure and high pest densities). The assessment of risk resulting from pesticide exposure is further complicated by the fact that previous insect collections have not always been designed to mimic the feeding behavior of predatory birds and thus do not accurately reflect their potential dietary intake. Insects are commonly captured by pitfall traps, which may seriously underestimate residue levels by sampling active insects; by sweep netting, which may overestimate the residue level on locusts from contact with contaminated vegetation; or by collecting dead insects that desiccate rapidly, thereby



increasing detected residue levels (Stafford *et al.* 2003). During spring and summer 2010, population increases of *Chortoicetes terminifera* led landholders, state authorities, and the Australian Plague Locust Commission (APLC) to call for extensive control operations throughout the states of New South Wales, South Australia, and Victoria. As part of this program, aerial spraying undertaken by the APPLC using the organophosphorus compound fenitrothion in New South Wales and Victoria provided the opportunity to quantify residue levels in *C. terminifera* nymphs for this pesticide across different physiological states and to document declining residues over time.

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## Materials and methods

### Study plots

Study plots were chosen from targets identified by APPLC staff to be aurally treated. These were Oakdale (70 ha; 34°24.3'S, 142°55.6'E, approximately 75 km east-southeast of Mildura, Victoria) and Koolaman (200 ha; 34°20.1'S, 143°04.5'E, approximately 86 km east-southeast of Mildura, Victoria) in New South Wales, and Karawinna (150 ha; 34°21.2'S, 141°42.3'E, approximately 42 km west-southwest of Mildura, Victoria) and Ouyen (120 ha; 35°10.5'S, 142°16.7'E, approximately 110 km south of Mildura, Victoria) in Victoria. Oakdale and Karawinna were part of larger spray blocks, whereas the other study sites were stand-alone targets.

All sites were located within grazing paddocks on farming properties practicing a 3-yr rotation farming cycle (cereal– fallow–sheep). Vegetation within the study sites was made up of a combination of native and introduced grasses with patches of higher grasses (20–30 cm) surrounded by low, sparse vegetation (<5 cm) and bare ground. Also present within the short vegetation were several unidentified medic species (*Medicago* spp.), with the highest densities of locust nymphs being found within these patches. Koolaman and Karawinna had the lowest vegetation density and height and, in part, were almost bare due to previous treatment with herbicides in preparation for the upcoming cropping season.

### Sampling sites

In each spray plot, several locust bands (marching groups of second to fifth instar nymphs) were located and flagged before spray (sample sites). Our sampling design called for 3 hopper bands (sample sites) to be sampled per spray plot, well separated from each other and under different flight passes of the spray aircraft. Locust samples for residue analysis were taken at 1 h, 3 h, 6 h, and 24 h postspray on both Oakdale and Koolaman plots, and additional samples were taken at 46 h and 70 h postspray on the Oakdale plot. We found that hopper bands were rather small and sometimes even fractioned before spray and that movements were rather limited. Being aware of their movements away from the initial sampling site, the surrounding area was searched to localize the new front at each sample period. This was, in most cases, less than 10 m from the initial site. Not all samples proved possible to obtain. For example, there were no dead nymphs to sample before 6 h at

Koolaman and 24 h at Oakdale, and dead nymphs were no longer visible in sufficient numbers to be sampled at 46 h postspray at Oakdale. Ouyen and Karawinna were sampled only once, 3 h and 6 h postspray, respectively.

### Pesticide application

Plots were sprayed cross-wind with ultralow-volume fenitrothion (1.23 kg active ingredient [a.i.] L<sup>-1</sup>; Sumitomo Chemical) by fixed wing aircraft (Cessna 188 for Ouyen and Brave for Oakdale, Karawinna, and Koolaman) using a targeted flying height of 10 m and a track spacing of 100 m. Spray aircraft were equipped with 2 Micronair® AU5000 rotary atomizers (Micron Sprayers) using a flow rate of 7 L min<sup>-1</sup>, an application rate of 0.210 L ha<sup>-1</sup>, and blade angles set at 50° giving 5000 rpm to 5500 rpm at 200 kph. This resulted in a mean dose rate of 260 g a.i. ha<sup>-1</sup> (APLC 2010). Planes were equipped with a Satloc® differential global positioning system (Hemisphere GPS) for spray guidance using a constant rate flow control. Flight and spray data were recorded by the Satloc system, and these dose rates were used in calculations in the present study.

### Sample collection

At each site, pooled locust samples were collected in 200-mL Schott glass vials precleaned with acetone. Caps were lined with aluminum foil. A subsample of the vials was tared before use. Locusts pertaining to 3 different physiological states (live, debilitated, and dead) were collected separately at the postspray intervals outlined in the *Sampling sites* section, above. The first sample was collected by sweep net (hereafter defined as live) and transferred as a single mass by inverting the net into a jar while minimizing contact between the net and the jar itself. The net was changed after each sample. A second sample of live individuals (hereafter defined as debilitated) was collected 1 individual at a time from the ground or from vegetation with fine tweezers. Attempts were made to preferentially catch any individual that appeared to be affected, moving erratically, or falling on its back when landing. The intent was to obtain a sample that closely approximated choices made by a foraging bird. A third sample consisted of freshly dead individuals (evident from state of dehydration, hereafter defined as dead) also picked with tweezers directly from the ground. Tweezers were cleaned with acetone between samples. Samples typically consisted of 25 individuals to more than 100 individuals, with a few exceptions. Samples were placed immediately in a portable Engel freezer (Sawafuji Electric) and transferred to a regular chest freezer each evening (see Table 3.2).

At both Oakdale and Koolaman, 3 locust nymph samples (13–20 g each) were collected by sweep netting immediately prior to spraying, spiked with 17  $\mu$ L to 17.4  $\mu$ L (Oakdale) and 16.6  $\mu$ L to 22  $\mu$ L (Koolaman) of the spray formulation, and treated in the same way as the other samples. Three control samples per site were also collected.

In the laboratory, a subsample of dead and debilitated locusts of the same instar from each of the sample plots was counted and weighed individually to correct for eventual water loss of the former. These weights also served to verify whether to correct for weight loss from sample storage until analysis.

During the collection of nymphs for residue analysis, burrowing nocturnal raspy crickets (Gryllacrididae, *Pareremus* sp.) were seen dead or debilitated on the surface at several of our sites, notably on the Oakdale and Karawinna spray plots. Several insectivorous bird species were seen to switch to this species as a food source, so we opportunistically sampled 6 crickets for fenitrothion and fenitrooxon analysis.

### Residue analysis

Locust samples were held in a  $-17^{\circ}\text{C}$  freezer until time of analysis. Whole samples were weighed in their original containers (container plus liner and locusts). Whole samples and liner foil rinses were used for analysis. The emptied, dried containers were weighed after rinsing with 10% acetone in hexane solvent added to the respective samples. The difference between the container plus liner and locusts and the dried empty container plus liner was recorded as the whole sample weight. The samples were macerated in 10% acetone in hexane solvent with dried sodium sulfate, using an Ultraturrax blender at approximately 13,500 rpm. Fenthion was added to the final solution as an internal standard.

Fenitrothion and fenitrooxon were analyzed using the Hewlett Packard HP6890 gas chromatography system coupled to a flame photometric detector, using  $\text{H}_2$  and  $\text{N}_2$  as the carrier and makeup gases, respectively. Appropriate dilutions of sample aliquots were made, and fenitrothion and fenitrooxon concentrations were determined by high-performance liquid chromatography (HPLC) with mass spectrometric detection using an AB/Sciex AP14000Q mass spectrometer (AB/Sciex Concord), equipped with an electrospray (TurboV) interface coupled to a Shimadzu Prominence HPLC system (Shimadzu). Separation was achieved using a 5 micron,  $150 \times 4.6$  mm Alltima  $\text{C}_{18}$  column (Alltech) run at  $40^{\circ}\text{C}$  and a flow rate of  $0.8 \text{ mL min}^{-1}$  with a linear gradient starting at 40% B for 2 min, ramped to 100% B in 4 min, held for 4 min, and then to 40% B in 0.2 min, and equilibrated for 4 min (A = 10% methanol/HPLC-grade water, B = 90% methanol/HPLC-grade water; both 5 mM in ammonium acetate). The mass spectrometer was operated in the positive ion, multiple reaction-monitoring mode using nitrogen as the collision gas.

The inlet was pulse-pressured at 200 kPa for 0.8 min and purge-flowed at  $95.7 \text{ mL min}^{-1}$  for 0.75 min. The column used was Agilent J&W DB-1701, 30 m,  $320\text{-}\mu\text{m}$  diameter, with  $0.25\text{-}\mu\text{m}$  film thickness using a nominal initial pressure of 33.6 kPa and average velocity of  $38 \text{ cm s}^{-1}$ . The oven was run at an initial temperature of  $50^{\circ}\text{C}$  for 1.5 min, at  $35^{\circ}\text{C min}^{-1}$  to  $150^{\circ}\text{C}$ , and then at  $20^{\circ}\text{C min}^{-1}$  to  $260^{\circ}\text{C}$  for a run time of 10 min. The detector was run at  $250^{\circ}\text{C}$  with  $\text{H}_2$  flow at  $150^{\circ}\text{C min}^{-1}$ , air (oxidizer) flow at  $110 \text{ mL min}^{-1}$ , and a constant column plus makeup combined flow of  $60 \text{ mL min}^{-1}$ .

### Statistical analysis

Because of slight variations in application rates between spray plots, all residue data were normalized to a  $1 \text{ kg a.i. ha}^{-1}$  application following the residue-per-unit dose principle (Hoerger & Kenaga 1972) and then log-transformed for normality. A paired  $t$  test was applied to test for water loss in dead individuals, those samples being paired with samples of debilitated nymphs taken at the same time and sample location. Paired  $t$  tests of log-

transformed residue-per-unit dose values were used to compare live, debilitated, and dead locust samples matched for time of collection and sampling sites. Because different sampling locations within sites showed different time courses of residue accumulation, we applied linear regressions to relative log residue-per-unit dose values over time between 3 h and 70 h postspray in debilitated locusts after setting log residue-per-unit dose to 0 at  $t = 3$  h and between 1 h and 70 h postspray for live nymphs after setting log residue-per-unit dose to 0 at  $t = 1$  h.

## Results

### Meteorology and spraying parameters

Temperature and wind were generally favorable for spraying (Table 3.1), and no rain fell during treatments or shortly thereafter. Spraying parameters used (Table 3.1) during the present trial were in line with the standard operating parameters employed by the APLC during its standard locust control operations (APLC 2010). Effective dose rates ranged from 0.261 kg a.i. ha<sup>-1</sup> to 0.307 kg a.i. ha<sup>-1</sup>, and the spray direction was generally perpendicular to the wind direction. Observed flying heights were variable because of the presence of mallee (*Eucalyptus* sp.) windbreaks; isolated stands of trees throughout the target area; forested areas bordering plots (e.g., Oakdale on all sides, Koolaman and Ouyen on 3 sides of the spray plot); sand dunes within a plot (Ouyen, Karawinna); or the presence of hazards such as trees, power lines, and property boundaries common in such a highly dissected agricultural area. Consequently, spray aircraft were forced to operate above the optimal 10 m flying height on several occasions while applying fenitrothion on the trial sites used in the present study.

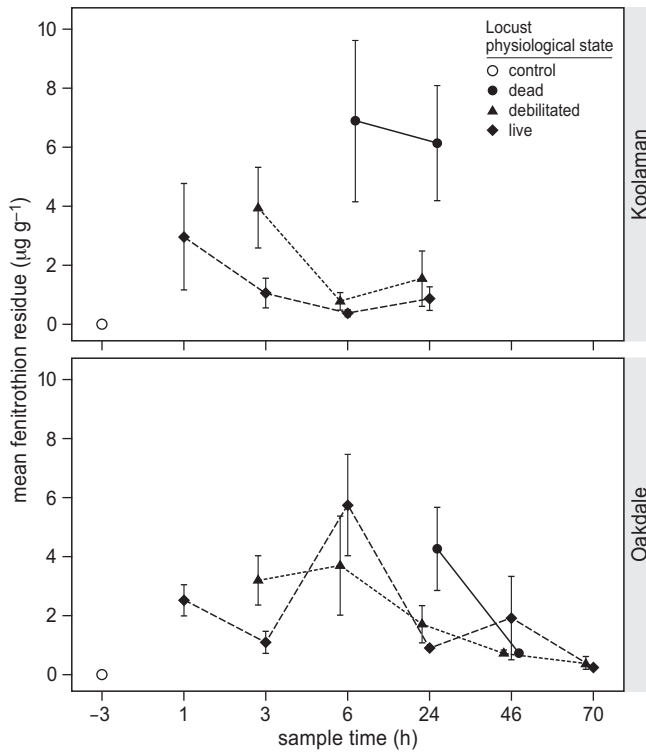
### Residue data

Residues in spiked samples ranged from 83.5% to 117.3% of expected values, and thus no adjustments for effects of transport, storage, or extraction efficiency were made to the residue values. Residues in control samples were below the limit of quantitation ( $<0.1 \mu\text{g g}^{-1}$ ) for the fenitrothion analysis.

Freshly dead locusts were found only at 6 h and 24 h postspray. The individual body

**Table 3.1.** Spray and meteorological parameters on the day of treatment for each study plot.

Property/ Location	Treatment date	Treatment start	Plot size (ha)	Dose rate (kg a.i. ha <sup>-1</sup> )	Spray height (m)	Temperature (min–max, °C)	Wind direction	Wind speed (m s <sup>-1</sup> )
‘Oakdale’	26-10-2010	12:07	70	0.284	15–20	7–29	NW	3.5
‘Koolaman’	28-10-2010	12:33	200	0.307	10–15	9.9–25.8	NNE	1–2
‘Karawinna’	02-11-2010	11:36	150	0.261	15–20	7.1–20	WSW	3.1–10
Ouyen	09-11-2010	10:00	120	0.291	15–25	10.5–31.2	E-N	2.5–5.6



**Figure 3.1.** Mean residue levels (adjusted for a 1 kg a.i. ha<sup>-1</sup> application rate) on the two main spray plots: 'Koolaman' (B4, B5 & B7 sites) and 'Oakdale' (L1, L3 & L7 sites) given with one SE. SE bars are offset for visual clarity. Residues in dead hoppers are corrected for moisture loss.

mass of a subsample of dead locusts was not different from that of debilitated individuals at 6 h postspray (paired *t* test;  $t = 0.719$ ,  $df = 4$ ,  $P > 0.05$ ), but at 24 h an average 24.7% reduction in body mass of dead locusts due to desiccation was apparent ( $t = -3.788$ ,  $df = 5$ ,  $P = 0.012$ ). Therefore, residues in dead locusts at 24 h were corrected for water loss when looking at the time course of residues (Fig. 3.1). Debilitated locusts were found at sampling times ranging from 3 h to 70 h postspray, and live locusts were present at all sampling times.

Fenitrooxon residues in locust nymphs were generally below the limit of quantitation ( $<0.02 \mu\text{g g}^{-1}$ ), with 2 exceptions – 1 live and 1 debilitated sample returning residues at the limit of quantitation. Four dead samples contained  $0.04 \mu\text{g g}^{-1}$ ,  $0.05 \mu\text{g g}^{-1}$ ,  $0.05 \mu\text{g g}^{-1}$ , and  $0.1 \mu\text{g g}^{-1}$  fenitrooxon (actual levels not normalized for application rate). Opportunistic samples in other insect species are reported below.

### Effect of plot and time

Residue levels were variable and did not always show a monotonic decrease over time, although some of this variation may have been caused by irregular sample sizes and there

was no clear correlation in the time taken to reach maximum residue levels between sampling sites (Table 3.2). Looking at the maximum residues per sampling site, there was a broad overlap of residue levels between the 2 main spray plots, Oakdale and Koolaman (Fig. 3.1) and, in some cases, wide variation across sampling sites within a spray plot (Table 3.2).

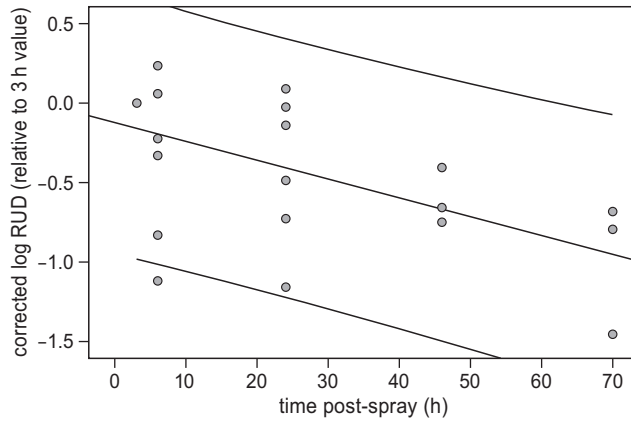
### Differences between live, debilitated, and dead locust nymphs

For this comparison, data from Karawinna and Ouyen spray plots were added to the two other, more complete data sets. These data cannot be used to infer maximum residue levels from these plots because only one collection was made. Residue levels were highest in dead nymphs, followed by debilitated and then live (Table 3.2). Paired *t* tests showed significant differences in log residue-per-unit dose values between live and debilitated locusts ( $t = -2.5767$ ,  $df = 28$ ,  $P = 0.02$ ), live and dead locusts ( $t = -4.3071$ ,  $df = 10$ ,  $P = 0.01$ ), and debilitated and dead locusts ( $t = -3.2641$ ,  $df = 10$ ,  $P = 0.01$ ). A comparison of the log residue transformed concentrations in dead or debilitated nymphs with those collected live shows an increase of 0.24 log concentration units for debilitated nymphs

**Table 3.2.** Fenitrothion residues ( $\mu\text{g g}^{-1}$ , adjusted for a 1 kg a.i.  $\text{ha}^{-1}$  application rate) on the two main spray plots: Koolaman (B4, B5 & B7 sites) and Oakdale (L1, L3 & L6 sites). Maximum residues at each sample site for live, debilitated or dead hoppers are in bold type. Residues in dead hoppers are not corrected for moisture loss.

Physiological state	Site	1 h	3 h	6 h	24 h	46 h	70 h
Live	B4	<b>21.11</b>	6.28	1.66	3.68		
Live	B5	<b>5.89</b>	3.45	1.50	4.59		
Live	B7	<b>1.89</b>	0.55	0.39	0.20		
Live	L1	5.82	2.50	<b>8.64</b>	3.21	1.02	1.16
Live	L3	8.88	29.33	<b>31.98</b>	2.75	16.71	0.60
Live	L6	<b>12.06</b>	5.11	11.14	3.46	2.54	0.78
Debilitated	B4		<b>21.67</b>	3.19	1.50		
Debilitated	B5		<b>7.87</b>	3.68	2.57		
Debilitated	B7		8.98 <sup>a</sup>	0.68 <sup>a</sup>	<b>11.16</b>		
Debilitated	L1		5.29	<b>6.06</b>	5.01	2.08	0.85
Debilitated	L3		14.46	<b>24.89</b>	10.47	2.57	3.00
Debilitated	L6		<b>14.10</b>	8.46	2.64	3.10	0.49
Dead	B4			<b>39.82</b>	26.12		
Dead	B5			<b>16.82</b>	7.32		
Dead	B7			10.61	<b>26.45</b>		
Dead	L1				<b>5.89</b>		
Dead	L3				<b>15.76</b>		
Dead	L6				<b>10.01</b>		

<sup>a</sup> Small sample weight representing fewer than 10 individuals. May be less representative.



**Figure 3.2.** Scatter plot of time course of residue concentrations (given as corrected log residue per unit dose (RUD) values (relative to 3 h value) in debilitated (tweezer-collected) *C. terminifera* nymphs, given with 95% CI.  $R^2 = 0.35$  ( $y = 0.12 + 0.01 \cdot x$ ).

(range =  $-0.81$ – $1.8$ ; standard deviation SD =  $0.50$ ) and  $0.80$  log concentration units for dead nymphs (range =  $0.07$ – $2.0$ ; SD =  $0.61$ ) over live samples. Returning these calculated values to a linear scale, the increased concentration in debilitated and dead (corrected for desiccation) nymphs represents an average increase of  $62\%$  and  $224\%$ , respectively, over the concentration measured on live locusts collected by sweep netting.

#### Maximum residue values at each sample site

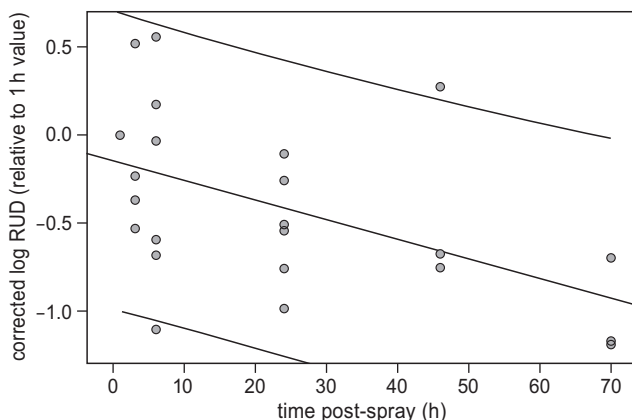
Peak residue-per-unit dose values in live nymphs at Koolaman or Oakdale ranged from  $1.9 \mu\text{g g}^{-1}$  to  $32 \mu\text{g g}^{-1}$ , whereas the range in debilitated nymphs was  $6.1 \mu\text{g g}^{-1}$  to  $25 \mu\text{g g}^{-1}$  (Fig. 3.2;  $n = 6$  sample sites). Two samples of dead locust nymphs collected at Karawinna  $6$  h postspray (not necessarily peak values) had residue-per-unit dose values of  $31.7 \mu\text{g g}^{-1}$  and  $16.7 \mu\text{g g}^{-1}$ , and when combined with the Oakdale and Koolaman values, the full range of residue-per-unit dose values for dead samples from  $8$  sample sites ranged from  $5.9 \mu\text{g g}^{-1}$  to  $39.8 \mu\text{g g}^{-1}$ .

#### Time course of residues in locusts, debilitated individuals

Relative residue-per-unit dose values were calculated by expressing each data point as a change relative to the 1st sample ( $3$  h postspray) taken on the sampling site. Forcing the regression through the origin, the reduction in log residue-per-unit doses over time is highly significant ( $F_{1,23} = 35.223$ ,  $P < 0.00001$ ) with a slope of  $-0.0146$  log units of concentration per hour, an approximate  $10$ -fold reduction in residues over approximately  $74$  h.

#### Time course of residues in locusts, live individuals

Maximum residues were found in nymphs from  $4$  sampling sites at  $1$  h postspray and in  $2$  sites at  $6$  h. One of the sites at Oakdale, however, peaking at  $1$  h, had similar values at  $6$  h;



**Figure 3.3.** Scatter plot for time course of residue concentrations (given as corrected log residue per unit dose (RUD) values (relative to 1 h value) in live (sweep-netted) *C. terminifera* nymphs, given with 95% CI.  $R^2 = 0.29$  ( $y = 0.15 + 0.01 \cdot x$ ).

and another at Koolaman that peaked at 1 h had only slightly lower values 24 h postspray. At the latter site, locust bands moved rapidly through the plot at a rate of 200 m to 350 m in the 4 d to 5 d prior to spraying. If they were sprayed in an area with low vegetation cover or even bare ground, they might have moved postspray into areas with a higher vegetation cover, which likely had intercepted higher spray deposits. Regardless of the actual reasons, more variation in the site-specific rate of loss was seen in live nymphs relative to debilitated ones (Fig. 3.3). Forcing the regression through the origin, the temporal reduction in log residue-per-unit doses was again highly significant, with an almost identical slope of  $-0.0145$  log units of concentration per hour, resulting in a 10-fold reduction of residues in approximately 75 h to 76 h ( $F_{1,29} = 30.472$ ,  $P < 0.00001$ ).

### Residues in insects other than locust nymphs

Postspray, a number of large (1.5–2 g) burrowing nocturnal raspy crickets (Gryllacrididae, *Pareremus* sp.) were collected for analysis from the Oakdale and Karawinna spray plots. Two opportunistic samples taken 24 h postspray had residue-per-unit dose values (uncorrected for desiccation, because only fresh specimens were collected) of  $7.85 \mu\text{g g}^{-1}$  and  $28.56 \mu\text{g g}^{-1}$ . A sample of dead nymphs collected at the same time and location as the latter had a residue-per-unit dose level less than half of that of the cricket ( $11.5 \mu\text{g g}^{-1}$ ). Another cricket collected 46 h postspray had a residue-per-unit dose value of  $2.79 \mu\text{g g}^{-1}$ , and a fourth sample had a residue-per-unit dose of  $15.2 \mu\text{g g}^{-1}$  (collection time not noted). Three of the cricket samples had measurable oxon concentrations ranging from  $0.03 \mu\text{g g}^{-1}$  to  $0.09 \mu\text{g g}^{-1}$ , uncorrected for application rate. Finally, a carabid beetle (0.5 g) found on the Oakdale plot 46 h postspray had a residue-per-unit dose of  $2.29 \mu\text{g g}^{-1}$  uncorrected for desiccation.



## Discussion

### Physiological state of locust nymphs and time course of residues

Residues in dead, debilitated, and live nymphs were all statistically different from one another, with dead samples containing the highest and live samples the lowest residues. Changing the collection method from sweep netting to picking out individuals with tweezers resulted in an average increase of 62% in residue concentration. Dead nymphs contained 224% more residues than paired sweep net samples.

We consider residue levels in debilitated locust nymphs to best represent the risk to avian predators postspray. Debilitated individuals certainly attracted our attention by their erratic movements and we believe that this conspicuousness, as well as the relative ease with which they could be captured, increases the possibility that affected nymphs could be picked out selectively by foraging birds.

Residues in dead insects reported in the literature may not represent the best choice for avian risk assessment as insects tend to desiccate rapidly after death (water loss of 25% between 6 h and 24 h postspray in the present study) and may be less attractive as a food source in a desiccated state. This is supported by a study addressing avian food preferences for live, freshly dead, or desiccated insects under laboratory and field conditions. That study concluded that, when given a choice, wild birds did not consume desiccated prey but had a strong preference for live insects, followed by freshly dead individuals (Stafford *et al.* 2003). In line with this observation, captive white ibis (*Threskiornis molucca*) have been shown to prefer live locusts over freshly killed or dried locusts (Cowling 1974). The latter two categories were consumed in higher quantities after the live locust supply was exhausted. However, the same author observed during spray trials that straw-necked ibis (*Threskiornis spinicollis*) appeared to favor freshly sprayed paddocks containing “sluggish” locusts over unsprayed areas. Once desiccated, however, sprayed locusts did not appear to be attractive to ibis. Observing birds that feed heavily on desert locusts (*S. gregaria*), Symens (1990) noted that those species appearing to favor dead locusts were the small-bodied ones. In line with that observation, a study using clay-colored sparrows (*Spizella pallida*) maintained in indoor cages and fed fresh dead and live grasshoppers (*Melanoplus sanguinipes*) simultaneously (Forsyth *et al.* 1994) concluded that *S. pallida* showed a preference for dead (over live) *M. sanguinipes* and that this resulted in a greater consumption of pesticide-exposed (over unexposed) grasshoppers. Although these studies were conducted under different conditions and degrees of rigor, the apparent disparity highlights the importance of appropriate consideration of the species under consideration, their energetic requirements, and feeding behavior when formulating risk assessments for gorge-feeding avian species.

Few freshly dead locusts were found after 24 h, despite intensive searches by the authors. We attribute this to high scavenging rates of dead and debilitated hoppers in the period when they contain the highest residues. Scavenging was most likely both by diurnal and, probably more importantly, by nocturnal scavengers/predators, reducing the number of nymphs available for birds. While other studies suggest that predators such as arachnids and ground beetles rapidly remove prey from fields (Stafford *et al.* 2003), it is likely that

small mammals and the significant number of reptile species present in Australia's arid and semiarid regions must also be contributing to scavenging of moribund and dead locusts (Story *et al.* 2005).

### Transfer of residues

Through scavenging, risks may be transferred from locusts to alternative prey, thereby extending exposure to the birds that feed on these scavenger species. Similarly, contamination of other invertebrates such as raspy crickets increases the scope of vertebrate exposure. Based on a single point of comparison, the crickets may accumulate higher residue levels than locust nymphs. The presence of measurable oxon levels in 3 of the 4 samples also contrasts with samples where the majority had nondetectable levels of the oxon.

### Secondary uptake

The loss of fenitrothion residues in live and debilitated nymphs was approximately 10-fold over the 70 h to 75 h postspray, or an apparent first-order dissipation half-life of approximately 48 h. This should not be equated to degradation of the chemical because of the dynamic sampling situation, the movement of locusts in and out of sample plots, and the gradual disappearance of dead and affected individuals. Locusts acquire insecticide residues both from direct droplet impingement and from uptake by touching and/or ingesting contaminated vegetation (Nguyen 1981). Residue levels at Koolaman tended to peak later, usually 3 h to 6 h postspray and, in a single sampling site, 24 h postspray. The state of the vegetation, ranging from almost bare to low grasses, probably was the main factor accounting for differences in secondary uptake and hence for insecticide residue peaking at different time intervals after spraying. In the context of risk assessment, such variation is likely to be important for gorge-feeding species.

### Avian risk assessments using invertebrate residue data

The geometric mean concentrations of residues (1 peak value per sampling site) normalized to a 1-kg a.i. ha<sup>-1</sup> application were 9.6  $\mu\text{g g}^{-1}$ , 12.6  $\mu\text{g g}^{-1}$ , and 14.8  $\mu\text{g g}^{-1}$  in live, debilitated, and dead nymphs, respectively. The residue-per-unit dose values reached maxima of 32  $\mu\text{g g}^{-1}$  in live and debilitated nymphs and 40  $\mu\text{g g}^{-1}$  in dead nymphs (fresh wt). There are surprisingly few published data with which to compare these values. Average residue-per-unit dose values of 2.3  $\mu\text{g g}^{-1}$  and 23.5  $\mu\text{g g}^{-1}$  for live and of 15.9  $\mu\text{g g}^{-1}$  and 29.5  $\mu\text{g g}^{-1}$  for dead grasshoppers in 2 yr of aerial spraying with carbofuran have been reported previously (Forsyth & Westcott 1994); and similarly, in a study using acephate (Stromborg *et al.* 1984), the average calculated residue-per-unit dose values in live and debilitated ground-sprayed locusts were 17.6  $\mu\text{g g}^{-1}$  and 22.8  $\mu\text{g g}^{-1}$ , respectively. Our values may therefore be low compared with other values for Orthoptera.

Residue values calculated from nomograms (Hoeger & Kenaga 1972, Fletcher *et al.* 1994) have long dominated the data contributing to avian risk assessments concerning pesticides. It was initially proposed that values for seeds could be used as a proxy for insects (Kenaga 1973). If we consider residue-per-unit dose values for large insects to be

applicable to acridids, these would range from  $2.7 \mu\text{g g}^{-1}$  to  $4 \mu\text{g g}^{-1}$  for mean (or typical) values to  $11 \mu\text{g g}^{-1}$  to  $13 \mu\text{g g}^{-1}$  for maximum values (following Hoerger and Kenaga (1972) and Kenaga (1973). The Ecological Committee on FIFRA Risk Assessment Methods (ECOFRAM) exercise (ECOFRAM 1999) used data from terrestrial field studies conducted by industry in the late 1980s and early 1990s, mainly based on two previous studies (Fischer & Bowers 1997, Brewer *et al.* 1997). Their range of  $3.7 \mu\text{g g}^{-1}$  to  $5.4 \mu\text{g g}^{-1}$  for large insects falls roughly within the nomogram range, as does the range recently given elsewhere (Luttik 2001) of  $7.5 \mu\text{g g}^{-1}$  (mean) to  $13.8 \mu\text{g g}^{-1}$  (90<sup>th</sup> percentile) in ground-dwelling arthropods without interception by vegetation. The latter values were derived to be used in European tier 1 avian risk assessments.

However, all proposed regulatory values underestimate residues found following acridid control. Possible reasons for this might be the use of ultralow-volume formulations and a finer droplet spectrum resulting in better interception by the insects, as well as the typical acridid habitat consisting of scattered and irregular vegetation structure. Furthermore, past insect collections have not always been designed to mimic the feeding behavior of insectivorous birds and therefore accurately reflect their potential dietary intake. It has been argued that sweep netting may remove residues from vegetation and transfer them to the insects (Luttik 2001). Our data show that locusts captured by sweep nets had lower residues than individuals picked by tweezers. If we unwittingly contaminated those samples by sweeping vegetation, then the difference between live and debilitated nymphs would be greater than we reported. It is also reasonable to assume that insects falling into pitfall traps (the other common method of collection) are the most mobile individuals and may therefore reflect the least affected portion of the exposed population, whereas insects affected by insecticide residues are likely to become immobilized. Yet, immobilizing insects under the spray or placing them in enclosures (Brewer *et al.* 1997) gave insect residue levels that were lower than those measured in pitfall traps (Fischer & Bowers 1997).

## Conclusions

The present study shows that locusts accumulate residues by secondary uptake before they become debilitated and that maximum residues are generally reached 3 h to 24 h post-spray. Therefore, it is unlikely that artificially immobilized insects reflect realistic maximum residue levels any more than pitfall traps can. Our data suggest that sampling of (debilitated) acridids for risk assessment should include mimicking predation and take place over a longer and different time course (preferably 3–24 h postspray) than sampling of vegetation (typically 1–2 h postspray). This is particularly important in arid or semiarid habitats, where vegetation is naturally patchy and residue uptake variable. The present study, in concert with previous research, shows that applying existing regulatory (European Food Safety Authority or US Environmental Protection Agency) standard values for residues in insects will likely underestimate the risk of pesticides applied for locust or grasshopper control and possibly other insects.

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# Does bird predation enhance the impact of Green Muscle® (*Metarhizium acridum*) used for grasshopper control?

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## Abstract

A study at Khelcom, Central Senegal from September 2008 till June 2009 tested two different dose rates (25 and 50 g conidia ha<sup>-1</sup>) of the mycoinsecticide Green Muscle® (*Metarhizium acridum*) and an untreated control, against grasshoppers in a field trial on nine 400-ha plots in three blocks. The study area was a deforested sylvo-pastoral reserve, 12.5% cultivated, whereas the remainder was fallow or grassland in succession. Grasshopper densities were calculated by executing transect and quadrat counts on plots. The grasshopper community structure was assessed by systematic sweep-net sampling on plots. From these data grasshopper biomass on plots was calculated for each sampling date. Bird numbers were counted on the same transects by the Distance Sampling technique and their densities calculated from Effective Strip Widths (ESW). Energetic requirements of individual bird species were calculated from digestibility-corrected Field Metabolic Rates (FMRs) and for acridivorous species their daily intake of grasshoppers was calculated. Grasshopper densities were very high, with up to 90 ind. m<sup>-2</sup> in September and 30–35 ind. m<sup>-2</sup> in October. Numbers and biomass decreased on treated plots as grasshoppers became infected, and remained significantly different from control plots for three months (until January). The relative importance of grasshopper consumption by birds increased between October (high grasshopper densities) and December (medium densities) from an initial 0.06 (± 0.03)% day<sup>-1</sup> to a ceiling of 1.6 (± 0.9)% day<sup>-1</sup>. Total grasshopper removal during the dry season was 70%, whereas during the rainy season this was < 1 %. Birds specifically captured large and medium-bodied grasshoppers, but rarely small-bodied species, whose numbers initially increased. These findings were corroborated by field observations and by analysis of regurgitated pellet contents of Montagu's Harriers, *Circus pygargus*, i.e., small-bodied grasshoppers were only 1.4–1.6% of all grasshoppers taken, whereas they constituted 61–68% of random samples from the field. Densities of acridivorous Palaearctic migratory birds, in particular White Stork, *Ciconia ciconia*, Montagu's Harrier and Lesser Kestrel, *Falco naumanni*, were very high and unprecedented elsewhere. Their numbers largely exceeded the 1% criterion for international importance.

## Introduction

Locust and grasshopper control in the Sahel still heavily relies on chemical insecticides. During the 2003–05 Desert Locust, *Schistocerca gregaria*, upsurge in western and northern Africa, over 13 million liters of organophosphate and pyrethroid compounds were used, but no biopesticides (Brader *et al.* 2006). This is surprising because the entomopathogenic fungus *Metarhizium acridum*, formerly *M. anisopliae* var. *acridum* (Bischoff *et al.* 2009) (Green Muscle®, further GM) has been commercially available for operational use since 1999 (Lomer 1999) and has been assessed for use against Desert Locust at a dose rate of 50 g conidia (spores) ha<sup>-1</sup> (PRG 2004). Its use in locust control has meanwhile been recommended by the FAO (Magor 2007, FAO 2009) and it obtained full registration from the "Comité Sahélien des Pesticides" (CSP-CILSS) in nine Sahelian countries in January 2010.

Despite the recommendations and registration, there is still very little operational use of GM in Africa because users a) consider the time lag before the onset of mortality as a constraint, b) find its price prohibitive at the recommended dose rate, c) consider the temperature dependency as a drawback and d) consider current oil-based formulations (OF) as too rapidly deteriorating under prevalent field conditions (*e.g.*, van der Valk 2007).

Meanwhile, it has been shown that the dose rate of GM in grasshopper control can be reduced to 25 g conidia ha<sup>-1</sup> (Mulli   & Gu  ye 2009) without compromising efficacy, making it directly competitive with chemical insecticides. Following these results, the Senegalese Crop Protection Directorate used GM successfully in 2009 in c. 50% of its treatments against the Senegalese Grasshopper *Oedaleus senegalensis* at a dose rate of 25 g ha<sup>-1</sup> (Khalifa Ndour, DPV Dakar, pers. comm.). New formulations with longer shelf-lives have also been tested and found to perform very well against Desert Locust under operational conditions (Ould Mohamed 2009).

Natural predation of locusts and grasshoppers by vertebrates can be so important that (chemical) control by man becomes redundant (Mulli   2009). Nevertheless, predation is rarely if ever considered in the decision-making processes applied to locust and grasshopper control.

The effect of entomopathogens on the predator-prey relationship is completely different from that of chemical insecticides. In a study design very much comparable with ours described hereafter, but by using the organophosphorous compounds fenitrothion and chlorpyrifos at two different dose rates, Mulli   & Keith (1993a) found that apart from direct mortality of 2–7% of the avian community due to anticholinesterase poisoning, bird numbers on transects decreased significantly by as much as 50% following treatments and colonies of Buffalo Weavers, *Bubalornis albirostris*, were deserted. This was caused by the impact of the organophosphates on nontarget arthropods such that insectivores faced an immediate depletion of their food resources (Mulli   & Keith 1991, 1993a, 1993b).

Biopesticides do not kill immediately, as the pathogens need time to develop after insects become infected (Langewald *et al.* 1999). Because mycopesticides are very selective,



no impact on nontarget species occurs (Lomer 1999, Peveling *et al.* 1999, Mullié & Guèye 2009) and birds neither leave sprayed areas, nor do they become intoxicated. Instead, there are indications that their numbers may temporarily increase (Mullié 2007). The insects become sluggish, and an easy prey for birds, when basking to induce behavioral fever, i.e., by altered thermoregulatory behavior raising body temperature in reaction to infection by a pathogen (Blanford *et al.* 1998). There is indeed field evidence of synergy between the impact of entomopathogens and predation (Chapter 5 of this thesis), but this has never been tested experimentally.

The current article addresses the question of whether birds do indeed enhance the impact of *M. acridum* and if so, to what extent and under which conditions. Medium sized plots (400 ha) were sprayed with GM in a field trial and grasshopper and bird densities monitored over an 8-mo period post treatment. Grasshopper consumption by birds over time was calculated for each of the treatments, based on energetic requirements and compared to available grasshopper biomass. Grasshopper removal rates were assessed to compare treatments.

## Methods

### Study area

The study took place from September 2008 until June 2009 at Khelcom, also known under the name of Mbégué, central Senegal (lat 14°28' – 14°43'N, long 15°22' – 15°36'W).

Between 1991 and 2004, 55,400 ha (as measured by GPS) out of the 73,000 ha Mbégué Sylvopastoral Reserve was gradually deforested to allow for groundnut production. However, in 2008 the total cultivated area was only 12.5%, of which about 60% consisted of groundnut and the rest of millet and some smaller surface areas that were grown with crops such as maize, tapioca and sesame. Typically, a field cultivated with groundnut would be sown with millet during the following rainy season one year later and thereafter left fallow for one or more years. As a consequence, Khelcom is now a mosaic of cropland, fallow and never-cultivated but deforested land, in various stages of succession, mainly with the shrub *Guiera senegalensis* ('Nger' in Wolof) and regrowth of *Combretum glutinosum* ('Rat').

Of special mention is the liana *Leptadenia hastata* ('Thiakhat'). This evergreen plant, forming large green patches in an otherwise barren environment, harbors reproducing Pyrgomorphidae throughout the dry season, and is also exploited by other grasshopper species, most notably *Cryptocatantops haemorrhoidalis*, *Metaxymecus gracilipes*, *Diabolo-catantops axillaris* and *Heteracris annulosa*. Hence stands of *L. hastata* are often favored by acridivorous birds during the dry season.

Except for 15 so-called Daras (small settlements each housing up to several hundred children who receive religious training and labor in the surrounding fields), there are no other permanent dwellings in the area. There are, however, dozens of temporary camps inhabited by semi-nomadic herders.

Wildfires occur annually at Khelcom between November and the end of the dry season. In Fig. 4.1, a map of Khelcom is given showing the layout of our experimental plots and of the areas being burnt.

### Floristic composition

The floristic composition of the study plots was assessed from the diagonals which also served as transects for counts of grasshoppers and birds (Fig. 4.1). In the middle of each sub-transect of 100 m the herbs present on a 10 × 10-m quadrat were identified with Berhaut (1967), Terry (1993) and Sankara (2008), and their coverage (%), height (cm) and the percentage of bare soil noted. Trees and shrubs present were identified with von Maydell (1990) and counted on a 100 × 100-m bloc and their height and number noted.

### Meteorology

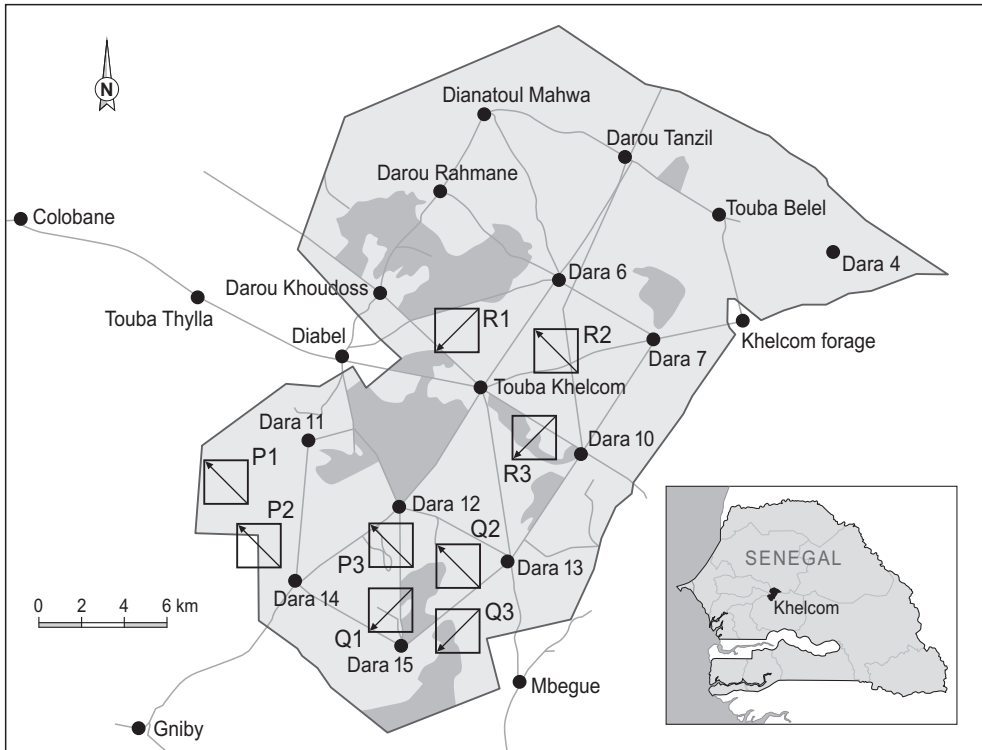
Data on rainfall, wind, temperature and relative humidity were obtained from the National Meteorological Station at Toubia Khelcom (lat 14°34'N, long 15°30'W; Mr Moussa Sy, National Meteorological Service, Khelcom, pers. comm.) situated in the middle of our study area, Fig. 4.1. Ambient temperatures (T, °C) and relative humidity (RH, %) values were also recorded with Hobo® Pro Series (Onset Computer Corporation, USA, 1998) weather recorders, placed on the ground in the center of each of our plots. The latter observations were believed to provide the best meteorological information of the grasshopper environment and thus for the action of the entomopathogen.

### Plots and treatments

Nine plots of 2 by 2 km, 400 ha per plot, in three blocks of three plots each were delimited. Each block, coded P, Q and R, received three replicate treatments of 0g (control; P3, Q3, R1), 25 g (P1 Q1, R3) and 50 g (P2, Q2, R2) conidia of *M. acridum* (strain IMI 330189) per hectare. Plots were at least one kilometer apart to avoid contamination during treatments (Fig. 4.1).

GM was available in two OF formulations, respectively with 2.5 and  $1.25 \times 10^{13}$  conidia l<sup>-1</sup> and was mixed in a 1:10 ratio with diesel fuel prior to treatment. Samples of the formulations before mixing were analyzed for viability and concentration of conidia. After calibration plots were treated with four Micronair™ AU 8115 rotary atomizers (Micron Sprayers Ltd, UK) mounted on four four-wheel drive pick-up vehicles, operating simultaneously at a speed of 10 km h<sup>-1</sup>, with a track spacing of 50 m, dose rate of 1 l ha<sup>-1</sup> and a flow rate of 833 ml min<sup>-1</sup>. Treatments took place between 9 and 11 October 2008 from 8 till 11 AM and again from 4 till 7 PM. To prevent clogging of the spray system by conidia, filters and hoses of all Micronairs were cleaned repeatedly after several passes.

Deposition of droplets was measured in the center of each of the spray plots by placing oil-sensitive papers over a length of 600 m perpendicular to the spray paths and facing the wind at 15-m intervals, 60 cm above ground level.



**Figure 4.1.** Map of the Khelcom study area. Squares are study plots with diagonals indicating transects and flashes (arrows) indicating the direction of the counts. Codes of plots sharing the same letter (P, Q and R) are in the same block. Areas that were burnt during the study are dark grey.

### Grasshopper availability

Two monitoring periods were distinguished. A period of intensive monitoring was applied from 4 d before until 18 d after treatment, at three-day intervals, during the month of October 2008. Extensive monitoring (once a month) was applied from November 2008 until May 2009, with the exception of April, when no observations were made. In September, three more series of counts, respectively at 20, 18 and 14 d prespray, were made in nine other plots in the same study area, which were to be sprayed aerially. We will also refer to some of the data obtained from these plots.

All observations were done on transects. One of the 2800-m long diagonals of each plot, usually SE-NW, in a few cases NE-SW (Fig. 4.1), was divided in three transects of 700 m, starting at 250 m from the corner of the plot, with a 100-m buffer between successive transects and again 250 m at the end of the third transect. Each transect was divided in seven sub-transects of 100 m to facilitate observations and to differentiate observations spatially. Observations on subsequent transects within the same plot were considered as being independent.

### Density

During the intensive monitoring period, two observers walked on either side of the transects, starting at about one to two hours after sunrise. On each sub-transect of 100 m, and spaced at 20-m intervals, five open quadrats of 1 m<sup>2</sup> were selected at random and total number of grasshoppers (nymphs plus imagos) counted. For each sub-transect of 700 m this produced 35 observations or 105 observations for each plot. The densities for the two observers were averaged for each paired quadrat and these figures used in calculations.

During the extensive monitoring, counts were done by a single observer. As it was anticipated that grasshopper densities and proportion of nymphs would drop during the dry season, making the use of quadrat counts inappropriate, during the last count of the intensive monitoring period, both quadrat and full-transect counts (Cressman 2001) were executed successively on the same transects. All grasshoppers seen within 50 cm on either side of the walked track were counted and totals for each subtransect of 100 m were noted. Average numbers per m<sup>2</sup> (x) were subsequently regressed against average numbers counted by the quadrat method (y) on the same subtransect. To make counts during both periods comparable, the regression equation ( $y = 1.0905x$ ;  $r^2 = 0.9388$ ) was used to convert densities counted by transect counts into densities calculated by the quadrat method during the period of intensive monitoring.

### Community structure

As the transect counts did not produce information on individual species, after each count insects were captured with sweep nets. Over the entire diagonal, between the 250-m limits from each corner, on three repetitions each of 150 m, grasshoppers were captured in a way as representative as possible: to assure representation of the various habitats available on the plot and to assure the capture of "difficult" stages, such as first instars (less mobile, difficult to capture when on the ground) and such as adults of the larger grasshoppers, the latter particularly difficult when temperatures were elevated and imagos were highly mobile. To reduce variability among samples and dates and to standardize sampling, the capture continued until at least 500 individuals per plot were trapped during each sampling. To prevent interaction with counts, grasshoppers were captured on an imaginary line which was c. 100 m away from and parallel to the transects.

Grasshopper imagos were identified with Lecoq (1979, 1988, 2008) Mestre (1988) Launois & Launois-Luong (1989) and Launois-Luong & Lecoq (1989). If necessary they were compared to specimens in the reference collection of the Crop Protection Directorate. Nymphs were identified with Popov (1989).

### Biomass

A subsample of the grasshoppers captured for establishing community structure, to which were added individuals captured between September and December 2009 during a follow-up study on the same plots, was used to take both fresh and dry body masses to calculate grasshopper biomass.

### Bird numbers

The same transects and sub-transects used for grasshopper counts (see under grasshopper availability) were used for bird counts. As birds were most active in the first hours of daylight and grasshoppers only after they had increased their body temperatures, the onset of bird counts on transects preceded those of grasshoppers, usually starting within the first hour after sunrise. The only exception was when heavy rains prevented an early start. We believe this did not influence the quality and comparability of the counts, as all three observers had the same delays and birds usually exhibited a peak of activity immediately following rains.

As the observers had only basic experience in field ornithology and in estimating distances, the senior author organized a three-day intensive practical training course in the field prior to the onset of the study. Individual performances, both on visual identification and by sound, were tested at the end of the training to assure that the most important and/or most common species could be properly identified. In the course of the monitoring period these training sessions were repeated several times to account for species having newly arrived, e.g., Palaearctic-African migrants. For visual identification of birds Brown *et al.* (1982), Urban *et al.* (1986, 1997), Fry *et al.* (1988,2000), Keith *et al.* (1992) and Borrow & Demey (2001) were used. For identification of bird song the CD collections of Chappuis (2000) and Barlow *et al.* (2002) were used.

To standardize counts temporally (monitoring per plot) and spatially (comparison with other plots), each sub-transect of 100 m was counted in 5 min. (*cf.* Mullié & Keith 1993a), or 35 min. per transect. All birds that could be seen with the naked eye or heard at unlimited distance on either side of the transect, were noted on field forms. Binoculars (8×40, 10×40) were used to identify species that otherwise were too distant to be properly identified, or to distinguish between similar species. It was decided not to apply a maximum observation distance *à priori*, because otherwise the sample of raptors and storks might become too small. However, during analysis (see under statistics) observation width was sometimes limited *à posteriori*.

For each observation, *i.e.*, a single bird or a group of two or more individuals close together, the number and the distance perpendicular to the transect was noted, according to the methodology of distance sampling (Buckland *et al.* 1993). Non-identified species were noted with a description. In many cases these species could later be identified. The Distance software (Thomas *et al.* 2006) allows for a calculation of true densities, as it accounts for the probability of detection: small or secretive species will only be observed at rather short distances, whereas flocks, large species or species with a conspicuous behavior tend to be noticed at larger distances.

### Grasshopper consumption by birds

Based on existing knowledge of the food and feeding behavior of the bird species present on our transects (*cf.* Morel & Morel 1978, Mullié & Keith 1993a, Mullié 2009), as well as on the basis of visual observations during the study, on gizzard contents of birds found dead, *e.g.*, as road victims in our study area, and on the contents of regurgitated pellets

(see also next paragraph) collected in roosts of communal species such as White Stork, Montagu's Harrier and Lesser Kestrel, *Falco naumanni*, observed species were classified as acridivorous or non-acridivorous.

As the total observation period, from September till June, included both a part of the rainy season and the major part of the dry season, some species were present in only a part of this period. Certain species such as the Village Weaver, nested and fed their young with insect prey. During the dry season they became granivorous. The Village Weaver is considered as one of the principal predators in Senegal of the Senegalese grasshopper *Oedaleus senegalensis* during the rainy season (Axelsen *et al.* 2009). Other common species, such as the Singing Bushlark, *Mirafraga cantillans*, and the White-billed Buffalo Weaver, are acridivorous during the rainy season, feeding themselves and their young with grasshoppers (Mulli  & Keith 1993), but become granivorous during the dry season (pers. obs. WCM). Other species may change the proportion of grasshopper prey in their diet throughout the season. As yet, we do not have sufficient information to estimate accurately for each species which proportion of its diet consists of grasshoppers throughout the year.

To be on the conservative side, we assume here that the diet of any species considered to be acridivorous does not consist by more than 50% of grasshopper prey, even if we know for certain species (Montagu's Harrier, Lesser Kestrel; Mulli  2009) or suspect for others (White Stork, Abyssinian Roller, *Coracias abyssinica*, Woodchat Shrike, *Lanius senator*) (this study) that the proportion by weight of grasshoppers in their diet may be as high as 80–90%. Furthermore we follow Axelsen *et al.* (2009) that any *breeding* granivorous species only feeds on grasshoppers during the rainy season and from November onwards only feeds on grains.

Individual field metabolic rates (FMRs) of birds were calculated by using an allometric analysis of log-log transformed data of fresh body mass ( $M_b$ ) in g and FMR in  $\text{kJ day}^{-1}$  of 229 vertebrate species ( $P < 0.007$ ,  $F_{1,227} = 547$ ;  $\text{FMR} = 2.25M_b^{0.808}$ ) (Nagy 2005). A database was developed in Excel (Microsoft Inc.) containing for each record, along with bird species name, number observed and distance, information on bird body mass, FMR, FMR corrected for food digestibility, effective strip width (ESW; see under statistics), origin (Palearctic or Afro-tropical) and detection probability (Table 4.1). From these data, bird densities  $\text{km}^{-2}$  and their grasshopper consumption were calculated.

To estimate the percentage of grasshopper biomass taken daily by the acridivorous bird community, the calculated daily consumption ( $\text{kg DM km}^{-2}$ ) was divided by the calculated daily total biomass of grasshoppers present + daily consumption ( $\text{kg DM km}^{-2}$ ) and multiplied by 100.

### Food remains in regurgitated pellets

Only data on pellet contents from Montagu's Harriers became available during the study; data from other species will be published elsewhere. All Montagu's Harriers feeding in Khelcom used up to 4 communal roosts within the area. During the extensive monitoring period regurgitated pellets were randomly collected in the roosts (c. 100 pellets per roost and per visit), individually wrapped in plastic foil and labelled for future analysis and

identification of the prey remains. As the main orthopteran remains in pellets are mandibles, a key had to be developed to identify individual species (Franck Noel, in prep.), taking into account the wear of the mandibles (cf. Chapman 1964, Zouhourian-Saghriet al. 1983, Gangwere & Spiller 1995, Smith & Capinera 2005). Prey remains per pellet were counted (using maxima of left or right mandibles, tibiae, femurs, and other identifiable parts) and biomass of grasshoppers calculated from data in Appendix 1.

### Statistics: grasshopper densities

Count data were log transformed before analysis. Because data were not independent in time, a BACI (Before-After-Control-Impact) design was applied (Stewart-Oaten *et al.* 1986). In this design, the effect parameter  $[\text{Log}(n+1)_{\text{treated}} - \text{Log}(n+1)_{\text{counted-before}}]$  was tested against  $[\text{Log}(n+1)_{\text{treated}} - \text{Log}(n+1)_{\text{counted-after}}]$ . It was thus implicitly assumed that the log difference of 'to be treated' and control plots was constant in time before treatments and that any change in this ratio, after spraying of GM, was caused by a treatment effect.

### Statistics: bird densities

The results from the transect counts were analyzed with the software Distance 5.0, release 1 (Thomas *et al.* 2006). For each individual species (for common species) or group of species (for less common species) Effective Strip Width (ESW) was calculated, a measure that depends on the probability of detecting a bird. To obtain a larger sample size, the less-common species were grouped into three categories: from 1 (low) to 3 (high) probability of detection. These categories corresponded approximately with detection distances of 0–100 m (1), 0–200 m (2) and 0–>400 m (3). For about half the number of bird species, a Hazard Rate model was used to calculate ESW, for the others a Half-normal with Cosine Expansion model. The chosen observation interval was manual in half of the cases and automatic in the others, whereas truncation of observation distance was applied in most cases to allow for a better fit of the chosen model. Details are given in Table 4.1.

To transform count data into densities, the following formula was applied: Density ( $\text{ind. km}^{-2}$ ) = number counted / ( $2 \times 2.1 \times \text{ESW}$ ). In this formula the factor 2 corrects for the two sides of the transect counted and the factor 2.1 is the total length in km of the three subtransects per plot. As an example we can take 9 Singing Bush-larks counted on a plot, which would give a density of  $9 / (2 \times 2.1 \times 0.0326) = 65.73 \text{ ind. km}^{-2}$ . Calculated densities were neither corrected for breeding females nor for nestlings, as was done by Axelsen *et al.* (2009).

Treatment effects were analyzed by Repeated Measures ANOVA (SPSS, v.13.0; SPSS Inc., Chicago, Illinois, USA). Missing values on day –1 for plots R1 and R2 were obtained by interpolation of count data on days –4 and +3.

**Table 4.1.** Information on body mass, (corrected) Field Metabolic Rates, origin, detection probabilities, ESW and model parameters used in calculations of species considered as acridivorous during at least a part of the study period.

Scientific name	N	Body Mass	FMR	corr. FMR	Origin	Detect.	ESW (m)			Details of model applied		
							avg.	95% conf. int.		Type	Interval	Truncation (m)
								min.	max.			
<i>Bubalornis albirostris</i>	405	75.4	199.4	269.4	A	ind.	61	55	69	HR	manual	1000
<i>Bubulcus ibis</i>	144	335.0	550.5	743.9	A	ind.	316	274	364	HN	manuel	2000
<i>Bucorvus abyssinicus</i>	1	4000.0	2979.8	4026.8	A	3	243	227	261	HR	manual	1175
<i>Centropus senegalensis</i>	14	170.0	346.8	468.7	A	2	71	68	75	HN	HN	375
<i>Chelictinia riocourii</i>		110.0	257.9	348.5	A	1	243	227	261	HR	manual	1174
<i>Ciconia ciconia</i>	28	3473.0	2706.5	3657.4	P	3	243	227	261	HR	manual	1175
<i>Circus aeruginosus</i>	763	627.5	844.0	1140.6	P	ind.	236	222	252	HN	automatic	-
<i>Circus pygargus</i>		315.5	528.5	714.1	P	ind.	236	222	252			-
<i>Cisticola juncidis</i>	662	7.7	42.2	57.0	A	ind.	20	19	22	HN	manual	140
<i>Cisticola</i> sp.		7.7	42.2	57.0	A	ind.	20	19	22			
<i>Coracias abyssinica</i>	542	114.0	264.2	357.0	A	ind.	102	93	112	HR	automatic	-
<i>Cursorius temminckii</i>	3	68.5	186.8	252.4	A	2	71	68	75	HN	manual	375
<i>Elanus caeruleus</i>	3	240.0	438.6	592.8	A	3	243	227	261	HR	manual	1175
<i>Eupodotus xavilei</i>	78	615.0	832.6	1125.1	A	2	71	68	75	HN	manual	375
<i>Falco naumanni</i>	422	152.5	322.1	435.3	P	ind.	233	214	255	HN	manual	2000
<i>Halcyon senegalensis</i>	56	56.9	164.6	222.4	A	ind.	59	40	87	HR	automatic	-
<i>Lamprotorornis caudatus</i>	74	121.0	275.1	371.8	A	ind.	80	64	99	HN	manual	600
<i>Lamprotorornis chalybaeus</i>	19	100.0	241.7	326.6	A	2	71	68	75	HN	manual	375
<i>Lanius meridionalis</i>	2	65.5	181.2	244.8	A	2	71	68	75	HN	manuel	375
<i>Lanius senator</i>	269	31.9	111.0	150.0	P	ind.	106	91	123	HR	manual	200
<i>Leptoptilos crumeniferus</i>	2	6325.0	4071.1	5501.5	A	3	243	227	261	HR	manual	1175
<i>Macropyx croceus</i>		47.6	145.8	197.0	A	1	36	34	37	HR	manuel	105
<i>Merops albicollis</i>	143	24.2	92.0	124.3	A	ind.	43	38	49	HR	automatic	200
<i>Milvus migrans</i>	11	704.0	912.8	1233.5	P	3	243	227	261	HR	manual	1175
<i>Mirafraga cantillans</i>	655	18.7	77.1	104.3	A	ind.	33	31	34	HN	automatic	-
<i>Motacilla flava</i>	5	17.6	74.0	100.0	P	1	36	34	37	HR	manuel	105
<i>Myrmecocichla aethiops</i>	7	56.6	164.0	221.6	A	2	71	68	75	HN	manual	375



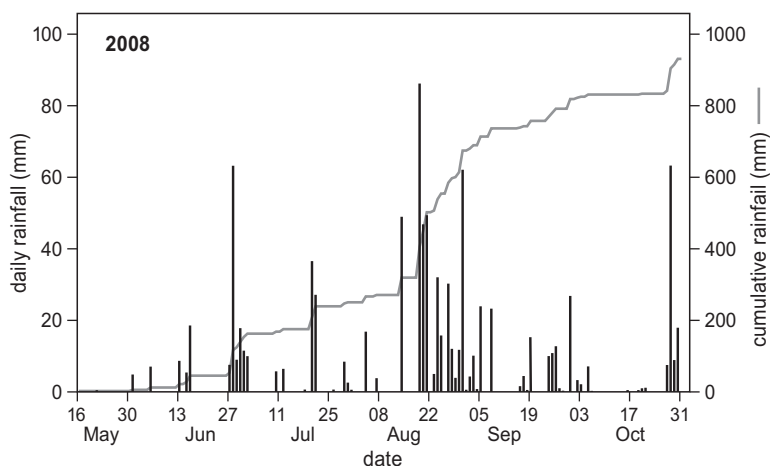
Scientific name	N	Body Mass	FMR	corr. FMR	Origin	Detect.	ESW (m)			Details of model applied		
							avg.	95% conf. int.		Type	Interval	Truncation (m)
								min.	max.			
<i>Neotis denhami</i>	1	4120.0	3040.4	4108.7	A	3	243	227	261	HR	manual	1175
<i>Oenanthe oenanthe</i>	111	23.2	89.3	120.7	P	ind.	54	47	62	HN	automatic	125
<i>Oenanthe</i> sp.		23.2	89.3	120.7	P	ind.	54	47	62			
<i>Passer griseus</i>	243	28.1	101.8	137.6	A	ind.	35	30	40	HR	automatic	-
<i>Ploceus cucullatus</i>	420	37.5	123.9	167.4	A	ind.	38	34	42			
<i>Ploceus</i> sp.		37.5	123.9	167.4	A	ind.	38	34	42	HR	manual	250
<i>Ploceus velatus</i>		24.2	92.0	124.3	A	ind.	38	34	42			
<i>Prinia subflava</i>	54	9.0	46.9	63.4	A	1	36	34	37	HR	manual	105
<i>Spreo pulcher</i>	34	64.2	178.7	241.5	A	ind.	61	47	79	HN	manual	170
<i>Thagra senegala</i>	88	53.9	158.6	214.4	A	ind.	53	38	74	HR	automatic	-
<i>Tockus erythrorhynchus</i>	65	146.0	312.7	422.6	A	ind.	87	68	112	HR	automatic	-
<i>Tockus nasutus</i>	106	208.0	397.9	537.7	A	ind.	36	23	57	HR	manual	800
<i>Turnix sylvatica</i>	4	23.2	89.3	120.7	A	1	36	34	37	HR	manual	105
<i>Upupa epops</i>	16	51.9	154.6	208.9	P	2	71	68	75	HN	manual	375
<i>Vanellus rectus</i>	417	112.5	261.8	353.8	A	ind.	67	59	75	HR	automatic	-
<b>Explanations</b> N Number of observations (not necessarily number of birds; some species occur in groups) Body mass Fresh Body Mass (g) (cf. Dunning 1993). FMR FMR in kJ/day = $10.5 \times \text{body mass (g)}^{0.681}$ (according to Nagy 2005). corr_FMR FMR corrected for digestibility (= (FMR/74) $\times$ 100)) (According to Petersen et al. 2006) origin A = Afrotropical, P = Palaearctic detect. Detectability: Ind. = individual ESW could be calculated. 1 = species with low detectability; 2 = species with medium detectability; 3 = species with high detectability (see text for details). ESW Effective Strip Width (Buckland et al. 1993, Thomas et al. 2006), see under Statistics. energy content grasshopper 22 kJ/g DW (Petersen et al. 2006) % water in grasshopper 71.6 (Petersen et al. 2006). Type: HR = Hazard Rate model, HN = Half Normal with Cosine Expansion model.												

## Results

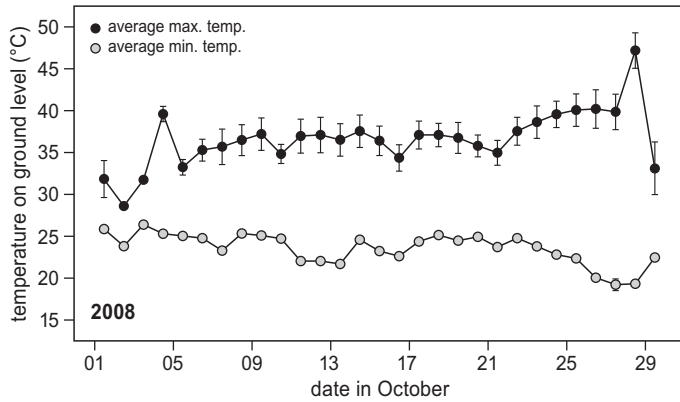
Detailed information on the quality and efficacy of the treatments, droplet deposition, germination and persistence of conidia of *M. acridum* and of meteorological conditions during treatments is given elsewhere (Mullié & Guèye 2009) and will be summarized here. Germination of spores just prior to the first treatments was 93.6 ( $\pm 1.8$ ) % ( $n = 14$ ) in the 500 g l<sup>-1</sup> formulation and 92.4 ( $\pm 1.5$ )% ( $n = 17$ ) in the 250 g l<sup>-1</sup> formulation (SenBiotech in litt.). Droplet distribution was regular and similar on all treated plots, with an average of 13 droplets cm<sup>-2</sup>. Efficacy of treatments, corrected according to Henderson-Tilton (1955) for grasshopper counts on control plots, was 83.8–87.1%. Median Lethal Times (MLT) were 8.24 (95% CL 5.97–10.07) and 8.19 (95% CL 6.58–9.74) days and LT<sub>80</sub> values were 15.85 (95% CL 11.99–28.00) and 13.06 (95% CL 10.93–17.11) days respectively for 25 and 50 g ha<sup>-1</sup>, with no differences between the two dose rates. The maximum effect of GM was between 6 and 12 d postspray. Mortality of >90% due to *Metarhizium* infection (confirmed by sporulation) of untreated grasshoppers placed in persistence cages on treated plots for 72 h, showed that *M. acridum* activity persisted for at least 18 d post-spray.

### Meteorological conditions

Daily and cumulative rainfall during the 2008 rainy season are given in Fig. 4.2. Minimum and maximum daily temperatures at ground level during October are given in Fig. 4.3. The 2008 rainy season at Khelcom was characterized by extremely wet conditions and a cumulative rainfall of 935 mm, which is well above normal and approximately twice the annual amount of the past 20 y in the same area. No rains were recorded during treatments and the following period of intensive monitoring. The interval of minimum and maximum daily temperatures during treatments and thereafter was favorable for a rapid develop-



**Figure 4.2.** Daily and cumulative rainfall (mm) during the 2008 rainy season at Touba Khelcom.



**Figure 4.3.** Average daily minimum and maximum temperatures ( $n = 7$ ) at ground level in study plots at Khelcom in October 2008.

ment of *M. acridum*, as the optimum ambient temperature range for the pathogen development is between 24 and 38°C (C. Kooyman, FAES Dakar, pers. comm.).

### Floristic composition

Average shrub density in our plots was 17.9 shrubs  $\text{ha}^{-1}$  and consisted of *G. senegalensis*, *C. glutinosum*, *Balanites aegyptica*, *Callotropis procera*, *Cassia occidentalis* and *Bauhinia rufescens*. Tree density was 1.3 trees  $\text{ha}^{-1}$ , the commonest tree being *C. glutinosum*. Locally stands of Baobabs, *Adansonia digitata*, had been saved to provide shade for man and livestock. More rarely *Tamarindus indica*, *Piliostigma reticulatum*, *Mitragyna inermis* (in temporary flooded depressions), *Acacia* (*Faidherbia*) *albida* or *Sterculia setigera* were still present. The few remaining trees are heavily exploited for firewood and browse.

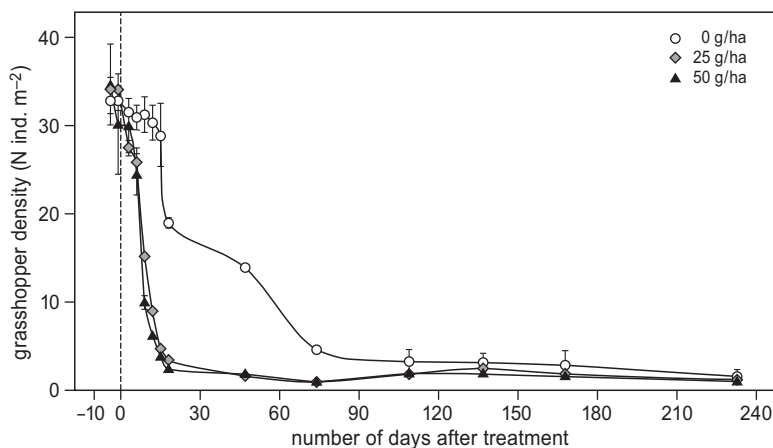
The herbaceous layer was mainly composed of Gramineae, such as *Andropogon* sp., *Cenchrus biflorus* ('Cram cram'), *C. tenium elegans*, *Eragrostis tremula*, *E. tenella* and *Digitaria* sp. Adventives such as *Mitracarpus villosus* and *Spermacoce* (*Borreria*) *radiata* dominated on sites which had been cultivated in recent years.

### Wildfires

In November 6046 ha of Khelcom was burnt, whereas throughout the dry season, but in particular during April-May, an additional 1307 ha fell prey to wildfires, making a total of 13.4 % of Khelcom being burnt during the study. Some of our plots were partially affected by the fires. Based on our monitoring, this concerned 5.3% of the transects in November, 13.2% from December till March and 18.0% in May.

### Grasshopper densities

Immediately prior to treatments, average densities of grasshoppers (all stages confounded) were between 30 and 35 ind.  $\text{m}^{-2}$  in all plots (Fig. 4.4). At the end of the second decade of September (not shown in Fig. 4.4), three weeks prior to treatments, average



**Figure 4.4.** Average density of grasshoppers (ind. m<sup>-2</sup>) per treatment in time. Bars indicate Standard Errors.

densities of 90 ind. m<sup>-2</sup> were even recorded. Densities remained at prespray levels in the control plots until 15 d postspray, after which date they gradually decreased due to natural factors related to the end of the rainy season.

Starting from day 6 after treatment, grasshopper densities in both the 25 and 50 g ha<sup>-1</sup> treated plots decreased rapidly as a result of exposure to *M. acridum* conidia, which was confirmed by sporulation of *M. acridum* on caged individuals.

Grasshopper numbers remained low in treated plots and were statistically different from densities in control plots until 74 d after treatment. Only from 109 d postspray onwards (January), densities on all plots were no longer different from each other (Fig. 4.4).

### Grasshopper community structure

In total, 79,480 grasshoppers were captured and identified. The grasshopper community consisted of at least 32 species. In addition, 5 taxa could only be identified to the genus level and at least one or more species remained unidentified. The latter category was only 0.3 % of the total number captured. For 31 species and seven stages (adult and 6 larval stages) fresh body mass (WW,  $n = 1731$ ) and dry body mass (DW,  $n = 908$ ) were taken (Appendix 1). For species and stages for which only fresh body masses were available, these were multiplied by 0.3 to obtain DW values (based on Appendix 1;  $n = 908$ , ratio DW/WW = 0.295). For two rarely captured species lacking field information on body mass (*Aiolopus simulatrix* and *Homoxyrhopes punctipennis*), a regression analysis was obtained from body length vs DW of the captured specimens and the regression equation applied to body-length data from the literature (Mestre 1988), to calculate corresponding DW (imagos only).

On the basis of numbers captured, the Senegalese grasshopper *Oedaleus senegalensis* (producing diapausing eggs at the end of the rainy season) dominated during the rainy

season (58–66%), and was absent from February onwards. Species with diapausing adults or continuous reproduction were present during the entire period (Fig. 4.5). Numerically the most important species were *Acorypha clara*, *Acrotylus blondeli*, *Ornithacris cavroisi*, *Pyrgomorpha cognata* complex and *P. vignaudi*. These species, together with *O. senegalensis*, comprised 75–85 % of grasshopper numbers in the community at any moment.

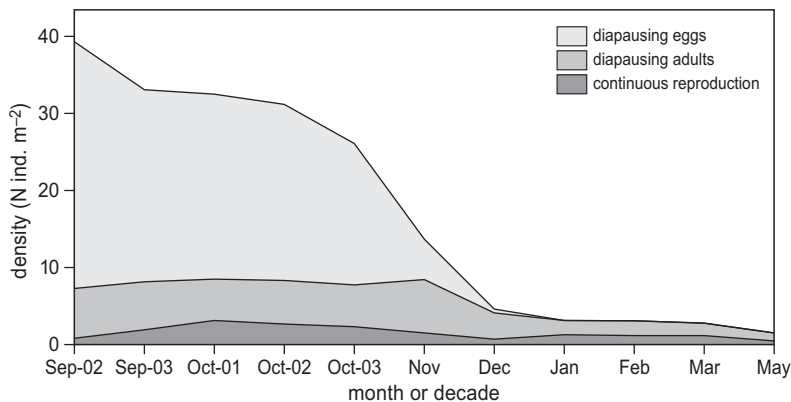
On the basis of body mass, the situation is slightly different. *O. senegalensis* dominated during the rainy season (47%), followed by *O. cavroisi* (14%), *Acorypha glaucoptis* (9%) and *A. clara* (7%). These four species represented 77% of the grasshopper community. During the dry season, from December onwards *O. cavroisi* (53%) *A. clara* (23%) and *Diaabolocatantops axillaris* (6%), represented 82% of the grasshopper community by biomass. From December onwards Catantopinae (*D. axillaris*, *Harpezocatantops stylifer*, *Catantops stramineus*, *Cryptocatantops haemorrhoidalis*) and Pyrgomorphidae replaced species laying diapausing eggs (*O. senegalensis*, *A. glaucoptis*). In Fig. 4.6, the grasshopper biomass in time in kg DW km<sup>-2</sup> is given.

### Birds

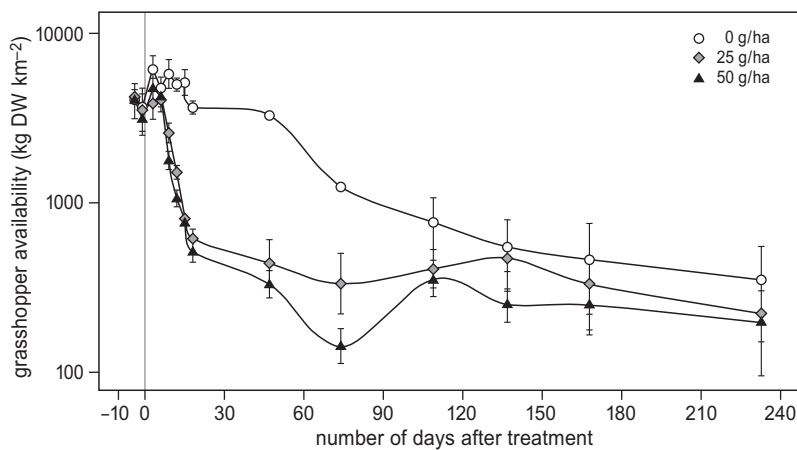
During transect counts, 83 bird species were identified. Of these, 42 were classified as acridivorous, at least during a part of the time (Table 4.1). In Table 4.1, details are also given on the various parameters used for calculating densities. Tables with detailed count results per plot and per species can be found in Mullié & Guèye (2009) and are summarized in Appendix 2.

The bird community consisted of a mix of Palaearctic-African (all migratory) and afro-tropical (both migratory and resident) species. Consequently, during the course of the study some species arrived, while others left our study area.

Commensalistic feeding associations were common and contributed to an efficient removal of grasshoppers from plots. Species implicated were White-billed Buffalo Weaver, White-throated Bee-eater *Merops albicollis*, White Stork, Lesser Kestrel, Cattle Egret *Bubulcus ibis* (Fig. 4.7) and Abyssinian Roller.



**Figure 4.5.** Densities (ind. m<sup>-2</sup>) over time of the grasshopper community according to life history strategy.



**Figure 4.6.** Average grasshopper biomass (kg DW km<sup>-2</sup>) over time per the three treatments. Bars indicate Standard Errors.

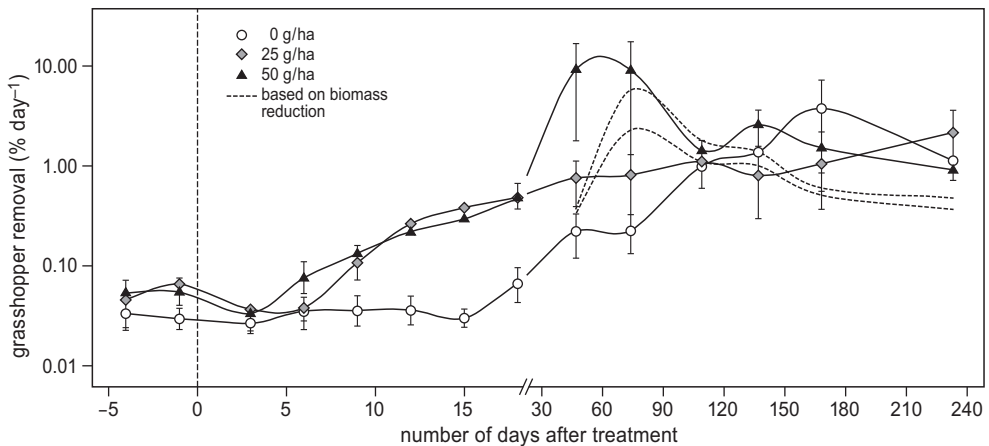


**Figure 4.7.** Cattle Egret with *Acorypha clara* as prey. Cattle Egrets, *Bubulcus ibis*, often in commensalistic feeding associations with Lesser Kestrels, exploited medium-sized grasshoppers in green patches of the liana *Leptadenia hastata*.

The results from the repeated measures ANOVA applied to acridivorous bird species on transects, showed a significant time effect ( $F = 8.582$ ,  $df = 4.562$  (Huynh-Feldt correction),  $P < 0.001$ ) but independent of treatment (N.S.): bird numbers gradually increased over time on all plots, with no differences between treatments (Mulli   & Gu  ye 2009).

Although bird numbers were not influenced by treatments, absolute grasshopper consumption by birds increased on all plots, whereas daily removal rates increased on treated plots only. From day -4 until day 6, treated and control plots were not different. Starting from day 9 until day 18, daily removal of the available grasshopper biomass on treated plots increased tenfold from 0.04–0.08% to 0.48%, whereas grasshopper consumption on control plots remained at a low 0.03–0.04% daily removal in the same period (Fig. 4.8).

The increase of grasshopper removal was due to a combination of two factors. Firstly, and most importantly, GM treatments caused a strong reduction of grasshopper densities,



**Figure 4.8.** Average percentage of the grasshopper community (by weight) removed per day per treatment by acridivorous birds. Bars indicate Standard Errors. The dashed lines indicate minimum and maximum biomass reduction, based on the difference in availability on successive monitoring dates. The time scale changes in the middle of the x-axis. See text for further explanation.

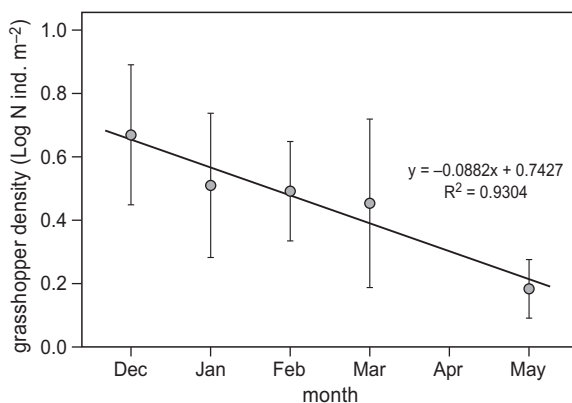
while densities of acridivorous birds did not significantly change. Secondly, although acridivorous bird densities remained in the same order of magnitude, the species composition changed due to migration, with generally heavier migrant species such as Montagu's and Marsh Harriers (*C. aeruginosus*) arriving and smaller species such as White-throated Bee-eaters and Woodland Kingfishers (*Halcyon senegalensis*) leaving (Appendix 2).

From 18 till 74 d post-treatment, treated and control plots remained significantly different. In this period both removal rates and consumption continue to increase, as more and heavier bird species arrived, White Storks in particular. From January (day 109) onwards, grasshopper removal rates level off at  $1.58 (\pm 0.25)\%$  daily removal and are no longer different between treatments.

To verify the validity of our approach, we also calculated grasshopper removal from monthly decreases in densities on control plots from December onwards (interval between dashed lines in Fig. 4.8). Control plots were taken to avoid confounding factors such as a prolonged mortality due to persistence of viable *M. acridum* conidia. The results are very much in agreement with the calculations based on energetic requirements, although the grasshopper removal rate is slightly lower at 1.07 ( $\pm 0.63$ )%.

As the community structure data did not provide any evidence for major grasshopper immigration to or emigration from the plots from December onwards, it was assumed that population declines on plots in this period were mainly resulting from predation. The daily population decline, as calculated by regression analysis on Log  $n$  ind.  $m^{-2}$  ( $y$ ) in time ( $x$ ): ( $y = -0.0882x + 0.7427$ ,  $r^2 = 0.9304$ ; ANOVA:  $df = 524$ ,  $F = 7.097$ ,  $P = 0.008$ ; Fig. 4.9) showed that the rate of decrease of grasshopper densities between December and May was constant. In December, average densities had fallen to below 5 ind.  $m^{-2}$ .

The composition of the bird community changed considerably during the course of the study. During the rainy season it consisted of about 90% acridivores, dropping to c. 10% in



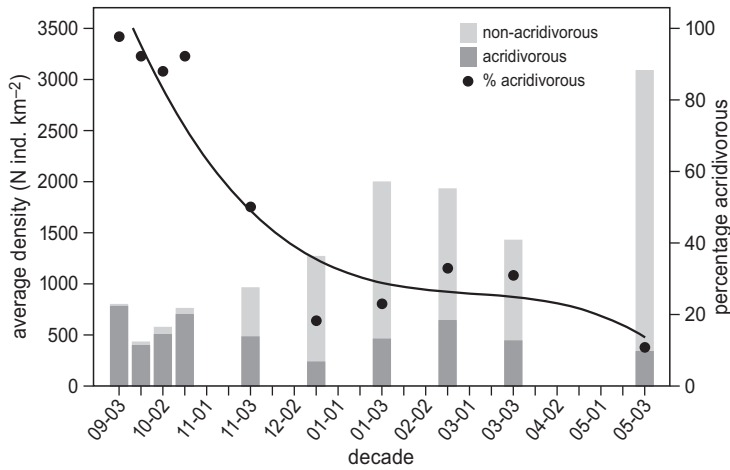
**Figure 4.9.** Decrease in grasshopper densities (Log  $N$  ind.  $m^{-2}$ ) on control plots between December and May. Bars indicate Standard Errors.

May (Fig. 4.10). However, this change was not caused by decreasing densities of acridivorous birds ((ANOVA;  $df = 9$ ,  $F = 1.671$ , N.S.), but by a very strong increase of granivorous species during the dry season, most notably Golden Sparrows (ANOVA;  $df = 9$ ,  $F = 48.456$ ,  $P < 0.001$ ).

### Pellet contents

Based on numbers of prey items recovered from Montagu's Harrier pellets in the period January to March, grasshoppers constitute 87.4–91.0% of the diet, whereas on the basis of biomass, they constitute 51.0–61.0% (Mullié & Koks unpub. data). In Table 4.2, recovered grasshopper species are given from high to low body mass. The harriers do not take grasshoppers proportionally to their occurrence ( $\chi^2$  (13,  $n = 1623$ ) = 3701.04,  $P < 0.0001$ ).





**Figure 4.10.** Average bird densities (ind. km<sup>-2</sup>) in time according to diet.

Common species (*i.e.*, > c. 5% of the community) with a body mass > 0.73 g are taken significantly more often than smaller species. The latter represent only 1.4–2.6% of all grasshoppers being taken by the harriers, whereas they represent 61–68% of the grasshopper community in the field. The three preferred prey species in order of importance are *Acorypha clara* (57–65%), *Ornithacris cavroisi* (23–26%) and *Diabolocatantops axillaris* (9–15%).

## Discussion

The 2008 rainy season at Khelcom was characterized by exceptionally abundant rainfall (935 mm) and very high grasshopper densities (up to 90 ind. m<sup>-2</sup> in September, 30–35 in October) showing a low mobility. This low mobility can be explained by the presence of a well-developed herbaceous layer, due to abundant rainfall and by the absence of favorable winds at the right moment for the second generation of the Senegalese Grasshopper to migrate north (J. Bak, National Environmental Research Institute, DMU, Denmark, pers. comm.). This resulted in two subsequent generations of *O. senegalensis* reproducing at Khelcom, leading to very high densities. The treatments with GM were executed against a population of *O. senegalensis* of which adults predominantly belonged to the second generation, and nymphs to the third generation.

The composition of the grasshopper community, consisting of 32+ species, was dominated numerically by the Senegalese grasshopper (58–66%) before treatments and during the intensive monitoring period at the end of the rainy season, but in terms of both numbers and biomass, species with diapausing adults such as *O. cavroisi* and *A. clara*, were common throughout the entire study period (Fig. 4.5). Their continuous presence is likely the main reason that they form an important part of the diets of many acridivorous birds

(Mulli   2009). Nymphs were the dominant life-form until mid-October. Starting from December onwards, only nymphs of continuously reproducing species were present.

In recent years Khelcom has become an important reproduction area for an array of grasshopper species, some of which are important pests to rain-fed agriculture. Until 1991, Khelcom was part of the 73,000-ha protected Mb  gu   Sylvo-Pastoral Reserve, of which 55,400 ha were gradually cleared until 2004 to make way for groundnut production. Grasshopper problems were unknown from the area before 1991 (data from Crop Protection Directorate). The resulting mosaic of cropped areas (not exceeding 12.5% of the surface area during the study), fallow and cleared land and partially regenerating former forest, has become an ideal habitat for grasshopper development. This in turn has attracted acridivorous bird species, such as Montagu's Harrier, characteristic for areas

**Table 4.2.** Species composition of grasshoppers captured in the field by sweep-net sampling and found as prey remains in regurgitated pellets of Montagu's Harriers, collected in night roosts, at Khelcom between January and March 2009.

Scientific Name	Wet Weight (g)	January		February		March	
		Field n=2655	M. Harrier n=1628	Field n=3037	M. Harrier n=971	Field n=3232	M. Harrier n=1632
<i>Acanthacris ruficornis citrina</i>	3.50	-	-	-	-	0.09	0.06
<i>Hieroglyphus daganensis</i>	3.41	-	-	-	0.10	-	-
<i>Ornithacris cavroisi</i>	2.66	15.07	25.55	10.41	25.75	9.13	22.61
<i>Truxalis johnstoni</i>	1.57	0.26	-	-	-	-	-
<i>Truxalis</i> sp.	1.57	-	0.06	-	-	-	0.18
<i>Acridoderes strenuus</i>	1.07	-	-	-	0.10	-	0.31
<i>Acrida bicolor</i>	0.91	0.45	-	-	-	-	-
<i>Acrida</i> sp.	0.91	0.26	-	-	-	-	-
<i>Acorypha clara</i>	0.90	18.57	61.18	14.55	57.16	15.16	65.32
<i>Heteracris annulosa</i>	0.77	-	-	-	-	2.20	-
<i>Diabolocatantops axillaris</i>	0.73	4.60	11.55	7.08	15.45	5.85	8.88
<i>Metaxymecus gracilipes</i>	0.64	0.45	-	0.82	-	-	-
<i>Acorypha picta</i>	0.57	5.46	1.66	7.41	1.34	6.13	2.27
<i>Oedaleus senegalensis</i>	0.53	0.23	-	-	-	-	-
<i>Catantops stramineus</i>	0.50	1.13	-	0.86	-	1.58	-
<i>Harpezocatantops stylifer</i>	0.30	0.56	-	1.98	-	3.96	-
<i>Chrotogonus senegalensis</i>	0.29	1.51	-	2.54	-	0.46	-
<i>Cryptocatantops haemorrhoidalis</i>	0.23	0.11	-	3.79	0.10	3.47	0.31
<i>Pyrgomorpha vignaudii</i>	0.19	19.47	-	17.42	-	20.20	0.06
<i>Zacompsa festa</i>	0.18	-	-	-	-	-	-
<i>Pyrgomorpha cognata</i> complex	0.09	19.66	-	21.24	-	21.60	-
<i>Acrotylus blondeli</i>	0.07	11.19	-	11.43	-	10.18	-
Unidentified species		1.02	-	0.49	-	-	-
<b>Total</b>		100	100	100	100	100	100

rather low in the succession cycle. Numbers of Montagu's Harriers that use Khelcom either for foraging or as a night roost are maximally 5000–6000 individuals (Mulli   & Gu  ye 2009). These numbers are unprecedented elsewhere and are among the highest recorded anywhere in the world (Clarke 1996). They constitute about 16% of the population of about 37,000 individuals estimated to migrate via Spain to the African mainland (B. Koks, SWGK, The Netherlands, *pers. comm.*) and 2% of the entire world population estimated at 300,000 individuals (C. Trierweiler, Groningen University, The Netherlands, *in litt.*).

Some of the other acridivorous species reach very high densities, rarely found in such concentrations elsewhere in the Sahel, e.g., White Stork (3,500 ind.; 1.75% of the flyway population) and Lesser Kestrel (5,000; 10%) (Mulli   & Gu  ye 2009). All these species exceed the 1% criterion for areas of international ornithological importance (Ramsar Convention-COP9 2005; [www.ramsar.org/key\\_criteria](http://www.ramsar.org/key_criteria)) and are protected under the Bonn Convention of which Senegal is signatory (CMS-COP9 2008).

The consistent data of the temporal composition of the structure of the community supports the hypothesis that grasshopper movements during the entire study period were rather limited and that continuously decreasing densities between December and May were largely caused by predation. They logarithmically decreased (all species combined) from 4.66 ind. m<sup>-2</sup> in December to 1.51 ind. m<sup>-2</sup> in May, or a reduction of 67.2%. A constant decrease of grasshoppers due to predation started at densities below c. 5 ind. m<sup>-2</sup>, which can be considered as a medium density. Total grasshopper biomass was reduced from 1,256 kg DW km<sup>-2</sup> in December to 352 kg DW km<sup>-2</sup> in May, a reduction of 71.9%. Some local displacements might have occurred as dead grasshoppers, in particular *Diabolocatanotops axillaris*, showing sporulation of *M. acridum*, were found up to 12 km from the nearest treated plots in October and November (Mulli   & Gu  ye 2009), and also the small increase of densities on treated plots between December and January may have been the result of either local redistribution of grasshoppers or of small-scale immigration.

An important finding of this study is that GM did not have an impact on bird numbers and densities; these changed significantly over time, but were unrelated to treatments. [Treatments of grasshoppers with the organophosphates (OPs) chlorpyrifos and fenitrothion in Senegal were shown to have large and significant negative effects on bird displacements, apart from direct mortality and reproduction effects (Mulli   & Keith 1993).]

While numbers of acridivorous bird species did not change, their biomass did when heavier migrant species arrived. This increased grasshopper removal rates on all plots, but more steeply on treated plots, because grasshopper densities had already been affected by GM. It is only at 3.5 mo postspray that grasshopper removal rates level off and are no longer different from controls. Hence by increasing the duration of the GM impact (Fig. 4.8), bird predation enhanced its action.

When taking body size into account, we observed that large (*O. cavroisi*) and medium-bodied (*A. clara*) species significantly declined, whereas small-bodied species (Pyrgomorphidae) first increased adult population levels, to decline only during the last count. Grasshopper remains from Montagu's Harrier pellets show the same tendency: small bodied grasshoppers (<0.73 g) represented < 2.6% of the prey by body mass, while they

represented 61–68% of the grasshopper community in the field. Montagu's Harriers preyed preferentially on *A. clara*, *O. cavroisi* and other medium bodied species such as *D. axillaris* [Lesser Kestrels also prey preferentially on *O. cavroisi* (Mulli   2009).]

Our data also confirm earlier findings of Branson (2005b) who reported that birds reduced the proportion of medium-bodied grasshoppers, while small-bodied grasshoppers increased in abundance. Belovsky & Slade (1993) found a predation-mediated reduction of large-bodied grasshoppers, whereas changes in abundance of medium and small-bodied species that they observed could not be explained by predation.

There was a large difference between grasshopper removal during the rainy season and during the dry season. Because most grasshoppers reproduced between September and November, predation by birds could not be calculated in the same way as during the dry season. Therefore, cumulative daily predation rates were integrated over time (September–November) to obtain an estimate of the total predation during this period. As vegetation development started at the onset of the first significant rains (i.e., > c. 20 mm), which was in mid-June (Fig. 4.2) and grasshopper reproduction soon afterwards, our data do not cover the entire reproductive period and the importance of predation may have been underestimated. Nevertheless, predation did not remove more than 1% from the community, which can be considered as insignificant. This is in contrast with modeling results presented by Axelsen *et al.* (2009) that birds reduce *O. senegalensis* populations in Senegal and Niger during the rainy season by 20–25%. It should be mentioned that bird densities in their study have been corrected for breeding females and for nestlings as well as for reduced reproductive output of the grasshopper population, but this alone does not explain the large difference.

Data from North American studies are much more variable. Predation rates of 30–50% have been reported at low and medium grasshopper densities (Joern 1986, 1992; Fowler *et al.* 1991; Bock *et al.* 1992). However, in some cases no measurable effect of predation was present (Joern 2000). Our data are corroborated by those of Branson (2005) who states that bird predation becomes less important at high grasshopper densities, which was also the case during the rainy season in our study. However, when grasshopper densities decreased to medium levels (c. 5 ind. m<sup>-2</sup>), bird predation became very important.

The removal of grasshoppers by birds was calculated under some broad assumptions about diet composition (maximum 50% of the diet of acridivorous species supposed to be composed of grasshoppers) and temporal aspects of predation (granivorous species which were known to feed on acridids during their breeding season were supposed not to do so from December onwards). As compared to calculations derived from grasshopper densities in successive months, calculations from bird consumption produced very similar results, supporting the idea that under conditions of less stable grasshopper populations, bird consumption of grasshoppers can be approximated by a few rough assumptions about predation rates, if the densities of acridivorous species have been assessed. The peak in consumption in November and December (days 47–74) in Fig. 4.8 is due to the presence of large concentrations of White Storks on some of the plots treated with 50 g conidia ha<sup>-1</sup>, which has a strong influence on calculated biomass removal. The shape of the curves

of grasshopper removal calculated from monthly biomass reduction (dashed lines in Fig. 4.8) strongly suggests that White Storks had also been present on control plots, but were not seen during the monthly count.

Commensalism maybe an advantageous strategy for feeding on orthopterans in particular, in dense vegetation and at high grasshopper densities. In addition to our observations, Mullié *et al.* (Chapter 5 of this thesis) observed lanners, *Falco biarmicus*, exploiting Desert Locust by following men and camels in dense *Schouwia thebaica* vegetation in Northern Niger and stooping on locusts being flushed. Jensen *et al.* (2008) very frequently observed lanners exploiting grasshoppers (probably *O. senegalensis*) flushed by Abdim's Storks, *Ciconia abdimii*, in SE Niger. In Waza National Park (Extreme North of Cameroon), Ralph Buij (*in litt.*) observed Yellow-billed Kites, *Milvus parasitus*, doing the same when people flushed grasshoppers in high grass. He also observed Northern Carmine Bee-eaters, *Merops nubicus*, with goats (they also travel on goatbacks) and Arabian Bustards, *Ardeotis arbabs*, and Piapiacs, *Ptilostomus afer*, with small livestock, and Abyssinian Rollers with Abdim's Storks at the start of the rains. Only once two adult Montagu's Harriers were observed flying amongst a herd of over 200 cattle catching flushed grasshoppers (Ralph Buij, *in litt.*).

Deforestation for expansion of groundnut production destroyed the formerly protected forest at Khelcom, reputedly with a high biodiversity (Schoonmaker-Freudenberger 1991). In turn it also created a habitat low in the succession cycle and rich in orthopterans and their predators. Some of them, such as the Montagu's Harrier, are so abundant that their numbers are unprecedented anywhere else in the world. The semi-arid Sahelian agricultural habitat is currently under a severe human pressure (Zwarts *et al.* 2009) and avian predators in the Sahel have faced a decline of over 90% in the last 30 years (Thiollay 2006a, b). Biopesticides and predators can play a vital role in controlling grasshoppers considered to be a pest to agriculture without compromising agro-ecosystem functioning. Therefore their complementary role should be exploited instead of neglected.

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## Appendices

**Table A1.** Fresh (WW) and dry body mass (DW) of grasshoppers captured on plots and used for calculations of grasshopper biomass.

Species	imago				L1				L2			
	N	WW	N	DW	N	WW	N	DW	N	WW	N	DW
<i>Atractomorpha acutipennis</i>	2	0.184	-	-	-	-	-	-	-	-	-	-
<i>Acrida bicolor</i>	32	0.912	20	0.307	1	0.005	1	0.001	8	0.042	2	0.006
<i>Acrotylus blondeli</i>	8	0.072	5	0.041	-	-	-	-	-	-	-	-
<i>Acorypha clara</i>	176	0.889	172	0.386	34	0.022	19	0.008	65	0.062	31	0.018
<i>Acorypha glaucopsis</i>	59	1.518	25	0.270	4	0.036	4	0.011	11	0.123	8	0.020
<i>Acorypha picta</i>	25	0.568	-	-	-	-	-	-	-	-	-	-
<i>Acanthacris ruficornis citrina</i>	-	-	-	-	-	-	-	-	2	0.707	-	-
<i>Acridoderes strenuus</i>	9	1.120	2	0.245	-	-	-	-	-	-	-	-
<i>Acrida sulphuripennis</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cataloipus cymbiferus</i>	5	4.356	-	-	-	-	-	-	-	-	-	-
<i>Cataloipus fuscocoeruleipes</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cryptocatantops haemorrhoidalis</i>	50	0.214	32	0.085	-	-	-	-	-	-	-	-
<i>Chrotogonus senegalensis</i>	16	0.289	9	0.112	-	-	-	-	4	0.041	2	0.008
<i>Catantops stramineus</i>	8	0.505	-	-	-	-	-	-	-	-	-	-
<i>Diabolocatantops axillaris</i>	54	0.729	10	0.288	-	-	-	-	-	-	-	-
<i>Duronia chloronota</i>	2	0.155	2	0.050	-	-	-	-	-	-	-	-
<i>Heteracris annulosa</i>	20	0.773	18	0.352	-	-	-	-	-	-	-	-
<i>Hieroglyphus daganensis</i>	3	3.407	1	1.429	-	-	-	-	2	0.084	2	0.025
<i>Harpezoatantops stylifer</i>	7	0.226	7	0.114	-	-	-	-	-	-	-	-
<i>Humbe tenuicornis</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Kraussella amabile</i>	46	0.426	7	0.068	-	-	-	-	7	0.060	1	0.030
<i>Kraussaria angulifera</i>	16	3.073	1	0.638	-	-	-	-	2	0.587	-	-
<i>Morphacris fasciata</i>	1	0.153	1	0.052	-	-	-	-	-	-	-	-
<i>Metaxymecus gracilipes</i>	2	0.422	-	-	-	-	-	-	-	-	-	-
<i>Ornithacris cavroisi</i>	175	2.661	163	1.177	25	0.029	16	0.010	40	0.071	25	0.025
<i>Oedaleus nigeriensis</i>	5	0.598	5	0.230	-	-	-	-	-	-	-	-
<i>Oedaleus senegalensis</i>	153	0.534	25	0.128	44	0.018	20	0.006	51	0.055	30	0.015
<i>Pyrgomorpha cognata complex</i>	26	0.088	26	0.041	2	0.007	2	0.002	2	0.016	2	0.005
<i>Pyrgomorpha vigneaudii</i>	12	0.195	2	0.047	2	0.006	2	0.002	2	0.010	2	0.003
<i>Truxalis longicornis</i>	2	1.568	-	-	-	-	-	-	-	-	-	-
<i>Truxalis sp.</i>	1	0.317	1	0.094	-	-	-	-	-	-	-	-
<i>Zacompsa festa</i>	9	0.181	2	0.034	-	-	-	-	-	-	-	-
<b>N total WW</b>	<b>924</b>				<b>112</b>				<b>196</b>			
<b>N total DW</b>			<b>536</b>				<b>64</b>				<b>105</b>	

L3				L4				L5				L6				N WW Total
N	WW	N	DW	N	WW	N	DW	N	WW	N	DW	N	WW	N	DW	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
2	0.092	2	0.027	8	0.343	1	0.038	5	0.808	2	0.283	-	-	-	-	56
-	-	-	-	1	0.034	1	0.010	-	-	-	-	-	-	-	-	9
40	0.193	18	0.038	32	0.383	7	0.072	15	0.638	3	0.181	-	-	-	-	362
9	0.265	5	0.076	15	0.722	4	0.170	6	1.064	6	0.339	-	-	-	-	104
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25
-	-	-	-	8	1.509	-	-	1	1.161	-	-	-	-	-	-	11
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9
-	-	-	-	-	-	-	-	1	0.631	1	0.170	-	-	-	-	1
5	0.078	2	0.045	1	0.046	-	-	1	0.505	1	0.148	-	-	-	-	12
-	-	-	-	2	0.473	2	0.158	1	0.236	1	0.068	-	-	-	-	3
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	50
7	0.067	4	0.024	9	0.127	7	0.037	6	0.099	6	0.035	-	-	-	-	42
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	54
2	0.105	2	0.035	2	0.159	2	0.048	1	0.205	1	0.063	-	-	-	-	7
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20
1	0.108	1	0.030	7	1.316	-	-	-	-	-	-	-	-	-	-	13
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
2	0.123	1	0.050	1	0.120	1	0.040	-	-	-	-	-	-	-	-	3
27	0.121	9	0.055	14	0.148	5	0.079	3	0.315	3	0.100	-	-	-	-	97
2	0.938	-	-	4	1.430	-	-	2	1.711	-	-	-	-	-	-	26
-	-	-	-	-	-	-	-	1	0.207	1	0.069	-	-	-	-	2
1	0.334	-	-	11	0.490	-	-	2	0.478	-	-	-	-	-	-	16
47	0.228	26	0.064	35	0.501	16	0.136	23	1.304	12	0.237	10	1.487	4	0.314	355
3	0.078	3	0.021	-	-	-	-	-	-	-	-	-	-	-	-	8
53	0.134	18	0.050	39	0.223	10	0.086	21	0.389	6	0.141	-	-	-	-	361
2	0.028	2	0.008	2	0.040	2	0.010	1	0.044	1	0.010	-	-	-	-	35
1	0.020	1	0.005	-	-	-	-	-	-	-	-	-	-	-	-	17
-	-	-	-	1	1.188	-	-	-	-	-	-	-	-	-	-	3
1	0.071	1	0.024	-	-	-	-	-	-	-	-	-	-	-	-	2
2	0.062	2	0.017	-	-	-	-	-	-	-	-	-	-	-	-	11
207				192				90				10				1731
		97				58				44				4		908

**Table A2.** Number of birds observed per species and per count from 18 d before until 233 d after treatment. Acridivorous and non acridivorous species are given separately.

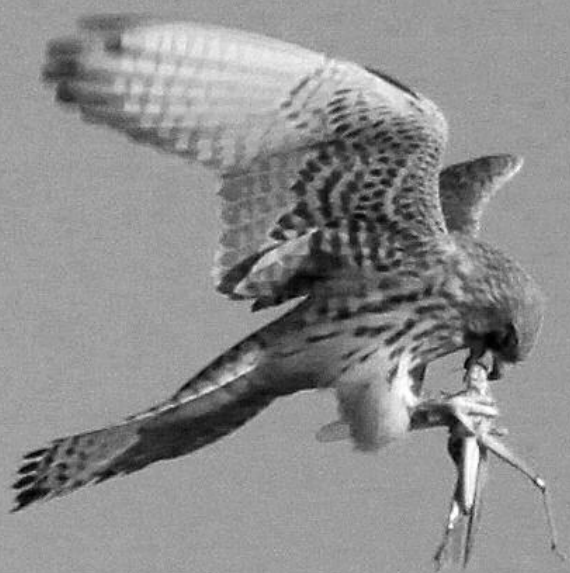
Scientific name	Number of days after treatment																Total
	-18	-14	-4	-1	3	6	9	12	15	18	47	74	109	137	167	233	
<b>Non acridivorous</b>																	
<i>Anthus</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	6	2	-	8
<i>Apus affinis</i>	-	-	-	-	-	-	-	-	-	-	-	12	11	10	-	-	33
<i>Apus apus</i>	1	-	15	1	-	-	-	-	-	-	-	-	-	-	-	-	17
<i>Aquila rapax</i>	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
<i>Ardea cinerea</i>	-	4	-	-	-	-	1	-	2	4	3	-	1	-	-	-	15
<i>Asio flammeus</i>	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	4
<i>Bubalornis albirostris</i>	-	-	-	-	-	-	-	-	-	-	288	116	489	81	212	56	1242
<i>Buphagus africanus</i>	-	-	-	-	-	3	7	9	12	12	10	13	17	3	7	17	110
<i>Calandrella brachydactyla</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	10	-	2	12
<i>Camaroptera brachyura</i>	-	1	-	1	-	-	-	-	-	-	-	-	-	-	1	1	4
<i>Cercotrichas podobe</i>	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	8	9
<i>Ciconia nigra</i>	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	2
<i>Cinnyris pulchellus</i>	1	1	-	-	-	-	-	1	-	-	-	14	-	2	-	8	19
<i>Circaetus beaudouini</i>	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
<i>Circaetus cinereus</i>	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	1
<i>Circaetus gallicus</i>	-	-	-	-	-	3	7	7	8	13	14	4	14	1	1	1	73
<i>Corvus albus</i>	-	-	-	-	-	-	-	-	-	-	-	-	2	1	-	4	7
<i>Grinifer piscator</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	2	3
<i>Delichon urbica</i>	-	-	-	-	7	2	12	3	3	-	-	-	-	-	6	2	45
<i>Eremopterix leucotis</i>	-	-	-	-	-	4	6	3	10	20	62	62	81	208	245	251	952
<i>Eremopterix nigriceps</i>	-	-	-	-	-	-	-	-	-	-	-	-	18	26	48	-	92
<i>Euodice cantans</i>	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	2
<i>Euplectes franciscanus</i>	-	-	9	-	1	-	6	2	2	-	-	-	-	-	-	-	20
<i>Falco ardosiaceus</i>	-	-	-	-	3	1	-	-	-	1	-	-	-	-	-	-	5
<i>Falco chicquera</i>	2	-	3	-	-	-	1	-	-	-	-	-	-	-	-	-	6
<i>Falco</i> sp.	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	2	4
<i>Ficedula hypoleuca</i>	-	-	3	3	3	1	-	-	1	-	-	-	-	-	-	-	11
<i>Gyps africanus</i>	-	1	-	-	10	-	-	2	2	8	6	-	5	8	-	-	42
<i>Gyps rueppellii</i>	-	-	-	-	-	9	4	9	7	-	-	-	10	-	-	-	39
<i>Gyps</i> sp.	-	-	-	-	-	-	-	-	-	-	28	22	20	24	25	21	140

Scientific name	Number of days after treatment																Total
	-18	-14	-4	-1	3	6	9	12	15	18	47	74	109	137	167	233	
<b>Non acridivorous</b>																	
<i>Hirundo rustica</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	-	12
<i>Hirundo senegalensis</i>	-	2	17	4	4	2	3	2	3	10	-	-	5	-	2	4	58
<i>Hoplopterus spinosus</i>	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-	4
<i>Lagonosticta senegalala</i>	-	-	1	-	-	-	-	-	-	-	-	150	128	176	6	-	461
<i>Laniarius barbarus</i>	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
<i>Macronyx croceus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	4
<i>Mirafra cantillans</i>	-	-	-	-	-	-	-	-	-	59	39	199	199	157	233	268	955
<i>Necrosyrtes monachus</i>	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1
<i>Oena capensis</i>	-	-	-	-	1	-	1	4	1	-	4	7	7	8	6	10	49
<i>Otus leucotis</i>	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
<i>Passer griseus</i>	-	-	-	-	-	-	-	-	-	42	67	8	1	3	1	-	122
<i>Passer luteus</i>	-	-	-	-	-	-	-	-	-	244	1210	1958	1677	1032	5128	-	11249
<i>Ploceus cucullatus</i>	-	-	-	-	-	-	-	-	-	97	144	26	-	-	-	-	267
<i>Polyboroides typus</i>	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	3
<i>Psittacula krameri</i>	-	4	-	1	4	20	18	19	23	27	26	14	8	26	3	-	193
<i>Pterodas sp.</i>	-	-	-	2	1	2	7	6	6	5	29	-	-	-	-	-	58
<i>Riparia riparia</i>	-	-	-	-	125	7	10	-	7	-	-	-	21	-	-	-	170
<i>Serinus leucopygius</i>	-	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8
<i>Spiloptila clamans</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
<i>Sporopipes frontalis</i>	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
<i>Streptopelia decipiens</i>	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
<i>Streptopelia senegalensis</i>	9	17	23	8	16	29	40	49	64	68	37	52	27	33	28	66	566
<i>Streptopelia sp.</i>	-	-	-	-	-	-	-	-	-	2	-	-	2	-	-	-	4
<i>Streptopelia vinacea</i>	8	7	9	7	6	5	11	10	15	15	28	25	20	12	5	13	196
<i>Sylvia sp.</i>	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	2
<i>Torgos tracheliotus</i>	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
<i>Uraeginthus bengalus</i>	-	-	1	-	-	2	1	4	1	2	1	-	-	-	-	-	12
<i>Urocolius macrourus</i>	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	6
<i>Vanellus senegallus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2
<i>Vidua orientalis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	2
unidentified species	1	-	-	-	-	-	-	-	-	-	22	5	4	-	-	1	33
<b>Total</b>	22	47	81	27	177	80	137	132	165	191	1008	1972	3089	2464	1907	5871	17362

Table A2. Continued.

Scientific name	Number of days after treatment																Total
	-18	-14	-4	-1	3	6	9	12	15	18	47	74	109	137	167	233	
<b>Acridivorous</b>																	
<i>Bubalornis albigrostris</i>	457	703	214	149	94	127	101	121	204	156	-	-	-	-	-	-	2326
<i>Bubulcus ibis</i>	-	-	-	-	1	-	3	-	-	15	10	53	1872	418	165	839	3376
<i>Bucorvus abyssinicus</i>	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	2
<i>Centropus senegalensis</i>	-	-	-	-	-	1	4	-	2	-	1	9	-	-	2	-	19
<i>Chelictinia riocourti</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3
<i>Ciconia ciconia</i>	1	-	-	-	-	-	-	-	-	-	1634	400	-	-	-	-	2035
<i>Circus aeruginosus</i>	6	11	29	11	19	24	22	31	41	37	18	8	3	2	6	-	268
<i>Circus pygargus</i>	11	24	37	19	18	28	33	42	34	36	87	46	124	139	48	16	742
<i>Cisticola juncidis</i>	35	26	52	40	77	101	155	169	200	168	46	22	14	2	-	-	1107
<i>Cisticola</i> sp.	-	-	-	1	7	3	2	3	-	1	-	-	-	5	23	25	70
<i>Coracias abyssinica</i>	2	8	4	14	15	24	49	57	46	49	46	118	148	157	93	75	905
<i>Cursorius temminckii</i>	-	-	-	-	-	-	-	-	-	2	-	-	2	2	4	5	15
<i>Elanus caeruleus</i>	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	5
<i>Eupodotus savilei</i>	3	5	10	9	4	9	9	10	5	6	8	-	1	-	6	-	85
<i>Falco naumanni</i>	11	10	7	3	4	2	3	5	-	3	1122	123	592	198	95	6	2184
<i>Halcyon senegalensis</i>	12	12	13	3	2	2	13	11	3	-	5	-	-	-	1	1	78
<i>Lamprolornis caudatus</i>	6	8	6	2	10	4	13	16	7	18	28	23	17	5	6	-	169
<i>Lamprolornis chalybaeus</i>	-	-	-	-	-	-	-	-	-	-	5	1	65	53	128	62	314
<i>Lanius meridionalis</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	4	2	-	6
<i>Lanius senator</i>	-	1	1	1	-	19	30	30	27	19	28	35	54	51	45	12	353
<i>Leptoptilos crumeniferus</i>	-	-	-	-	-	1	-	-	-	1	-	-	-	-	-	-	2
<i>Macropyx croceus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	3
<i>Merops albicollis</i>	47	160	78	39	79	18	25	-	-	-	-	-	-	-	-	62	508
<i>Milvus migrans</i>	-	-	-	-	-	-	-	-	-	-	17	-	-	1	-	-	18
<i>Mirafra cantillans</i>	28	44	55	40	24	68	118	232	136	104	-	-	-	-	-	-	849
<i>Motacilla flava</i>	-	-	-	-	-	-	-	-	-	-	-	-	10	-	-	-	10
<i>Myrmecocichla aethiops</i>	-	-	-	-	-	-	-	-	-	-	-	10	1	-	-	83	94
<i>Neotis denhami</i>	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	2
<i>Oenanthe oenanthe</i>	-	-	-	-	-	-	3	-	2	8	27	17	39	49	16	5	166
<i>Oenanthe</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	2

Scientific name	Number of days after treatment																Total
	-18	-14	-4	-1	3	6	9	12	15	18	47	74	109	137	167	233	
<b>Acridivorous</b>	23	36	40	33	14	12	76	49	49	105	-	-	-	-	-	-	437
<i>Passer griseus</i>	118	373	89	26	112	43	105	3	-	88	-	-	-	-	3	-	960
<i>Ploceus cucullatus</i>	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	2
<i>Ploceus sp.</i>	60	52	53	46	85	82	68	89	180	81	-	-	-	-	-	-	796
<i>Ploceus velatus</i>	-	1	2	-	3	22	10	12	6	7	-	-	-	-	-	-	63
<i>Prinia sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	4
<i>Prinia subflava</i>	-	-	-	-	-	2	-	-	-	3	4	8	9	750	459	29	1264
<i>Spreo pulcher</i>	2	-	5	5	7	10	6	19	16	19	-	-	-	-	-	-	89
<i>Tchagra senegala</i>	4	8	6	3	2	-	7	-	1	1	22	18	15	27	22	9	145
<i>Tockus erythrorhynchus</i>	-	-	1	-	2	49	33	82	21	58	21	12	17	49	17	30	392
<i>Tockus nasutus</i>	-	-	-	-	-	-	-	-	-	-	-	5	1	-	-	-	6
<i>Turnix sylvatica</i>	1	-	-	-	-	-	-	-	3	-	1	3	4	5	2	1	20
<i>Upupa epops</i>	41	51	43	30	64	73	49	63	50	52	95	142	192	203	165	186	1499
<i>Vanellus tectus</i>																	
<b>Total</b>	868	1533	745	474	643	726	941	1044	1033	1036	3231	1053	3182	2120	1311	1453	21393





# Increased and sex-selective avian predation of Desert Locusts *Schistocerca gregaria* treated with *Metarhizium acridum*

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## Abstract

The entomopathogenic fungus *Metarhizium acridum* in oil-based formulations (Green Muscle® (GM)) is a biopesticide for locust control lacking side-effects on biodiversity, unlike chemical insecticides. Under controlled conditions, GM-treated locusts and grasshoppers attract predators, a complementary advantage in locust control. We assessed avian predation on a population of desert locusts in northern Niger aerially sprayed operationally with GM with 107 g viable conidia ha<sup>-1</sup>. Populations of adult locusts and birds and vegetation greenness were assessed simultaneously along two transects from 12 days before until 23 days after treatment. Common kestrels *Falco tinnunculus* and lanners *F. biarmicus* were the predominant avian predators. Regurgitated pellets and prey remains were collected daily beneath “plucking posts” of kestrels. Locusts started dying five days post-spray and GM had its maximum effect one-two weeks after the spray, with 80% efficacy at day 21. After spraying, bird numbers increased significantly ( $P < 0.05$ ) concurrent with decreasing desert locust densities. Locust numbers decreased significantly ( $P < 0.001$ ) with both time since spraying and decreasing greenness. Before spraying, kestrel food remains under plucking posts accounted for  $34.3 \pm 13.4$  prey items day<sup>-1</sup>, of which  $31.0 \pm 11.9$  were adult desert locusts (90.3%), reducing post-spray to  $21.1 \pm 7.3$  prey items day<sup>-1</sup>, of which  $19.5 \pm 6.7$  were adult desert locusts (92.5%), attributable to decreased use of the plucking-posts by the kestrels rather than an effect of the spray. After spraying, kestrels took significantly ( $P < 0.05$ ) more larger female (75–80 %) than smaller male (20–25 %) locusts. Avian predation probably enhanced the impact of the GM on the desert locust population, especially by removing large adult females. No direct or indirect adverse side-effects were observed on non-target organisms including locust predators such as ants and birds. These substantial ecological advantages should also be considered when choosing between conventional chemical and biopesticide-based locust control.

## Introduction

Most locusts, including the desert locust *Schistocerca gregaria*, are usually controlled by synthetic pesticides such as the organophosphates fenitrothion, chlorpyrifos and malathion, e.g. during the 2003-2005 desert locust upsurge (Brader *et al.* 2006) and during the present desert locust outbreak in Eastern Africa, the Arabian peninsula and SW Asia with over 2 million hectares being sprayed between 1 January 2019 and 31 March 2020 (FAO 2019). These pesticides, if applied correctly kill sprayed locusts within hours, however, they also kill or debilitate natural enemies of locusts, such as birds (Mulli   & Keith 1993) and insects including Coleoptera, Hymenoptera and Diptera (Van der Valk 1990, Everts *et al.* 1997-2002). This reduces the efficiency of the overall control by diminishing the impact of the natural predators and parasitoids, and by inducing secondary pests (Van der Valk *et al.* 1999). Also, other beneficial insects are killed, such as pollinators, thus increasing detrimental long-term ecological effects of locust control (Leach *et al.* 2008). The need for alternative approaches that do not kill or debilitate the natural allies was already being advocated decades ago for sustainable locust control (Krall *et al.* 1997) and remains a pressing need during the current outbreak in eastern Africa, the Middle East and Pakistan (Roussi 2020).

An alternative to chemical pesticides is the application of biological agents such as entomopathogenic fungi. Formulations of the aerial conidia of isolates of the deuteromycete fungus *Metarhizium acridum* in oil-based suspensions were found to be effective, even in dry environments (Lomer & Prior 1992, Bateman *et al.* 1993, Lomer *et al.* 1997). Following developments by the LUBILOSA program (CABI Bioscience, Ascot, UK) (Lomer 1999), formulations of *M. acridum* were registered as ‘Green Muscle<sup>  </sup>, (GM) for use against the brown locust *Locustana pardalina* in South Africa in 1998 and against desert locusts and grasshoppers in nine Sahelian countries in 2001. The genus *Metarhizium* can kill a wide range of insects, but GM is based on a specific isolate which targets locusts and grasshoppers (Lomer 1999). This specificity is an important feature because the product has little or no adverse environmental impact, benefiting not only humans but also other animals, including the natural enemies of the pests. The Food and Agriculture Organization of the United Nations (FAO), based on the latest recommendation of its Pesticide Referee Group (PRG 2014) considers biopesticides based on *M. acridum* to be the most appropriate option for locust control, but many African countries still lag behind in the registration process. A second strain of *M. acridum* (EVCH 077), marketed as NOVACRID, was registered in November 2019 by the Comit   Sah  lien des Pesticides for use in the Sahel.

During trials of GM against hoppers of desert locusts in Mauritania and Algeria, very few dead or dying hoppers were found after the treatment. This was attributed to predation by birds attracted to high densities of sick and sluggish prey (Kooyman & Godonou 1997, Kooyman *et al.* 2005). The same phenomenon was observed after a trial with GM against adult red locusts *Nomadacris septemfasciata* in Tanzania (Kooyman *et al.* 2003), suggesting that there might be an interaction between GM and enhanced avian predation

on surviving or impaired locusts. Indications of increased bird densities after application of biopesticides also came from trials with the microsporidium *Nosema locustae* (Bomar *et al.* 1993). Predation by Hoopoe *Upupa epops* on pupae of the pine processionary caterpillar *Thaumetopoea pityocampa* was enhanced when the prey was infected with *Beauveria bassiana* (Battisti *et al.* 2000). Increased densities after application of fungal pesticides have also been reported for invertebrate predators that are apparently unaffected by the fungi, thus enhancing pest mortality (Alma *et al.* 2007). The seemingly enhanced contributions by wild birds to pest reductions upon application of GM contrast with the excess avian mortalities caused by ecotoxicological effects associated with the indiscriminate use of synthetic pesticides (Ritchie & Dobson 1995, Mineau 2002).

At least 537 species of birds from 61 different families are known to attack acridids in Africa, of which 146 species are known to feed on hoppers and adults of the desert locust, especially as predators of locust swarms (Mullié 2009). Field data on bird predation on hopper bands and swarms show that birds can regulate locust populations at low and medium densities (Mullié 2009). Also, experimental studies with grasshoppers using bird exclosures showed the potential of birds to regulate orthopteran populations. It was found that grasshopper densities outside exclosures were 33% lower in a year with average rainfall, and similar in a dry year with low densities (Fowler *et al.* 1991), whereas a 27.4% reduction in grasshoppers in plots subjected to 40 days of bird predation was reported (Joern 1986). In another study, an average annual bird-induced reduction of 25% in a three year experiment was reported (Joern 1992). At the end of a 4-year bird exclusion field experiment, adult grasshopper density was 2.2 times higher, and nymph density 3 times higher in exclosures (Bock *et al.* 1992). In the only bird exclosure study done in Africa (Mullié & Guèye 2010b, Mullié 2011), the authors found that grasshopper densities in Senegal outside exclosures at the end of the rainy season were 30% lower than inside, 37 days after the start of the experiment.

This study quantifies the effects of an operational application of GM against an isolated population of desert locusts on avian locust predation. Our hypotheses were:

- (1) avian predation complements the impact of the fungal insecticide to control the locusts, and
- (2) birds prefer the larger females of the desert locust over the males given that, in general, birds preferentially take larger species of grasshoppers or locusts or, within a species, the larger sex (Kaspari & Joern 1993, Belovski & Slade 1993, Buij *et al.* 2012).

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## Materials and Methods

### Study area

Potential breeding sites for the desert locust were selected during a helicopter survey between Agadez and Arlit in Northern Niger in September 2005. At Aghéliough (18°46'N, 7°31'E), approximately 15 km NNE of Arlit and West of the Aïr mountains, an extensive stand of 530 ha of c. 80% green *Schouwia thebaica* was found supporting an isolated

population of hoppers and immature adults ( $>3000$  ind.  $\text{ha}^{-1}$  on 15 September 2005) of transiens phase desert locusts. Freshly laid egg-pods were found. At the site dozens of black-crowned finch larks *Eremopterix nigriceps* and golden sparrows *Passer luteus* were breeding and adults were seen taking hoppers to feed their young.

On 23 October, two weeks before treatment, about 45% of the vegetation was still green. Two perpendicular transects, respectively 3.55 and 3.25 km long, across the area were chosen (Fig. 5.1, resp. A and B) to survey the locust and bird populations.

As both birds and locust numbers were expected to be influenced by vegetation density and greenness, the percentage of the surface covered by green vegetation in a 50 m wide stretch on either side of the transects was classified on a 5-digit scale: 0 - 0% green (vegetation had died and turned brown), 1 - 1-25% green, 2 - 26-50% green, 3 - 51-75% green and 4 - 76-100% green.

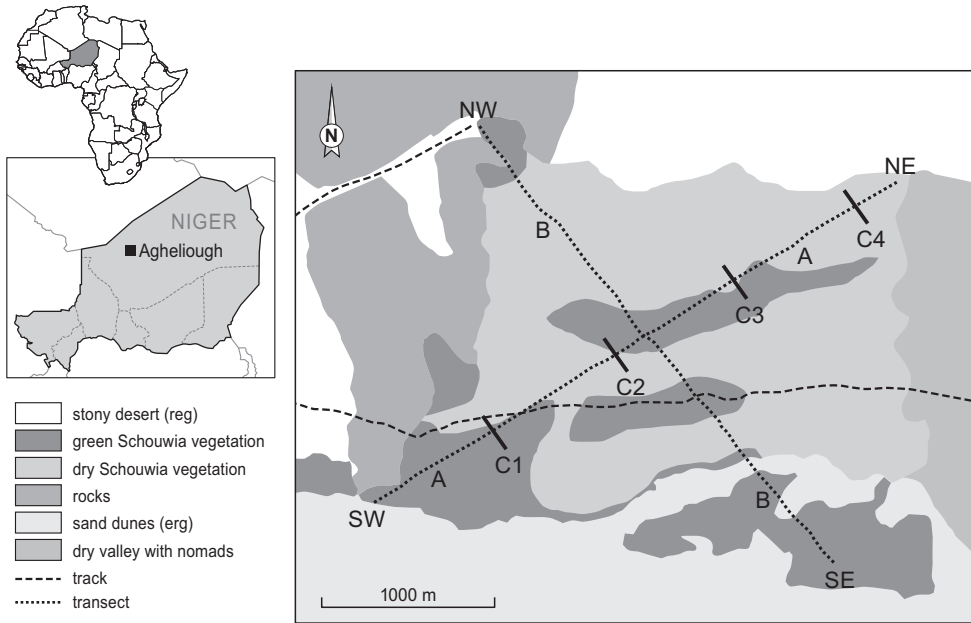
### GM treatment and locust surveys

The study area was aerially treated with GM (Biological Control Products SA (Pty) Ltd) on 5 November 2005 (08:00 – 11:00 hrs) with a Cessna 188 fixed wing aircraft, fitted with four Micronair® type AU5000 atomizers (Micron Sprayers Ltd) and the spray tracks recorded by a GPSmap 60CS (Garmin Ltd). The operational application dose of GM was  $107$  g viable conidia  $\text{ha}^{-1}$ . Spray deposit distribution and intensity (N droplets  $\text{cm}^{-2}$ ) were assessed with oil sensitive papers at 5 m intervals, placed 1.5 m high along three lines (C1-C3 on Fig. 5.1), each 200 m long and 800 m apart, perpendicular to diagonal A (SW-NE) of the plot. A fourth line with spray papers (C4) was just outside the sprayed area which was retained for the study. Densities of immature winged desert locusts were estimated every other day by counting all seen within 1 m on either side along each 100 m stretch of the transects, using a tally counter, while walking at a standard pace of c.  $0.6$  m  $\text{s}^{-1}$  ( $2.3$  km  $\text{h}^{-1}$ ). Pre-treatment counts took place from 24 October until 3 November, and post-treatment from 6 until 28 November. In parallel with our study, the efficacy of the treatment on locusts and the infectivity of *M. acridum* with locusts captured in the field and transferred to cages in the lab was assessed (Ouambama *et al.* 2006). Relevant outcomes of that study will be referred to in the results section of this paper.

Light traps were set up on 19, 20 and 21 November and operated from 19:00 until 03:00 hrs at  $18^{\circ}45'33.8''\text{N}$ ,  $007^{\circ}33'50.9''\text{E}$ , 2.4 km E of the SE starting point of transect B (Fig. 5.1). This was to determine whether any locusts were flying away from the treated area and thus contributing to a reduction in numbers on the study site. While driving to and from the light trap, activity of nocturnal locust predators was monitored. This was the only area outside the study site which still contained green vegetation but without desert locusts.

### Locust morphometrics

Before (27 October) and after (10 November) spraying, 164 adult locusts were randomly collected in the vegetation in the early morning while they were still inactive, sexed, weighed and body sizes measured when still fresh.



**Figure 5.1.** Map of study site and location of transects A (SW-NE) and B (SE-NW). The greenness of the Schouwia vegetation is the situation by mid-November. The lines C1-C4 indicate the position of the spray papers.

### Bird censuses and behavioral observations

The same transects that were used for the locusts were surveyed for birds by recording all birds seen within 50 m on either side of the transect. Surveys were conducted according to the same schedule as used for the locusts. Binoculars (10 × 40), and a telescope (10-30× zoom) were used whenever needed. Special attention was given to the behavior of falcons (kestrels *Falco tinnunculus* and lanners *F. biarmicus abyssinicus* and *F. b. erlangeri*), the most obvious locust predators present. The behavior of other avian locust predators, both diurnal and nocturnal, was only assessed qualitatively.

Between 09:00 and 10:00 hrs on each day from 27 October until 29 November, all pellets and remains of locusts found were collected from beneath two *Acacia raddiana* trees near the crossing of the transects used as “plucking posts” by falcons to consume their locust prey (designated KTREE1 and KTREE2). Before starting the daily collection under KTREE1 and KTREE2, all pellets and plucking remains were removed. Locust species in both the remains and the pellets were identified. Each daily sample was considered representative of what the birds had been consuming there during the previous 24 hour period.

Dry elytra, femurs and tibia recovered were stored and later measured to determine the sex of the locusts taken by the falcons. The drying of body parts slightly reduced their

sizes, complicating direct comparison with measurements taken on live locusts. As no fresh desert locusts were available to determine the relative size reductions, 15 females and 18 males of the slightly larger grasshopper species *Ornithacris cavroisi*, later captured at Khelcom, central Senegal, were used to quantify reduction of tibia length after 48 h oven drying at 70°C. Tibia were chosen because these were sufficiently common in the prey remains to allow for appropriate calculations.

### Statistical procedures and data analysis

All statistics were analyzed with R (R Development Core Team 2011). Acridivorous bird numbers and corresponding desert locust densities per 100 m transect were analyzed with a Linear Model (multiple regression) taking greenness of the vegetation and time after treatment as covariates. Data from transect sections with zero locusts recorded were excluded from the analysis.

To test the null hypothesis that the larger desert locust females would be preferentially taken by the falcons, analysis of the length – frequency data of tibia and elytra (as a proxy for body size) of desert locusts was performed with the likelihood ratio test for bimodality in univariate two-component normal mixtures from (Holzmann & Vollmer 2008). The package allows for equal as well as distinct variances, and accounts for finite sample corrections based on penalizing variance estimates under the alternative.

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## Results

### Quality of treatment and direct effect of GM on locusts

GM spray deposits on oil sensitive papers were evenly distributed (range 5–60, mean. c. 15 droplets cm<sup>-2</sup> along the first three lines, C1-C3 (Fig. 5.1)). As the fourth line (C4) was just outside the sprayed area, it was only slightly exposed, showing the precision of the treatment. As shown in the parallel study (Ouambama *et al.* 2006), mortality of locusts started five days after treatment, which was confirmed with caged locusts and by the characteristic red color appearing in freshly dead infected individuals. The maximum effect (mortality rate) was between one and two weeks post-spray. Overall efficacy on day 21 post-spray was 80% and > 90% at the end of the study (Ouambama *et al.* 2006).

### Locust morphometrics and densities

Males ( $n = 30$ ) were smaller than females ( $n = 24$ ) with their mean fresh weight (WW) being 58% of that of females. Dry weight (DW) of males was 55% and DW of females was 56% of WW after air drying. Mean WW for combined sexes was  $2.25 \pm 0.92$  g (Table 5.1, which includes morphometric data of all 164 captured individuals).

The parallel assessment of efficacy (Ouambama *et al.* 2006) showed that throughout the study >99 % of the population consisted of adults, the remainder being third to sixth instar nymphs, predominantly fifth. Therefore, the locust density calculations are further based on adult locusts.

The number of adult desert locusts (means and maxima ha<sup>-1</sup>) before and after the treatment are presented in Table 5.2. Maximum densities ranged from 2250 to 11700 individuals ha<sup>-1</sup> before spraying. Starting from about five days post spray, locust numbers declined rapidly and became as low as 13 ind. ha<sup>-1</sup>, with a maximum of 200 ind. ha<sup>-1</sup> at the end of the study.

**Table 5.1.** Morphometrics (mm) and body mass (WW, g) for desert locusts collected at the study site on 27 October and 10 November 2005.

Sex		Body Mass (WW, g)	Femur length F (mm)	Elytron length E (mm)
Males	mean (±s.d.)	1.70 (0.47)	25.40 (1.07)	53.11 (2.05)
	n	30	85	85
Females	mean (±s.d.)	2.93 (0.88)	29.51 (1.23)	62.24 (2.20)
	n	24	79	79

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**Table 5.2.** Number of adult desert locusts counted on two transects and estimates of mean (± s.d.) and maximum number of desert locusts per ha before and after treatment.

Date	Transect B (SE-NW)				Transect A (NE-SW)			
	n counted	mean n ha <sup>-1</sup>	s.d.	max n ha <sup>-1</sup>	n counted	mean n ha <sup>-1</sup>	s.d.	max n ha <sup>-1</sup>
<b>Before treatment</b>								
24-Oct-05	803	1147	1530	5500	858	1226	1987	7750
26-Oct-05	756	1080	1422	6750	749	1070	1125	4000
28-Oct-05	307	439	608	2250	709	1013	1514	6000
30-Oct-05	442	631	1000	4200	1726	2466	3289	11700
1-Nov-05	948	1354	2057	8850	1123	1604	1964	6350
3-Nov-05	625	893	1577	7800	748	1069	1377	4450
<b>After treatment</b>								
6-Nov-05	485	693	1016	3200	622	889	1277	5550
8-Nov-05	456	651	1176	5300	660	943	1175	5650
10-Nov-05	528	754	1025	3450	763	1090	1165	5050
12-Nov-05	257	367	542	1700	510	729	867	3550
14-Nov-05	124	177	239	750	456	651	739	3250
16-Nov-05	115	164	240	900	296	423	528	2400
18-Nov-05	34	49	119	500	72	103	156	600
20-Nov-05	30	43	100	450	68	97	150	650
22-Nov-05	21	30	82	450	56	80	158	700
24-Nov-05	9	13	33	150	41	59	96	350
26-Nov-05	13	19	43	200	26	37	56	200
28-Nov-05	9	13	28	100	20	29	59	200

### Observations of affected locusts and of avian locust predation

Searches for dead locusts revealed very few, and it was obvious that dead insects rapidly disappeared. Presumably nocturnal predation and scavenging activity was responsible for removal of dead or dying insects whereas ants, in particular, took care of the remains. Sluggish dying animals, which moved to the upper vegetation layer to bask, to induce behavioral fever (Blanford & Thomas 1999) as a reaction to the *M. acridum* infection, were rarely encountered.

The two falcon species were regularly observed preying on adult locusts. Examination of photographs showed that, although never more than six different lanners were ever observed on the same day, some individuals disappeared and new ones appeared. *F. b. abyssinicus* was the more abundant subspecies before treatment, whereas *F. b. erlangeri* became more numerous afterwards. Lanners dived from >30m to snatch adult locusts flying about 1 m above the *Schouwia* vegetation and exploited locusts that were disturbed into flight by people, camels and goats. Kestrels hovered to detect their prey.

Other bird predators of locusts included crested larks *Galerida cristata*, wheatears *Oenanthe* spp. and southern great grey shrikes *Lanius meridionalis leucopygos*. The latter impaled locusts on thorns of *Acacia raddiana*.

### Bird censuses

The number and species of birds recorded along the two transects before and after spraying are listed in S1 Table (in Supporting Information). In total, 28 species of birds were recorded. Many of these were irrelevant to the locust study as they were aerial feeders (e.g. swifts and swallows) while some acridivorous species such as nubian bustards *Neotis nuba* were never recorded during transect counts. Details of the 16 relevant acridivores are given in Table 5.3.

The numbers of acridivorous birds recorded along the transects varied from 0.2–20.3 individuals ha<sup>-1</sup> and interestingly the highest numbers were recorded about 10 days after the treatment. As can be seen in Table 5.2, this coincided with the peak of the GM effectiveness in terms of the greatest reduction of numbers of locusts counted (between 10 and 24 November). Multiple regression analysis showed significant increases in bird numbers with decreasing locust densities ( $P < 0.05$ ), decreasing locust densities with decreasing greenness ( $P < 0.001$ ) and decreases in locust densities with increasing time after the spray ( $P < 0.01$ ). The model was:  $\text{Log (locusts)} = 2.26 (\pm 0.091) - 0.125 (\pm 0.061) \text{ log (total birds)} - 0.084 (\pm 0.006) \text{ days after the spray} + 0.2745 (\pm 0.045) \text{ greenness}$  (parameter values  $\pm$  standard errors) ( $F = 83.44$  (3 & 504 DF),  $P < 0.00001$ ). In absolute terms, days after the spray had the greatest effect on locust numbers.

After the spraying, on the days that the light trap was operated, some nocturnal predation was observed. In the light of a vehicle's headlights, a lanner was seen hunting, as were spotted thick-knee *Burhinus capensis*, gerbils *Gerbillus* spp. and pale sand fox *Vulpes pallida*. Another nocturnal predator, a wild cat *Felis silvestris libyca* was seen in broad daylight during the spray.



### Indirect observations of avian predation of locusts

The total and daily average prey numbers taken by the kestrels using the plucking posts KTREE1 and KTREE2 before and after the treatment are given in Table 5.4 and S2 Table (in Supporting Information). Six larger pellets in the bulk material removed before daily collection started were significantly different from the kestrel pellets (Welch two-sample t-Test:  $t = 9.6049$ ,  $df = 7.796$   $P = 1.367e^{-0.5}$ ), but not different from lanner pellets containing bird and desert locust remains from Egypt and Sudan (Goodman & Haynes 1989) (Welch modified two-sample t-Test:  $t = 1.9773$ ,  $df = 37.449$ , N.S.). No such pellets were found during the daily collection of food remains. Therefore, the pellets and prey remains that we recovered were considered to be exclusively from kestrels.

As pellets and plucking remains potentially concerned the same locusts, pellet information was only used to calculate daily consumption when the total numbers of desert locusts were higher in pellets than those in the plucking remains. Coleopteran, mole cricket and bird remains were only present in pellets. Before the spray, in the Kestrel food remains  $31.0 \pm 11.9$  prey items  $\text{day}^{-1}$  out of a total of  $34.3 \pm 13.4$  or 90.3% were adult

**Table 5.3.** Total number, frequency and mean  $\pm$  s.d. of the most common acridivorous birds observed along the transects.

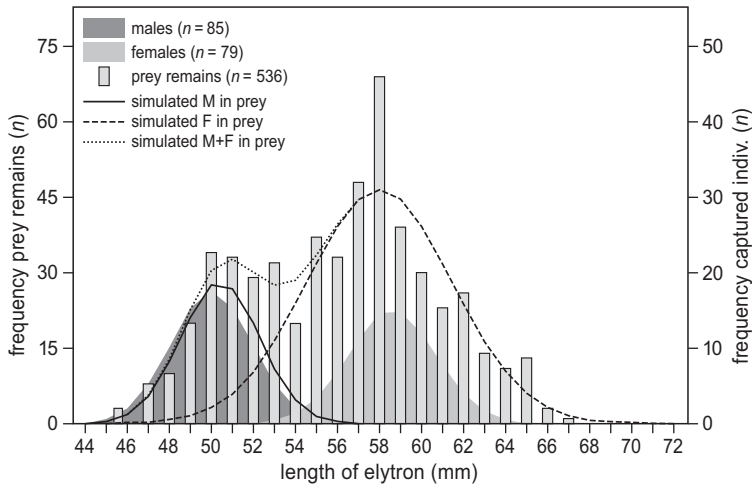
Species	Scientific name	Before treatment ( $n = 5$ counts)				After treatment ( $n = 12$ counts)			
		Total	Freq. $n \text{ ha}^{-1}$	Mean	s.d.	Total	Freq.	Mean	s.d.
Common Kestrel	<i>Falco tinnunculus</i>	30	1.00	6.0	1.4	40	0.83	3.3	2.8
Lanner	<i>Falco biarmicus</i>	3	0.60	0.6	0.5	33	0.67	2.8	2.8
Common Quail	<i>Coturnix coturnix</i>	not observed				2	0.08	0.2	0.6
African Hoopoe	<i>Upupa epops senegalensis</i>	5	0.60	1.0	1.2	not observed			
Crested Lark	<i>Galerida cristata</i>	73	1.00	14.6	4.4	244	0.92	20.3	16.0
Desert Lark	<i>Ammomanes deserti</i>	1	0.20	0.2	0.4	not observed			
Greater Short-toed Lark	<i>Calendrella brachydactyla</i>	37	0.80	7.4	5.5	21	0.25	1.8	4.9
Greater Hoopoe Lark	<i>Alaemon alaudipes</i>	2	0.40	0.4	0.5	not observed			
Black-eared Wheatear	<i>Oenanthe hispanica</i>	1	0.20	0.2	0.4	not observed			
Desert Wheatear	<i>Oenanthe deserti</i>	43	1.00	8.6	4.8	109	0.92	9.1	8.7
Isabelline Wheatear	<i>Oenanthe isabellina</i>	not observed				71	1.00	5.9	3.1
Wheatear sp.	<i>Oenanthe</i> sp.	not observed				76	1.00	6.3	4.6
Cricket Warbler	<i>Spiloptila clamans</i>	12	0.80	2.4	1.9	4	0.17	0.3	0.9
Fulvous Babbler	<i>Turdoides fulva</i>	6	0.40	1.2	2.2	not observed			
Southern Grey Shrike	<i>Lanius meridionalis leucopygos</i>	15	1.00	3.0	2.5	36	0.92	3.0	1.8
Chestnut-bellied Starling	<i>Lamprolornis pulcher</i>	2	0.20	0.4	0.9	4	0.25	0.3	0.7
Sudan Golden Sparrow	<i>Passer luteus</i>	38	0.60	7.6	9.1	57	0.58	4.8	6.6

**Table 5.4.** Kestrel prey remains (including from pellets; see text) by number and their calculated biomass (as total wet wt) collected under KTREE1 and KTREE2 before ( $n = 9$  days) and after ( $n = 24$  days) treatments.

<i>Taxa by number</i>	Before treatment ( $n = 9$ days)					After treatment ( $n = 24$ days)				
	Freq.	Total	Daily avg	Std	%	Freq.	Total	Daily avg	Std	%
Desert locust (ad.)	1.00	290	32.22	11.99	90.6	1.00	468	19.50	6.71	92.5
Desert locust (5th instar)	0.11	10	1.11	3.33	3.1	0.04	1	0.04	0.20	0.2
Tree locust	0.56	7	0.78	0.83	2.2	0.71	28	1.17	1.09	5.5
Unidentified grasshopper	0.22	7	0.78	1.72	2.2	0.04	3	0.13	0.61	0.6
Mole cricket	0.22	4	0.44	0.88	1.3	0.00	0	0.00	0.00	0.0
Coleopteran sp.	0.11	1	0.11	0.33	0.3	0.17	4	0.17	0.38	0.8
Small bird	0.11	1	0.11	0.33	0.3	0.08	2	0.08	0.28	0.4
<b>Total</b>		<b>320</b>	<b>35.55</b>	<b>13.13</b>	<b>100.0</b>		<b>506</b>	<b>21.08</b>	<b>7.26</b>	<b>100.0</b>
<i>Biomass (g wet wt)</i>										
	BM (g)	Total	Daily avg	Std	%	BM (g)	Total	Daily avg	Std	%
Desert locust	2.25	652.50	72.50	26.97	90.2	2.25	1053.00	43.88	15.10	87.3
Desert locust (5th instar)	1.10	11.00	1.22	3.67	1.5	1.10	1.10	0.05	0.22	0.1
Tree locust	3.90	27.30	3.03	3.25	3.8	3.90	109.20	4.55	4.25	9.1
Unidentified grasshopper	0.68	4.76	0.53	1.17	0.7	0.68	2.04	0.09	0.42	0.2
Mole cricket	2.00	8.00	0.89	1.76	1.1	2.00	0.00	0.00	0.00	0.0
Coleopteran sp.	0.10	0.10	0.01	0.03	0.0	0.10	0.40	0.02	0.04	0.0
Small bird	20.00	20.00	2.22	6.67	2.8	20.00	40.00	1.67	5.65	3.3
<b>Total</b>		<b>723.66</b>	<b>80.41</b>	<b>27.56</b>	<b>100.0</b>		<b>1205.74</b>	<b>50.24</b>	<b>17.38</b>	<b>100.0</b>

**Table 5.5.** Mean length (mm)  $\pm$  s.d. of tibiae and elytra of male and female desert locusts captured alive and in prey remains. For length adjustments because of drying of prey remains, see text. The bimodal distribution of prey remains over the sexes has been separated by applying the likelihood ratio test (Holzmann & Vollmer 2008).

	Captured alive				Prey remains				
	$n$	Length (mm)	Proportion	Adjusted length (mm)	$n$	Length (mm)	Proportion	Likelihood ratio	$P$ -value
<b>TIBIA</b>	54				287			3.729	0.0267
Males	30	23.1 $\pm$ 0.89	0.55	21.7 $\pm$ 0.84		22.8 $\pm$ 0.89	0.23		
Females	24	27.3 $\pm$ 1.38	0.45	25.7 $\pm$ 1.29		25.9 $\pm$ 1.83	0.77		
<b>ELYTRA</b>	164				526			5.030	0.0125
Males	85	53.1 $\pm$ 2.05	0.52	50.0 $\pm$ 1.90		50.5 $\pm$ 1.90	0.26		
Females	79	62.2 $\pm$ 2.20	0.48	58.6 $\pm$ 2.10		58.0 $\pm$ 3.39	0.74		



**Figure 5.2.** Frequency distributions of elytra of captured male ( $n = 85$ ) and female ( $n = 79$ ) desert locusts. Superimposed are frequency distributions (bars) of elytra ( $n = 536$ ) recovered beneath kestrel plucking posts, separated into two complementary normal distributions (males solid line and females broken line). The dotted line is the sum of the male and female normal distributions. The distribution of lengths of elytra from captured locusts was significantly bimodal,  $P = 0.012$  (Holzmann & Vollmer 2008).

desert locusts. After spraying this was 92.5%. In terms of biomass this was 89.8 % before and 87.3 % after spraying (Table 5.4).

The frequency distributions of lengths of tibia and elytra recovered under KTREE1 and KTREE2 are significantly bimodal (Likelihood ratio test tibia  $P = 0.03$ , elytra  $P = 0.012$ , Table 5.5; Fig. 5.2 shows the data for elytra). After separating them into two normally distributed components, the resulting distributions overlap with those of the sample of males and females which were collected during the study. Therefore, we attribute the two distributions to males and females. The bimodality test shows that kestrels took significantly more female than male locusts. Based on the larger sample of elytra lengths the proportion of females taken as prey was 0.74 while this proportion in the sample of live captured adult desert locusts was 0.48 (Table 5.5).

## Discussion

The results showed that Green Muscle® containing the entomopathogenic fungus *Metarhizium acridum* had strongly reduced locust numbers starting about five days after spraying (Table 5.2), without any negative impact on acridivorous bird numbers (Table 5.3) which continued feeding on locusts, including impaired individuals.

Our finding that acridivorous birds were not affected by GM, bird numbers being even higher after treatment than they were before spraying, is in accordance with the finding that chicks of ring-necked pheasant *Phasianus colchicus* fed with *M. acridum* infected

grasshoppers were unaffected (Smits *et al.* 1999). An increase of acridivorous birds in plots treated with entomopathogens against grasshoppers has been reported before (Bomar *et al.* 1993, Mullié & Guèye 2010a). This is in sharp contrast with reports from application of synthetic pesticides such as chlorpyrifos and fenitrothion, widely used for desert locust control. Such conventional chemical insecticides kill a large proportion of the target as well as non-target species within a short period (usually <24 hours) (Everts *et al.* 1997-2002). This includes natural locust predators or parasitoids of locusts or their eggs, such as birds, hymenopterans, coleopterans and arachnids. The dead insects remaining rapidly decay and become unattractive as prey or carrion (Stafford *et al.* 2003). Only a few insects not killed by the spray are available as prey and birds temporarily leave the treated zones (Mullié & Keith 1993). In plots treated with chlorpyrifos and fenitrothion, approximately 50% of the bird numbers present just before treatment had moved away within 24 hours post spray (Mullié & Keith 1993). This means that newly arriving or surviving locusts hardly encounter natural enemies anymore, potentially leading to a secondary pest outbreak (Van der Valk *et al.* 1999). In addition, synthetic pesticide applications can also damage other ecosystem functions and insecticide residues may pose risks for the avian and even human food chain (Story *et al.* 2013, Van Huis *et al.* 2014). Many desert locust treatments are carried out in remote arid areas and side effects on birds and other beneficial organisms are rarely monitored (Mullié 2009) despite the existence of FAO recommendations (Van der Valk *et al.* 2005). This will probably result in underestimates of the impacts of synthetic pesticides applied for locust or grasshopper control (Story *et al.* 2013) in the longer term.

A fundamental difference between the synthetic and selective biopesticides is that the latter do not kill instantly. While the fungal infection develops further, the affected insects become sluggish and eventually attach themselves to the upper layers of vegetation to bask and induce fever as a response to the infection, a type of behavioral thermoregulation (Blanford & Thomas 1999). This, however, exposes them to predators (Arthurs & Thomas 2001). In trials in Algeria and Mauritania avian predators were shown to eliminate GM treated desert locust hopper bands within a few days, whereas untreated nymphs persisted (Kooyman *et al.* 2005, Kooyman *et al.* 2007). Birds attacked hoppers even before any external effect of the entomopathogen was seen by the observers.

The reduction of locust numbers after GM treatment in our study took several weeks (Table 5.2), clearly longer than would be the case with synthetic insecticides, but the number of acridivorous birds even increased. These natural enemies clearly continued to feed on locusts, including those affected by the spray. The fact that GM neither kills nor debilitates natural enemies of locusts or contribute to their temporary emigration implies that these remain in the system and continue consuming locusts, including new arrivals.

Few studies have reported how natural predators continued to prey upon locusts affected by *Metarhizium*. Ants and beetles were observed to take cadavers of red locust tinted red by *M. acridum* infection 12–15 days after a GM application in Katavi National Park in Tanzania (Kooyman *et al.* 2003, Price & Mitchell 2003). Within three days 40 dead red locusts, that were placed in the Iku plains treated with 50 g ha<sup>-1</sup> of GM, almost all

disappeared. Sick and dying grasshoppers and locust hoppers, rendered sluggish by the *Metarhizium*, were also seen being taken by birds and frogs (R. E. Price, pers. comm., November 2005). Locust remains found in droppings indicated that some mammals also had eaten many locusts (Kooyman *et al.* 2003, Price & Mitchell 2003).

In the present study it was very difficult to recover dead or dying locusts in the dense *Schouwia* vegetation, although ants were observed to drag locust remains to their nests from out of this vegetation. In an earlier study it was also stated that finding cryptically colored locust cadavers amongst the dense grass was almost impossible (Kooyman *et al.* 2003, Price & Mitchell 2003). Probably most predation remained unseen because it took place at night. A lanner was seen hunting by night, a behavior which has been seen in kestrels (Cramp 1980) and suggested for lanners (Del Hoyo *et al.* 1994).

Based on our daily observations, we are confident that only two kestrels used the plucking posts before spraying, where remains of at least 20–59 locusts were recovered daily, and it is likely that the kestrels were regularly satisfying their daily energy requirements from locusts alone (Table 5.4). After the treatment, up to two lanners frequently used the trees as roosts. During our daily monitoring of the plucking posts, lanners were never seen plucking prey in the trees, but frequently did so elsewhere on the ground or in the air. The presence of these larger falcons may have restricted the use by kestrels of the plucking posts by kestrels, thereby limiting the number of remains that could be recovered. Pellets were not always found on daily searches and the number of desert locusts in the plucking remains fluctuated widely: 5–34 individuals day<sup>-1</sup> after the spray. Numbers of prey derived from the remains should be considered as minima and the difference between pre- and post-spray items rather as an indication of changes in the use of the trees by kestrels than of changes in food intake.

Based on observations, we estimated that at least 10 kestrels and six lanners were daily present on the plot throughout the study. The number of locusts eaten by these birds initially only represented a small proportion of the locusts present before spraying but became more important when locust numbers decreased after treatment and predation continued. In contrast to the kestrels, the lanners only took locusts on the wing, presumably the insects least affected by the biopesticide.

The remains of locusts beneath the plucking posts reveal that kestrels preferentially caught females (Table 5.5, Fig. 5.2), given their larger size this means energy maximization of their attacks (Kaspari & Joern 1993), confirming our initial hypothesis. In experimental studies of grasshoppers, it has been shown that birds specifically select the larger species, or within a species the larger sex (Kaspari & Joern 1993, Belovski & Slade 1993, Mullié & Guèye 2010b), unless the behavior of the males exposed them more than the females (Belovski *et al.* 1990).

Our study revealed that GM can be used effectively in controlling adult desert locusts, with the important added advantage, in contrast with classical synthetic insecticides, that it does not kill or debilitate birds and other locust predators but rather facilitates them. The lethal effect of *M. acridum* is delayed, but its infectivity was found to last for up to two months under field conditions in Senegal at the end of the rainy season (Mullié & Guèye

2010b ). This implies that newly arriving (sub)adult locusts and locust hatchlings emerging in treated fields within that period may also become infected. This strongly contrasts with synthetic insecticides which rapidly decay on vegetation (Story *et al.* 2013) and do not guarantee any longer lasting effect on newly arriving locusts or emerging nymphs, while causing ecotoxicological side-effects and potentially human health risks. The observed combination of significant increases in acridivorous bird numbers without having a negative impact on other locust predators, both diurnal and nocturnal, has the potential to further enhance the long-lasting effect of GM treatments, offering substantial additional advantages for selective locust control operations.

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## Supporting information

S1 Table. Birds recorded at Aghéliough (plus records from Arlit only).

S2 Table. Prey remains found under plucking posts K1TREE and K2TREE

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## Chapter 6

# General discussion

FROM BIRD KILLS AS COLLATERAL DAMAGE BACK TO BIRDS  
AS NATURAL ALLIES

Wim C. Mullié

## Introduction

In 2020, spraying chemical insecticides was still the major strategy for attempts to control upsurges of locusts and grasshoppers, also referred to as acridids, which are major pests of agriculture and pasture and a threat to livelihoods. Ever since Rachel Carson's (1962) "*Silent Spring*", scientists have warned about the adverse effects of chemical control of pests on their natural predators including birds, mammals, reptiles, insects and spiders. Acridid control has meanwhile been documented to lead to secondary outbreaks, either within the same (Van der Valk *et al.* 1999) or the next year (Lockwood *et al.* 1988). Safer alternatives such as the fungi *Metarhizium acridum* (Lomer *et al.* 2001) and *Beauveria bassiana* (Jaronski & Goettel 1997, Danfa & Van der Valk 1999) and the microsporidian *Paranosema locustae* (Bomar *et al.* 1993, Tounou 2007) have been tested experimentally and used operationally. However, large scale introduction of such biopesticides, in particular for use on Desert Locusts *Schistocerca gregaria*, has been hampered by perceived higher costs, a lack of trust in their efficacy and speed of action (Table 6.3), exacerbated by a lack of long term vision, preparedness and a strategy to apply biocontrol of acridids in time.

This is not the case in some other regions worldwide. Since 2000 the use of *M. acridum* (as Green Guard®) has been part of an Integrated Pest Management (IPM) system for gregarious locusts in about 10–12% of the treatments in Australia (Hunter 2010; Dave M. Hunter *pers. comm.*), in particular because it is compatible with organic beef production (Story *et al.* 2005). However, since 2010 when the study outlined in Chapter 3 was executed, Australia has been in the grip of extended drought and no significant treatments have been executed since (Paul G. Story, Australian Plague Locust Commission (APLC), *pers. comm.*). In China there has also been substantial use of *M. acridum* and *P. locustae*. While such products were used there in only 5% of treatments during 2004, their use has increased to over 30% in recent years, which amounts to more than 100 000 ha per year sprayed with these products. These applications of biopesticides against locusts and grasshoppers were more than all of the rest of the world combined (Zhang and Hunter 2017).

This thesis compares the impact on acridivorous birds of two contrasting strategies to control acridids. The first strategy is spraying the organophosphorous insecticides fenitrothion or chlorpyrifos, two commonly used organophosphate insecticides in acridid control up to the present (Chapters 2 and 3). The second strategy is spraying viable conidia of the entomopathogenic fungus *Metarhizium acridum* (Chapters 4 and 5). The focus is on comparing the short- and medium-term impacts of both strategies on acridivorous birds, natural enemies of acridids, so functionally human allies.

The main emphasis is on the impact of control of grasshoppers, in particular the Senegalese Grasshopper *Oedaleus senegalensis*, in the Sahel Region of Africa and of Desert Locust control. In the introduction, Chapter 1, the development in locust control methods since the beginning of the 20th century and how this has changed the views on the role of birds as acridid predators is introduced. Chapter 2 assesses the gross effects of fenitrothion and chlorpyrifos on birds, both direct effects through exposure to these cholinesterase

inhibitors and indirect effects through prey depletion. In Chapter 3 the exposure of avian predators is assessed based on collecting hoppers for residue analysis. The importance of this exposure route for regulatory risk assessment is emphasized in particular with reference to gorge-feeding, currently not considered in risk assessment. The effects of treatment of acridids with *M. acridum* is the opposite of that by chemical insecticides, by providing non-toxic prey abundance for the acridid predators. Chapters 4 and 5 reveal sex- and size-mediated avian predation as a potentially complementary impact on the efficacy of acridid control. In the current chapter these findings will be discussed in the context of their environmental impact by re-evaluating the role of birds as acridid predators. The perspectives of future increased use of the biopesticide *M. acridum* is discussed as an at least partially viable alternative to chemical control. In this Chapter 1 will successively answer the research questions which were posed in Chapter 1.

### Field studies show the negative effects of organophosphorous insecticides on birds

**RQ 1** -What are the consequences of different application rates of the organophosphorous insecticides fenitrothion and chlorpyrifos for locust and grasshopper control on birds in the short and medium term.

The detailed study on the impact on birds of an operational scale application in a controlled experiment of fenitrothion and chlorpyrifos in acridid control in the savannah of Northern Senegal (Chapter 2), was one of the first of its kind undertaken in Africa. Other field studies had concentrated on side effects of spraying against Tsetse fly *Glossina* sp. (Koeman *et al.* 1978, Mueller *et al.* 1981, Everts & Koeman 1987, Douthwaite & Tingle 1994) and granivorous birds (in particular *Quelea quelea*) (Bruggers *et al.* 1989, Mullié *et al.* 1999, Mullié 2000, McWilliam & Cheke 2004, Cheke *et al.* 2012), using other pesticides, but not on control of locusts and grasshoppers. Both compounds were shown to have several adverse impacts on the avian community, with fenitrothion showing a stronger and longer lasting, dose dependent, impact than chlorpyrifos. This was in terms of both direct effects (mortality and morbidity caused by cholinesterase inhibition) and indirect effects (birds leaving sprayed areas due to an imminent lack of prey, impaired breeding performance and loss of body mass). Reduced reproductive success was identified as potentially having the greatest impact on population levels in the long-term.

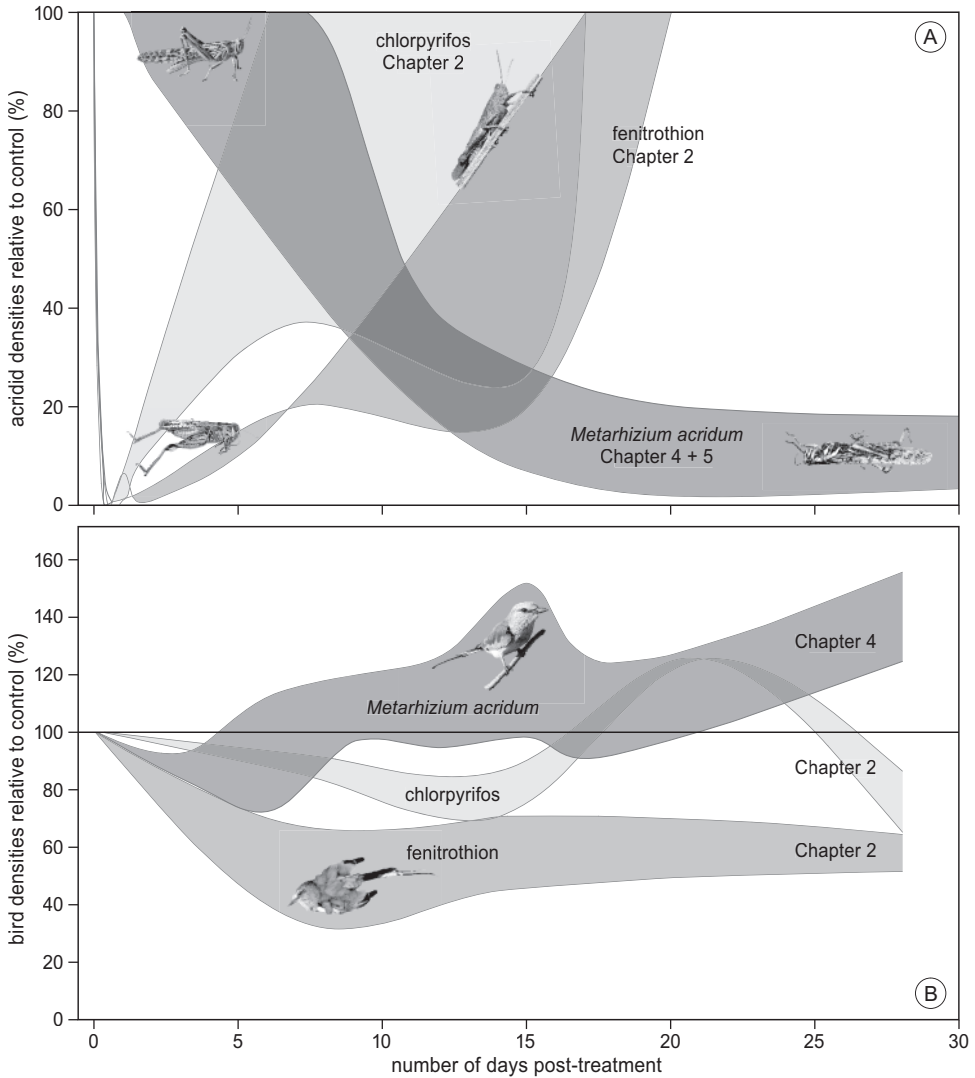
Both fenitrothion and chlorpyrifos have a broad-spectrum action affecting all insects, not only acridids. The ability of broad spectrum insecticides to cause indirect effects by reducing the prey of acridid predators is up to now notably absent in any existing avian risk assessment scheme, including the Pesticide Referee Group (2014) scheme applied by the Food and Agriculture Organization of the United Nations (FAO) for acridid control. Brain cholinesterase levels were generally back to pre-treatment levels after three weeks, but it cannot be excluded that new individuals had meanwhile entered the sprayed areas which would have hidden a longer lasting impact. Indeed, Mineau and Peakall (1987) demonstrated a strong collection bias in forest birds leading to an underestimation of individuals that had been severely exposed to aerial fenitrothion or acephate sprays. Contrary

to rapid recovery of plasma cholinesterase (ChE) levels after fenitrothion exposure, usually in the range of 1–2 days (Holmes & Boag 1990), brain ChE levels take much more time to recover. Maximum inhibition of brain ChE due to fenitrothion exposure has been found to be as long as 5–11 days post-spray in forest habitats (Hamilton *et al.* 1981), which was confirmed by Fildes *et al.* (2006) after locust control and in our study. Monitoring for at least three weeks post-spray is required to establish how long fenitrothion residues remain sufficiently high to affect birds (e.g. Chapter 2; Fildes *et al.* 2006).

Birds in fenitrothion treated plots did not recover at the same speed as their grasshopper prey. Grasshopper densities in plots treated with either fenitrothion or chlorpyrifos had fully recovered three weeks after treatments (Niassy 1990; Fig. 6.1A), however, four weeks after sprays, bird numbers were still significantly lower in the fenitrothion plots than in control plots (Figs. 2.2 and 6.1B). This was most obvious in insectivorous, ground feeding birds in open savannah habitat (grassland with low shrubs) and less so in granivorous, arboreal species in the more wooded depression habitat (Fig. 2.3). After an initial decrease in the first two weeks post-spray, bird densities in chlorpyrifos plots increased three weeks post-spray, due to an influx of Buffalo Weavers, to decrease again after four weeks. For a graphical representation of the ranges of the main findings see Fig. 6.1.

Keith (1994) suggested that the differences in mortality rates in the Senegal savannah (lower) and forests in Canada (higher) after spraying of fenitrothion at comparable dose rates using ULV equipment could have been due to the higher temperatures, minimal vegetative cover (less interception of droplets), greater thermal activity and therefore lower dermal exposure of birds in Senegal. Birds in the open savannah could have escaped direct exposure by flying away from the spray path of the plane. As shown by Driver *et al.* (1991) in a laboratory study, by using crops in wind tunnels to simulate natural conditions, dermal exposure had the greatest contribution to brain cholinesterase inhibition (28–42%) 24–48h post-spray, whereas food ingestion peaked 24 h after treatment and contributed only 10% to the exposure.

Acute mortality by anticholinesterase poisoning, although seemingly low based on our results, is still expected to cause at least hundreds of thousands, and maybe more than a million, of birds to die across the vast areas sprayed with chlorpyrifos and fenitrothion during major locust upsurges or plagues. Bird mortality, in particular of smaller species, is difficult to assess quantitatively and needs considerable effort and manpower (see Chapter 2), which is rarely applied after operational locust control in Africa. An exception is in Madagascar where in 2013/14 nine terrestrial chlorpyrifos 240 UL treatments were monitored. However, seven were largely inaccessible because of a tropical cyclone and of the remaining two, one was severely overdosed and a moribund snake was found (Everts 2015). High dermal chlorpyrifos toxicity to reptiles (*Acanthodactylus* lizards) has been documented previously with mass mortality in Mauritania (Sy 1996, Mullié *et al.* 1998, Peveling 2001) and by population declines and mortality in Niger (Mamadou 2015).



**Figure 6.1.** Generalized graphical summary of some of the ranges of the main results presented in this thesis. **A.** Densities of acridids in the first month following sprays with either chlorpyrifos and fenitrothion (Chapter 2) or *Metarhizium acridum* (Chapters 4 and 5). The organophosphates led to a rapid and >95% acridid decline followed by complete recolonization by 3 weeks post-spray, whereas *M. acridum* took 10–15 days to reach 70–90% decline, and kept acridid densities below 20% of pre-treatment values.

**B.** In fenitrothion treated plots bird densities decreased within days and remained up to 50% below pre-spray numbers four weeks post-spray, whereas in the same time in *M. acridum* treated plots bird numbers had increased 25–50%. It cannot be excluded (but has not been studied) that due to long lasting effects on acridids, other herbivorous arthropods increased in numbers due to reduced competition thereby providing an alternative food source to the birds. Bird numbers in chlorpyrifos treated plots took an intermediate position, and temporarily increased at three weeks post-spray due to an influx of Buffalo Weavers, but were still about 25% lower than pre-treatment levels at the end of the month. All numbers are expressed relative to controls set at 100 % at  $t = 0$ .



**Conclusions regarding RQ1** - The organophosphorous insecticides fenitrothion and chlorpyrifos caused direct mortality (2–7% by anticholinesterase poisoning) and total bird number decreases of up to 50% due to movements out of sprayed plots in reaction to a reduction of their arthropod food. Grasshopper numbers were depressed 90–95% immediately following spray but by three weeks post-spray numbers were the same as in the control. Fenitrothion impact on birds was more pronounced in the highest of the two dosages tested and was also stronger than that of chlorpyrifos. Nest desertion and morbidity of fledglings was found in some common species and reduced reproductive success was identified as having the greatest potential for medium and long-term effects on bird populations.

### Gorge-feeding is important and needs an adapted sampling protocol

**RQ 2** -What is the potential exposure of avian predators via consumption of locust nymphs sprayed with organophosphates, and how can the toxicological risk of this indirect exposure during gorge-feeding be addressed in regulatory avian risk-assessments?

Under operational conditions some bird species are expected to gorge on the overly abundant prey available during outbreaks (Mineau & Story 2009). This phenomenon is well documented for North America (Criddle 1920, 1924; Bradshaw 1934, Knowlton 1937, 1941; York 1949) and Africa (Moreau & Sclater 1938, Meinertzhagen 1959, Conley 1969). Therefore, under field conditions behaviour mediated oral uptake of toxic residues is much more likely to cause (sub)lethal cholinesterase poisoning than suggested by the wind tunnel experiments of Driver *et al.* (1991) who used Northern Bobwhite *Colinus virginianus*, which were not gorge-feeding in the experiment.

The study on residues on locust nymphs (Chapter 3) showed that residue levels peaked 3–24 h after the spray, most likely because they picked up secondary residues from contaminated vegetation and through their food. Therefore, the contribution of oral intake to total avian exposure will be underestimated if invertebrates for residue analysis are

**Table 6.1.** Food intake of grasshoppers by four intoxicated Abyssinian Rollers *Coracias abyssinicus* as a percentage of Body Mass (BM) and Field Metabolic Rate (FMR) leading to acute mortality through cholinesterase inhibition and calculated acute toxic dose of pesticide in mg kg<sup>-1</sup>. Further details can be found in Chapters 2 and 3 and in Everts (1990b, Annex XII.4).

Insecticide	Dosed rate (g ha <sup>-1</sup> )	Bird		Food intake		Intake as		Pesticide Intake (mg kg <sup>-1</sup> )	AChE Inhibition (%)
		BM (g)	FMR (kJ)	# grass-hoppers	g WW	% of BM	% of FMR		
chlorpyrifos	387	100	326.6	32	17.1	17.1	27.6	2.1–4.7	45.3
		80	280.5	29	15.5	19.4	29.1	1.8–4.1	n.d. <sup>1</sup>
		92	308.5	51	27.2	29.6	46.6	3.2–7.1	56.1
fenitrothion	485	91	306.2	35	18.7	20.5	32.2	2.8–6.2	86.5

<sup>1</sup> not determined



collected 1–2 h after spray, which is usually done. This is of particular importance when assessing the risks of gorging.

For example, the four Abyssinian Rollers *Coracias abyssinicus* found dead after both chlorpyrifos and fenitrothion sprays (Chapter 2) most likely had been gorge-feeding on intoxicated acridids given the much higher number of grasshoppers in their gizzards compared to other rollers which were examined. Their intake from probably a singly meal was up to almost 50% of their Field Metabolic Rate, and pesticide intake ranged from 1.8–7.1 mg kg<sup>-1</sup> for chlorpyrifos and 2.8–6.2 mg kg<sup>-1</sup> for fenitrothion (Table 6.1). The residues on locust nymphs from Chapter 3, irrespective of their physiological state, were used as follows: for minima the highest geometric mean of all mean values for any sampling time was used, whereas for maxima the highest geometric mean of maximum values for each site, within 1–24 h post-spray, was used which was at 6 h post-spray. Data were back transformed from RUDs to measured values. The calculated values are in the range of the HD5 (50%) for both compounds individually (Mineau *et al.* 2001). Maximum cholinesterase inhibition in rollers shot on plots 48 h till 7 d post-spray was resp. 56 and 44%, thus in the same range as those found dead. This indicates that rollers had been feeding on contaminated prey and potentially would have died if left in the field. It further suggests that true mortality on plots was higher than was established, but remained undetected. This is corroborated by the collection bias reported by Mineau and Peakal (1987) resulting in underestimation of birds that had been severely exposed to aerial sprays of organophosphate insecticides. This provides a strong argument to include gorge-feeding in avian risk assessments of chemical insecticides used for acridid control, and to perform intensive searches for dead or debilitated birds for at least three-four days post-spray.

**Conclusions regarding RQ2** - The results of the present study suggest that sampling of acridids for risk assessment should include mimicking predatory behaviour and be over a longer time course (preferably 3–24 h post spray) than sampling of vegetation (typically 1–2 h post spray). Current regulatory frameworks may underestimate the risk of pesticides applied for locust or grasshopper control. Gorge-feeding appears to pose a high risk from oral uptake of toxic residues leading to acute anticholinesterase poisoning, confirmed by our own data, which should be included in risk assessment procedures for locust and grasshopper control.

### The biopesticide *M. acridum* supports rather than limits birds

**RQ 3** -What are the consequences of application of the entomopathogen *Metarhizium acridum* for locust and grasshopper control on avian predators in the short and medium term?

The strain IMI 330189 of the entomopathogenic fungus *Metarhizium acridum* became commercially available at the end of the 1990s, under the trade name Green Muscle® (GM). It was licensed by CAB International (CABI) to commercial producers between 2002 and 2016 and then again from 2019 onwards when a second product (Novacrid®), containing a different strain of *M. acridum*, was also marketed. The latter product was registered in November 2019 by the Comité Sahélien des Pesticides (CSP) of the Comité permanent

inter-États de lutte contre la sécheresse dans le Sahel (CILSS) in nine Sahelian countries.

Chemical control and biological control with an entomopathogenic fungus are fundamentally different from one another, as is summarized in Table 6.3. Chemical compounds are easily accessible, their uses are well known, and rapid knock-down effects lead to over-reliance on them and under-representation of biological control methods, with dramatic environmental consequences. *M. acridum* has favourable characteristics from an environmental point of view and it deserves a more prominent place in locust and grasshopper control as will be outlined in this chapter. *M. acridum* is especially useful for well-trained acridid control organizations who understand its mode of action as it is not a fast acting product. Under certain circumstances, when the relatively long lag phase is not acceptable, its use is not recommended. For such cases chemical insecticides with a lower avian toxicity than chlorpyrifos and fenitrothion are available and have been assessed by FAO's Pesticide Referee Group (2014).

GM was successfully used in 2009 on 10 000 ha in the Iku-Katavi National Park, the Lake Rukwa plains and the Malagarasi River Basin in Tanzania against Red Locusts *Nomadacris septemfasciata* (FAO 2009). The rapid intervention markedly reduced Red Locust infestations, thereby preventing a full-blown invasion that could have affected the food crops of around 15 million people in the region. In 2013/14 it was used against Malagasy Migratory Locust *Locusta migratoria capito* (1660 ha on a total of 1.2 million ha treated; Everts 2015). In addition, within the Regional Project for Integrated Grasshopper Control in the Sahel (PRéLISS; Kooyman 2006) grasshopper treatments with GM were successfully executed in the Sahel. Some sprayed applications were a mix of reduced GM dose rates with equally reduced dose rates of the pyrethroid cyhalothrin for rapid knock-down and reduced costs (Douro Kpindo *et al.* 2008). Although successful, this would reduce GM to a partially chemical insecticide, removing its advantages over chemical control. Still, in general locust control departments in the various African countries were not eager to use GM on a large scale for reasons summarized in bold in Table 6.3 and operational use until now remains very low.

Chapter 4 describes how GM was applied in a series of experimental treatments using two different dosages to treat *O. senegalensis* and an untreated control in central Senegal. The habitat, a deforested former sylvo-pastoral reserve of >50 000 ha, was optimal for grasshopper development. Bird and grasshopper densities were assessed before and after treatments. Grasshopper densities in the control plots were very high, with 35–39 (up to 90) individuals m<sup>-2</sup> (of which 3–5 adults) in September and 30–35 individuals m<sup>-2</sup> in October (of which 10–19 adults). On the treated plots grasshopper numbers and biomass decreased significantly as they became infected and died. Overall spray efficacy at day 21 was 80%, and the effect was still statistically significant as compared to unsprayed control plots after three months. Birds specifically captured large and medium bodied grasshoppers, but rarely small-bodied species, numbers of which increased initially. This might have been an indirect effect of predation by changing survival rates and competition in the grasshopper community (cf. Belovski and Slade 1993).

An important finding of this study is that, contrary to our results with fenitrothion in particular (Chapter 2), *M. acridum* did not reduce acridivorous bird numbers and densities. These fluctuated over time, but were unrelated to treatments until four weeks post-spray when densities in treated plots were up to 50% higher compared to controls (Fig. 6.1). While relative numbers of acridivorous bird species compared to other bird species did not increase, their biomass did when heavier migrants, such as White Storks *Ciconia ciconia*, arrived. The grasshopper removal rates increased on all plots, but more steeply on treated plots where grasshopper densities had already been reduced by *M. acridum*. It is only at 3.5 months post-spray that grasshopper predation rates in sprayed plots level off and are no longer different from controls. Hence by increasing the duration of the *M. acridum* impact, bird predation enhanced its action.

A year after this study had been finalized, bird exclosures, permeable to grasshoppers, were placed in the same area, on both plots treated with *M. acridum* and untreated control plots, to quantify predation of grasshoppers during the rainy season, the main reproductive period of the species studied. Grasshopper densities inside the cages were  $31 \pm 2$ , 4% (max. 35%) higher than outside cages (repeated measures ANOVA ( $F(1,26) = 17.81$ ,  $P < 0.001$ )), irrespective of treatments, confirming the importance of predation (Mullié 2011).

The second study into the impact of *M. acridum* on avian predation was executed in the desert of northern Niger (Chapter 5). In this case an experimental aerial treatment in dense *Schouwia thebaica* vegetation was executed under operational conditions and populations of adult Desert Locusts and birds and vegetation greenness were assessed simultaneously along two transects. Common Kestrels *Falco tinnunculus* and Lanners *F. biarmicus* were the predominant avian predators and prey remains of the former species were analyzed from pellets and plucking remains. After spraying, kestrels took significantly ( $P < 0.05$ ) more of the larger female locusts (75–80%) than the smaller males (20–25%). The ranges of the main findings of the studies are graphically represented in Fig. 6.1.

The predation on the larger female Desert Locusts corroborated our finding in Chapter 4 that birds preferred the larger sized acridids which in this case were female Desert Locusts. It also confirms earlier findings of Branson (2005) who reported that birds reduced the proportion of medium-bodied grasshoppers, while small-bodied grasshoppers increased in abundance. Belovsky & Slade (1993) found a predation-mediated reduction of large-bodied grasshoppers, whereas changes in abundance of medium and small-bodied species that they observed could not be explained directly by avian predation, but were rather the consequence of indirect effects of the partial removal of large-bodied grasshoppers by changing survival rates of the remaining grasshoppers and competition between them.

By removing large adult females, avian predation probably enhanced the impact of *M. acridum* on the Desert Locust population by reducing reproductive potential. As Lanners captured locusts on the wing, these were probably the least affected ones or had been unaffected by the pathogen. No direct or indirect adverse side-effects were observed on non-target organisms including locust predators such as ants and other insects, birds,

small mammals and reptiles. The falcons continued hunting even after their prey became substantially depleted, an important finding corroborated by similar observations on grasshopper predation by Cattle Egrets in Nigeria (Amatobi *et al.* 1987). These substantial ecological advantages should also be considered when choosing between conventional chemical and biopesticide-based locust control.

**Conclusions regarding RQ3** - Both the grasshopper complex dominated by *O. senegalensis* and a population of Desert Locusts were successfully treated with *M. acridum*. Efficacy took two to three weeks to reach its maximum. Numbers and biomass remained significantly different from control plots for three months. The relative importance of grasshopper consumption by birds increased between October (high grasshopper densities) and December (medium densities) from an initial  $0.06 (\pm 0.03) \% \text{ day}^{-1}$  to a ceiling of  $1.6 (\pm 0.9) \% \text{ day}^{-1}$ . *M. acridum* only affected acridids and none of their predators, including birds, numbers of which increased on treated plots. Total grasshopper removal during the dry season was 70 %, whereas during the rainy season this was  $< 1 \%$ . Birds specifically captured large and medium-bodied grasshoppers, but rarely small-bodied species, whose numbers initially increased. Bird numbers also increased after treatment of Desert Locusts. Analysis of the food of Common Kestrels, one of the principal avian predators, showed that kestrels took significantly more, larger, female (75–80 %) than smaller male (20–25 %) locusts. Avian predation probably enhanced the impact of the GM on the desert locust population, especially by removing large adult females. No direct or indirect adverse side-effects were observed on non-target organisms including locust predators such as ants and birds.

### **Chemical pesticides come with external costs which make *M. acridum* competitive**

In the previous sections it has been argued that biopesticides, such as *M. acridum*, have clear environmental advantages over the chemical insecticides fenitrothion and chlorpyrifos. However, as has been shown in Table 6.3, the costs of *M. acridum* are frequently mentioned as a negative factor when it comes to choosing between chemical insecticides and biological control alternatives.

If we look in more detail at the costs of chemical control, it becomes obvious that there are important external costs or externalities, which are not taken into account. Externalities are defined as economic effects of one agent's behaviour on another agent's well-being, where such effect is not reflected in market transactions (Little and Mirrlees 1974). External costs of pesticides include monitoring for contamination of soil at loading sites, drinking water or food, poisonings of applicators, pickers and consumers, impacts on non-target organisms such as bees, beneficial insects, fish, birds, etc. (Leach *et al.* 2008). These costs are external to the individual decision maker because they are usually absorbed by society (Baumol and Oates 1988, Pretty *et al.* 1999). Pesticide externalities include features commonly found across the agricultural sector: 1) their costs are often neglected; 2) they often occur with a time lag; 3) they often damage groups whose interests are not represented; 4) the identity of the producer of the externality is not always known (Pretty

*et al.*, 1999, Leach *et al.* 2008). Pesticide externalities result in sub-optimal economic and policy solutions (Leach *et al.* 2008).

A pilot study in Senegal of externalities provided an estimate of the full costs of pesticides used in the Desert Locust control campaign in 2003–2005. As pointed out by Leach *et al.* (2008) this is not a cost benefit analysis (but see hereafter). Instead, by concentrating on the costs of a locust control campaign, it aims to provide transparency and accountability of public investments for a “public bad” like Desert Locust outbreak events. Hence the study can be seen as a practical application in accordance with the series of Desert Locust Guidelines of the FAO and thus should provide a tool for decision-makers confronted with Desert Locust control campaigns to better take cost-effectiveness considerations into account (Leach *et al.* 2008).

Based on official figures the area treated by insecticides during Desert Locust control campaign in 2003–2005 was about 750 000 ha and the total pesticide externality estimates were €8,054,448 (Leach *et al.* 2008). When combining the costs in different categories and applying a novel methodology called Pesticide Environmental Accounting developed by Leach and Mumford (2008), it was possible to attach monetary figures to each individual pesticide. Results for fenitrothion 500 UL and chlorpyrifos 240 UL, the formulations studied in Chapter 2, are shown in Table 6.2. For comparison the price of *M. acridum* in a 50 g.l<sup>-1</sup> Green Muscle® OF formulation during the 2003–05 campaign was €14.51.

Even if these are the results of a single study, which has not been repeated since, it is clear that, when taking external costs into the equation, there was hardly a price difference between Green Muscle® and chlorpyrifos (GM being 15% more expensive) whereas the cost related to fenitrothion was 50% higher than that of GM, giving *M. acridum* a clear advantage.

**Table 6.2.** Per hectare costs (€) of pesticide externalities in the 2003–2005 Desert Locust campaign in Senegal (Leach *et al.* 2008).

Type of effects	Chlorpyrifos	Fenitrothion
Applicators	0.84	3.48
Local inhabitants	1.45	3.01
Consumers	1.46	3.04
Ground water	0.27	2.21
Aquatic life	0.80	0.42
Birds	0.52	0.54
Bees	1.54	3.20
Beneficial arthropods	0.29	0.61
Total externalities (€)	7,15	16,51
Pesticide price l <sup>-1</sup> (€)	5,56	5,07
<b>Total cost ha<sup>-1</sup> (€)</b>	<b>12.71</b>	<b>21.58</b>
<b>Extern./direct cost ratio</b>	<b>1,29</b>	<b>3.26</b>

**Table 6.3.** Specific traits of chemical insecticides and the fungal insecticide *Metarhizium acridum* in acridid control. Issues highlighted in bold are frequently used by critics to justify use of chemical insecticides. OP = Organophosphorous compounds; IGR = Insect Growth Regulators; ULV = Ultra-Low Volume.

Issues	Chemical insecticides. In particular, but not limited to, fenitrothion and chlorpyrifos	<i>Metarhizium acridum</i> (trade names Green Muscle® and Novacrid®)
Legislation and use	Reviewed for efficacy and side-effects by Pesticide Referee Group, widespread registration and use by government agencies to control acridids. Catastrophe scenarios and use of non-recommended pesticides resulting from ill-preparedness lead to overdosing.	Until 2020 hardly used for locust control and minor use for grasshopper control in Africa despite proven efficacy. Since Nov. 2019 only registered as Novacrid® in nine Sahelian countries (CILSS).
<b>Speed of action</b>	Rapid knockdown and kill (hours) post-treatment leaving massive quantities of dead arthropods strengthening confidence of users.	No knock-down, mortality 80–90% in 2–3 weeks post-treatment, undermining confidence of users. Predators and scavengers remove dead and debilitated insects as they become available, which strengthens the natural allies.
Persistence	Rapid degradation (days) of OPs under field conditions. IGRs and fipronil persist longer and are recommended for barrier treatments only.	Viable spores persist from seven days to two months. Barrier treatments have shown promising potential. Other studies found some carry-over effect of viable spores into the next year.
<b>Stability and storage</b>	Formulations well developed with known shelf-lives, storage, transport and use without problems. Some formulations corrosive for ULV spray equipment or carriers (planes, vehicles).	Much shorter development history of formulations. Formulated product may settle on bottom of containers, clogging micronair® sprayers leading to frequent cleaning/loss of time, some complaints about short shelf-life of formulated product. Dry spores have known shelf-life and can be stored without problem and formulated <i>in situ</i> .
Non-targets	Most kill non-target arthropods, some also birds (2–7% in this study) and other vertebrates and/or deprive them of their arthropod prey. Fipronil and neonicotinoids are extremely toxic for social insects.	No negative impact on non-target species except on other Orthoptera. Numbers of acridivorous birds remain stable or increase post-spray.
Use on locust hopper bands	During large outbreaks and plagues most products (except IGR and fipronil) are not efficient because of lack of residual action. Therefore new hatchlings need new treatments, especially as natural enemies will have been reduced by the earlier spray.	Very effective and efficient on hoppers, remains infectious during weeks to even months. In this way, together with increased predator action, newly hatched hoppers will be attacked within the infectivity period of <i>Metarhizium</i> and natural predation including by birds.
Use on adult locusts and grasshoppers	Widely used for control of acridids under all conditions. Indiscriminate use of chemical insecticides can cause upsurges later in the season or the next year because egg predators have been killed.	Proven efficacy against adults, can be used in ecologically sensitive areas and where adult groups hardly move or are situated far from crops. Not currently used against (highly mobile) large groups of adults during outbreaks.
<b>Pesticide costs and externalities</b>	Relatively cheap when supplied through FAO but hidden direct and indirect costs from side-effects (externalities such as reduced pollination or human intoxications not normally seen as costs of treatments and paid by society).	Product 2–3 times more expensive per hectare per treatment than chlorpyrifos and fenitrothion but no externalities. Therefore costs of biological insecticides are equal to or less than costs per hectare sprayed than of chemical insecticides, without the negative side-effects of the latter.

Table 6.3. Continued.

Issues	Chemical insecticides. In particular, but not limited to, fenitrothion and chlorpyrifos	<i>Metarhizium acridum</i> (trade names Green Muscle® and Novacrid®)
Human toxicity	Slightly to moderately hazardous, sublethal effects and casualties have been reported. Should not be sprayed on crops, withholding times apply, killed acridids should not be eaten. Use of PPE compulsory.	Unlikely to present hazard under normal use. Can be sprayed on crops. No withholding time. Use of PPE less stringent, although recommended.
Livestock and (organic) farming	Slightly to moderately hazardous. Livestock should be removed prior to spraying. Not compatible with organic farming. Most products not registered for use on crops. Withholding times apply.	Unlikely to present hazard under normal use. No particular safety measures required, although direct spray on livestock should be avoided. Fully compatible with organic farming, no withholding times.
Protected and sensitive areas	Should not be used in protected areas and buffer zones are applicable for environmentally sensitive areas which limits its use.	Safe to be used in protected areas and near environmentally sensitive areas such as wetlands. No negative impacts of spraying near water bodies known.

A cost-benefit analysis of grasshopper control strategies in Senegal with both fenitrothion and *M. acridum*, but without taking externalities into account, as part of the development of integrated pest management (IPM) of grasshopper populations in the Sahel, was done by Fisker *et al.* (2007). Their simulation study showed that the most cost-effective strategy was a combination of annual *M. acridum* treatments of first generation nymphs, when densities were below a threshold of 40 ind. m<sup>-2</sup> in S. Senegal and treatment of 2nd generation adult grasshoppers in N. Senegal. Their results also showed it to be cost-effective for grassland. Chemical treatments were cost effective at densities above the threshold. When combining their results with the outcomes of the externality study cited above, *M. acridum* is also economically superior to fenitrothion above 40 ind. m<sup>-2</sup>.

### Considering birds as allies to regulate acridids with reduced chemical control

**RQ 4** - How do chemical- and bio-pesticide applications compare with respect to effects on avian predators and what does this mean for locust and grasshopper control in the shorter and longer term?

In recent decades the world has faced a dramatic loss of a large proportion of its insect fauna, primarily if not entirely through man-made causes. Dirzo *et al.* (2014) developed a global index for invertebrate abundance that indicated a 45% decline over the last forty years. However, as also stated by Dirzo *et al.* (2014) not all insects have reacted in the same way and several considered as pests of agricultural commodities increased. Indeed, acridid species such as *Oedaleus senegalensis* and *O. asiaticus* have actually increased and became pests of agriculture, and only since the first half of the 1970s (Cease *et al.* 2015).



When taking the Sahel as a relevant example, ongoing and severe land degradation (Ibrahim 2017, UNCCD 2019), deforestation and land clearing (Chirwa and Adeyemi 2019) and continuous grazing far beyond the carrying capacity (UNEP 2012) have created new opportunities for acridids to invade the newly created environments. Concurrently, bird populations in the Sahel have greatly suffered from habitat degradation (Cresswell *et al.* 2007, Zwarts *et al.* 2009, Stevens *et al.* 2010) and the accelerated removal of mature thorny trees has been suggested to play a major role in unprecedented bird population declines (Zwarts & Bijlsma 2015, Zwarts *et al.* 2015, Zwarts *et al.* 2018). As many bird species depend on acridids and other insects during at least a part of their annual cycle, e.g. even nestlings of most granivorous birds entirely depend on insect proteins for growth and development, the gap between the availability of acridid prey and the predation capacity of birds may have widened, in particular during upsurges.

As described in Chapter 1, before the Second World War birds were widely considered as important acridid predators, a view which changed with increases in the application of chemical insecticides since then. However, despite their dwindling numbers, birds are still important as predators capable of limiting acridid developments at low and medium densities, which in turn can prevent acridids from reaching economic thresholds (ETs), a conclusion which was also reached by Kirk *et al.* (2006). In combination with the wise use of biopesticides such as the entomopathogenic fungi *M. acridum* and *B. bassiana*, and the microsporidian *P. locustae* birds should be considered as important allies for controlling locusts and grasshoppers, in particular nymphal stages, in situations before these may develop into a threat to crops, pastures and livelihoods. This might even get an impetus after restoring degraded habitats in known outbreak areas by re-greening (Rey & Winterbottom 2015) and by decreasing or modifying grazing pressure, thereby both offering opportunities for increases in bird numbers while reducing habitats favoured by acridids.

Grazing management in the northern Great Plains of the USA mitigated grasshopper outbreaks on native rangeland (Onsager 2000). Key practices were the deliberate variation in timing and intensity of grazing events, the preservation of canopy during critical periods of grasshopper development, and reductions in areas of bare soil (Onsager 2000). Reducing grasshopper food abundance or availability at critical stages of the insects' life cycle can often be achieved by restoration of degraded savannah habitat that formerly was unsuitable for mass grasshopper development. These goals can also be achieved by habitat manipulation (Belovsky *et al.* 2000) and by increasing the abundance of natural enemies (Belovsky *et al.* 2000, Joern 2000), see also hereafter.

Several such initiatives are already under way (e.g. Hughes 2020) but in order to be really effective for the management of acridid populations, they should be scaled up by the involvement of rural populations who would directly profit from farmer-managed natural regeneration to restore degraded semi-arid agrosystems and landscapes in Africa (Reij and Garrity 2016). When linking these initiatives to knowledge about acridid and bird ecology, the role of birds as "natural allies" may become even more pertinent.



In Figs. 6.2 and 6.3 flow-charts are presented for control of Desert Locusts and grasshoppers respectively under different scenarios. They indicate in which situations:

1. Birds are expected to regulate grasshoppers to below Economic Thresholds (ETs) or prevent locusts from developing swarms;
2. *M. acridum* alone or in combination with Insect Growth Regulators (IGRs) might be used to keep or bring acridids below ETs and
3. Application of selected chemical insecticides, assessed by the PRG (2014), might be considered for this purpose in case (1) and (2) are insufficiently reducing acridid populations below ETs.

Bird predation and *M. acridum* are fully compatible, whereas bird predation might be constrained by the use of chemical insecticides, such as fenitrothion and chlorpyrifos, as shown in this thesis. However, the current PRG (2014) risk assessment procedure falls short when it comes to:

1. modelling probability of bird mortality in the field, e.g. by incorporating relative dermal to oral toxicity and  $HD_{50}(50\%)$  rather than  $LD_{50}$  values as an indicator for avian oral toxicity (cf. Mineau 2002a and b);
2. including indirect effects such as prey depletion, and
3. including behavioural aspects, most notably gorge-feeding in exposure estimation.

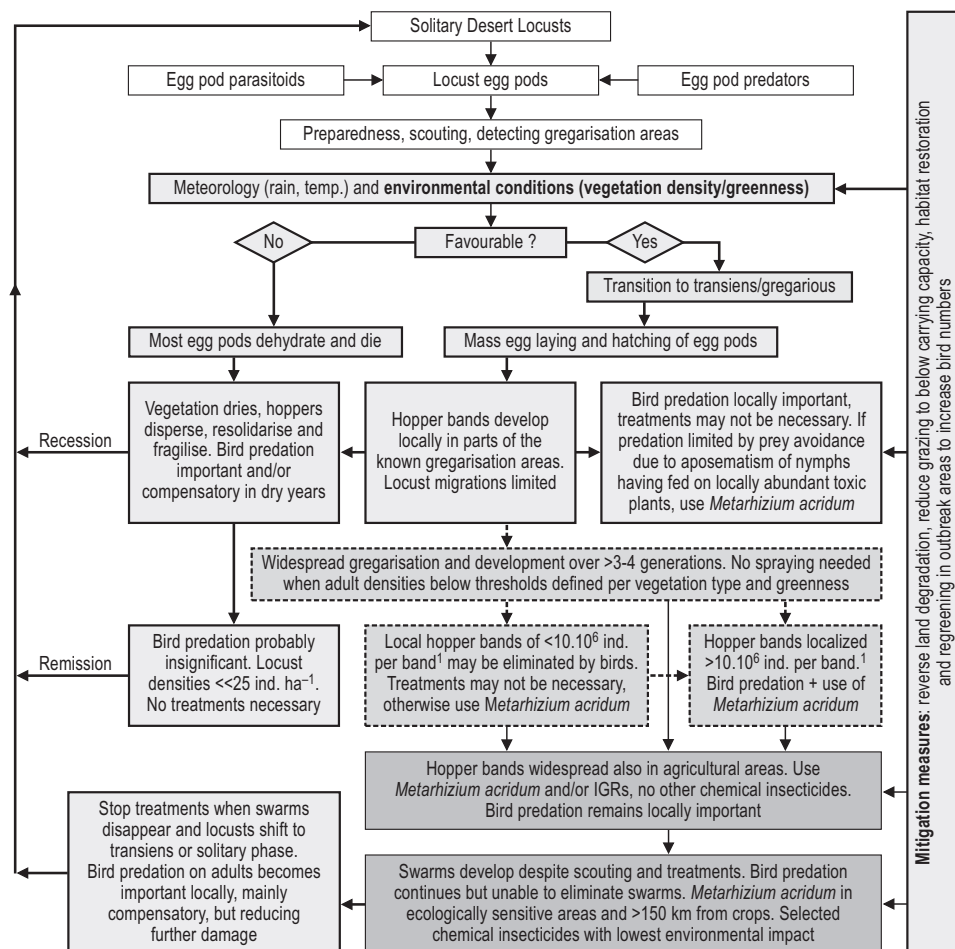
To more realistically assess avian risks of insecticides currently used or intended for future use in locust and grasshopper control, these aspects need to be taken into account.

The capabilities of birds regulating acridid populations as indicated in Figs. 6.2 and 6.3 are not hypothetical but based on my own (this thesis) and previous research. Kirk *et al.* (2006) used published data to calculate that the potential removal of grasshoppers by the breeding bird community in Saskatchewan and Alberta grasslands, Canada, could be as high as over four times the economic threshold of 50,000 ha<sup>-1</sup> cited by McEwen (1987). Even if these data are too optimistic, it shows that bird predation on grasshoppers is of substantial value to farmers (Kirk *et al.* 2006).

The use of thresholds has also been advocated for Desert Locust gregarisation risk. Cissé *et al.* (2013) argue that a locust control centre that would accept a 5% risk of gregarisation could avoid to treat a dense and developing vegetation holding 200 adults ha<sup>-1</sup>. On the contrary, gregarisation is more likely to happen if locusts are in a drying vegetation with a density of 300–400 adults ha<sup>-1</sup> (Cissé *et al.* 2013). Applying such criteria in combination with quantitative observations of bird predation would be of great value to make practical use of natural factors that regulate acridids.

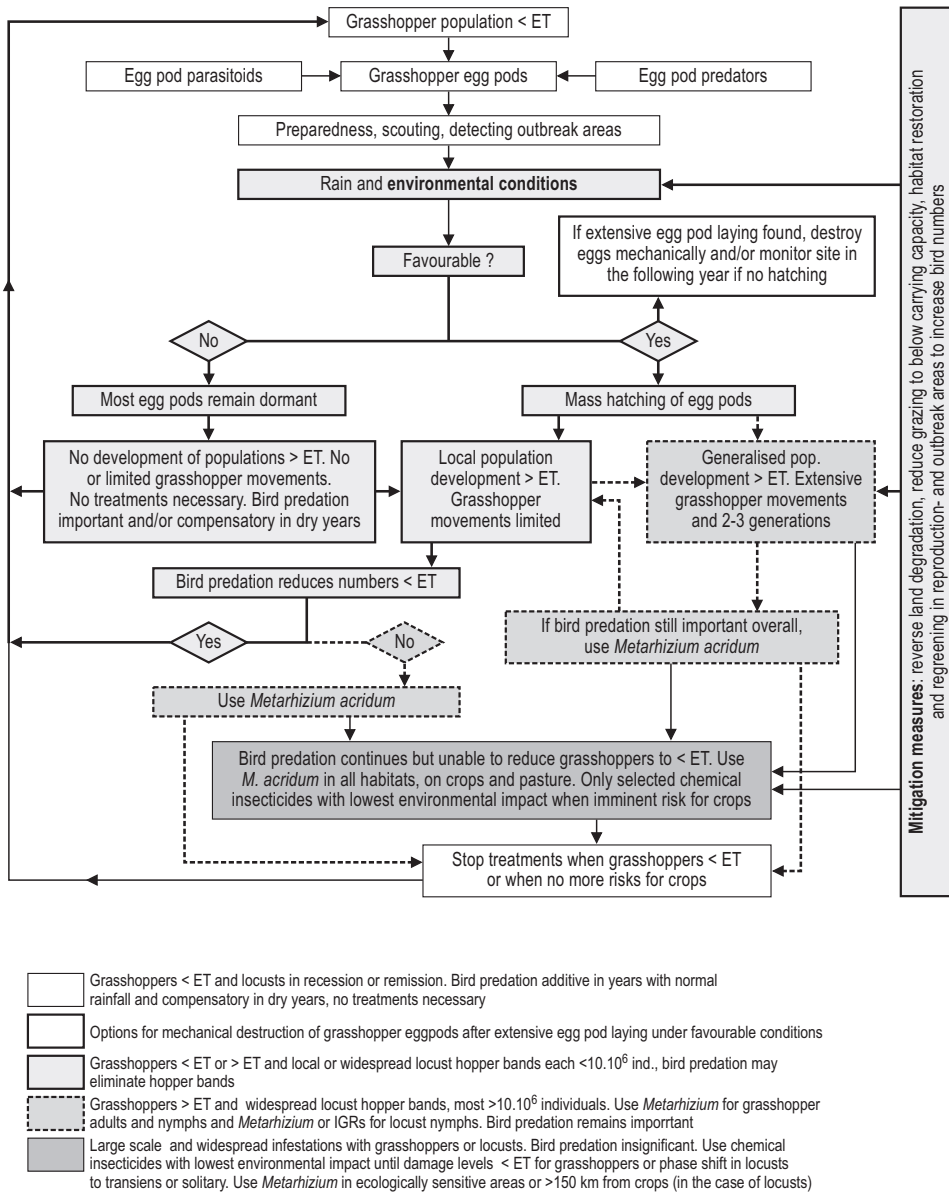
Several experimental exclusion studies of up to four years in the USA showed the potential of avian predation in regulating grasshopper populations. When densities were low in dry years no differences between plots with and without birds were detected (Fowler *et al.* 1991, Joern 1992). In years with normal rainfall, reductions of 25–33% were detected (Joern 1986, Bock *et al.* 1992). Although adult grasshopper density was >2.2 times higher, and mean nymph density >3.0 times higher, on plots from which birds were excluded,

Bock *et al.* (1992) concluded that birds still failed to qualify as "keystone predators" (*sensu* Paine 1966, 1969), at least in the short term. However, the unexpected increase in small-bodied grasshoppers as a result of modifications of competitive interactions between the different size-classes caused by avian predation in an enclosure study by Belovsky and Slade (1993) led them to conclude that birds were indeed keystone predators.



<sup>1</sup> From  $15.2 \cdot 10^6$  to  $5.2 \cdot 10^6$  individuals in 14 days, or a reduction of  $10 \cdot 10^6$  individuals by all avian predators, Eritrea (Ashall & Ellis 1962)

**Figure 6.2.** Flow chart of Desert Locust development scenarios depending on environmental and meteorological conditions. Birds can eliminate hopper bands under specific conditions reducing the need for biological or chemical interventions. Birds may be unable to eliminate hopper bands locally and then *M. acridum* and/or IGRs are the preferred strategy. If action was taken too late or environmental and meteorological conditions become overwhelming and swarms develop, chemical treatments may become necessary. Mitigation measures in regular outbreak areas to reduce suitability for locust development and improve conditions for birds to re-establish themselves are needed in all scenarios. For the legend see fig. 6.3.



**Figure 6.3.** Flow chart of grasshopper (notably *O. senegalensis*) development scenarios depending on environmental and meteorological conditions. Birds can eliminate grasshopper concentrations under specific conditions to below an Economic Threshold (ET) reducing the need for biological or chemical interventions. Birds may be unable to eliminate grasshopper populations locally to below the ET and then *M. acridum* is the preferred strategy. If widespread and massive grasshopper populations develop, chemical treatments may become necessary. Mitigation measures in outbreak areas to reduce suitability for grasshopper development and improve conditions for birds to re-establish themselves are needed in all scenarios.

A simulation study of the role of birds and egg-pod predators on Senegalese Grasshoppers (Axelsen 2009, Axelsen *et al.* 2009) showed that the combined natural predation caused reductions of between 60 and 75%, of which some 25–30% were by birds (Jørgen Axelsen, *pers. comm.*). These reductions are of the same order of magnitude as those found in the above-mentioned experimental exclusion studies and the exclusion study in Senegal (Mulli   2011). As a preliminary follow-up exercise, Halvorsen & Axelsen (2014) used the grasshopper density data from Chapter 4 in this ecosystem model, to assess control response timing and the contribution of birds with different runs of the model. Their initial results show that correct timing of control with *M. acridum*, about five to 10 days earlier than usual, will largely increase the impact of bird predation particularly in the dry season. The results of Halvorsen & Axelsen (2014) further suggest that actively supporting recovery and increase (doubling) of acridivorous bird population levels would be the most effective option. In Figs. 6.2 and 6.3 this is indicated under the mitigation measures of habitat restoration and re-greening.

The option to increase bird populations locally to enhance avian acridid predation potential is not a novel approach, as it has been applied in China for centuries (Yu 1988a), both by improving breeding conditions such as erecting rock piles for nesting, planting preferred shrubs and digging water holes for the Rose-coloured Starling *Sturnus roseus* (Yu 1988b) and by temporarily introducing massive herds of domestic fowl, such as ducks and chickens (Jun *et al.* 2014, Sun *et al.* 2016, Su *et al.* 2018). In some areas of India and Pakistan, Rose-coloured Starlings were claimed to have eliminated 50% or more of Desert Locust hopper bands in 1941, 1943 and 1951 (Khan 1953). China is one of the few countries where bird predation, either natural or by herded ducks and chickens, is actively used as a very important contribution to biological locust control (Zhang and Hunter 2017, Shi *et al.* 2019).

The Rose-coloured Starling is one of the most cited bird species as an acridid predator in the Middle East and Asia. When this species was present, average densities of grasshoppers during autumn migrations were 3.3, 3.6 and 3.0 times higher in bird exclosures than in open plots in 2005, 2006 and 2007, respectively (Ji *et al.* 2008). They concluded that the impact of *S. roseus* is sufficient to reduce grasshoppers to an acceptable level. However, their recommendation to artificially increase population numbers so that they "*can function as a biological control agent for insect pests in breeding areas worldwide*", is probably neither realistic nor to be followed. Like other starling species Rose-coloured Starlings are socially organized and shift prey according to the season which leads to their appreciation by man shifting equally from being hugely advantageous to being a pest. Baddeley (1940) in "*The rugged flanks of Caucasus*" recounts that Rose-coloured Starlings were called Birds of Mahomet or Holy Birds because of their locust-devouring qualities, but Devil Birds when they devastated fruits in orchards and vineyards two months later.

Ironically, several granivorous birds such as *Quelea quelea*, "Africa's feathered locust" (Mundy and Jarvis 1989), *Ploceus* weavers and Golden Sparrows *Passer luteus* which are perceived as serious pests to irrigated and rainfed agriculture in sub-Saharan Africa and destroyed by the millions, are also very important acridid predators when they are

breeding and feeding their young. Both in Niger and in Mauritania, Golden Sparrows were observed to be important predators attacking Desert Locust nymphs (Chapter 5, Kooyman *et al.* 2008), while Wilps (1997) found elimination rates of 95–99.5% of Desert Locust hopper bands containing 0.15–1.1  $\cdot 10^6$  individuals by Golden Sparrows, Desert Sparrows *P. simplex* and Cream-coloured Coursers *Cursorius cursor*. Village Weavers *P. cucullatus* were found to be the main predator of Senegalese grasshoppers in Senegal (Axelsen *et al.* 2009). The deforestation of Khelcom, the study site in Chapter 4, which created an ideal habitat for grasshopper development on >50 000 ha also attracted, at least for a number of years and during the northern winter, massive numbers (>10<sup>4</sup>) of Palearctic acridivorous birds such as Montagu's Harriers *Circus pygargus*, Lesser Kestrels *Falco naumanni* and White Storks (Mulli   2009). Therefore, habitat destruction and -restoration does not always lead to expected outcomes.

**Conclusions regarding RQ 4** - Chemical and biopesticide applications are fundamentally different in their impact on both acridids and their avian predators. The organophosphates fenitrothion and chlorpyrifos were found to be widely used, acted within hours but degraded within days, had sufficient shelf life, killed non-targets including birds, were not suitable for treatment of hopper bands, had high external costs, were slightly to moderately toxic to humans and livestock and not suitable for use in or near protected and environmentally sensitive areas. The biopesticide *M. acridum* was hardly used, had a two-three week lag phase before it achieved its peak efficacy, but had a sustained action for at least three weeks and possibly up to three months, has sufficient shelf life as dry spores but not as formulated product, has no impact on vertebrate or invertebrate non-targets, is very effective to both adults and nymphs and could be used in barrier treatments, has no external costs (and therefore economically competitive with chemical insecticides) and is safe to humans and livestock and can be used in protected and environmentally sensitive areas. It was found that in most situations bird predation alone or combined with the use of biopesticides would reduce acridids, in particular grasshoppers, both adults and nymphs, to below Economic Threshold levels. In those cases where this objective would not be achievable, chemical pesticides with the lowest environmental footprint and assessed by FAO's Pesticide Referee Group should be used, with the exception of fenitrothion and chlorpyrifos. Preventive measures by using biopesticides and large scale habitat restoration and grazing management to reduce suitability for acridid development and to increase bird numbers concurrently were identified as achievable goals with the highest probability of success.

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## Future perspectives and recommendations

In 2020, for the first time during a Desert Locust outbreak the biopesticide *Metarhizium acridum* was used operationally. In Kenya it was used on 140 ha which, however, constituted less than one percent of the total surface area treated from January till July 2020. In Somalia, since the start of control operations in January until July 2020, 34 225 hectares

have been treated using biopesticides in Somalia's key breeding areas (FAO Somalia newsletter 2020 update 6). This is therefore, to date, the most significant contribution of a biopesticide to Desert Locust control, but it also shows that, despite its huge environmental advantages, its total use is still less than 5% of the total surface area treated in the Greater Horn of Africa.

As has been argued in paragraph 6.3 and shown in Figs. 6.2 and 6.3, in many cases avian predators and other natural enemies will be able to eliminate individual hopper bands or large patches of grasshoppers without the need for human interference. If natural enemies are not expected to sufficiently reduce acridid numbers within a given period when crops or pastures are being threatened or preventing them from migrating, treatment with *M. acridum* is by far the preferred strategy as *M. acridum* has both economic and environmental advantages over chemical control. Furthermore, as has been shown in Chapter 4, it has no negative impact on birds and avian predation on acridids and even extends the impact of *M. acridum*. However, national and regional locust and grasshopper control organizations have generally little knowledge about birds and/or their importance as acridid predators and unless this is actively mitigated by training, documentation and involvement in research, it will continue to hamper a change in attitude. The publication (in French) on the role of birds in acridid control (Petersen and Axelsen 2009) in the series operational acridology of Agrhymet Regional Centre is certainly a promising starting point.

Widespread use of biopesticides, removal of fenitrothion and chlorpyrifos from the Pesticide Referee Group list of assessed insecticides for locust and grasshopper control, development of more appropriate risk assessment procedures including secondary effects, use of appropriate toxicity endpoints and gorge-feeding, large scale bird habitat protection and restoration in the Sahel, in particular of formerly forested areas, the protection and planting of thorny trees in savannah habitat (Zwarts *et al.* 2018) and a reduction or modification of livestock grazing in these areas, will offer opportunities for bird populations to recover and exert more of an impact on acridid populations and, depending on their scale, will concurrently reduce suitable habitats for grasshoppers. These are supposedly the measures most easy to implement helping to bring back our natural allies to previous numbers and densities and concurrently improve livelihood conditions for, and resilience of, subsistence farmers and livestock owners in affected semi-arid regions of Africa.

The increasing place of biological locust and grasshopper control in China, both by using biopesticides and by attracting acridivores by improving breeding conditions, should serve as an example to change the chemical oriented views prevailing up to the present in Desert Locust and Senegalese Grasshopper control.

Perhaps most importantly, politicians should be made aware of the economic benefits of preparedness, applying biological locust and grasshopper control, regulating grazing pressure and restoring degraded lands. If not, the next major upsurge will again trigger panic reactions with massive use of chemical insecticides and, potentially, famine.

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## Addenda

# Summary

*“Don't kill your allies, unless they're annoying.” – A Mercenary's Guide to Prosperity*

*“Welcome allies with open arms.” – The Unofficial Guide to Heroism...*

Eve Langlais - 2014 - Heroic Abduction: Alien Abduction #5 - Page 19

Before the large-scale introduction of chemical insecticides after the Second World War (WW2), birds were considered worldwide as welcome natural allies to control acridids. Until then, mechanical control e.g. by harvesting grasshoppers with ingenious dozers or digging trenches to interrupt marching hopper bands were chemically supplemented by massive use of arsenicals. The large scale introduction of dieldrin in locust control around 1955 marked a turning point as for the first time a persistent chemical became available to treat hopper bands while providing toxic residues which killed hoppers emerging afterwards. The high toxicity for man and wildlife and the persistence of dieldrin lead to a worldwide ban twenty years later, but in locust control it took until 1989 before its use eventually was discontinued. Organophosphorous insecticides had dominated the acridid arena since WW2 with varying contributions of carbamates, pyrethroids and some other chemical families. The interest in birds as natural allies had faded because of a widely held believe that the insecticides did a better job. In addition, the impact on birds of their widespread and large-scale use had neither been studied in Africa, nor its potential consequences for acridid control.

It was against this background that studies of the effects of applications of locust insecticides on birds were conducted in the savannah of northern Senegal in 1989 (Chapter 2). The organophosphorus insecticides studied were fenitrothion at 485 and 825 g ha<sup>-1</sup> and chlorpyrifos at 270 and 387 g ha<sup>-1</sup>. Total bird numbers decreased on all treated plots. Decreases in three of the most abundant species were significant on the fenitrothion plots. Some of the decrease was due to bird mortality, but most bird reduction seemed to be due to movements of birds in reaction to a reduction in their arthropod food. The reduction in grasshoppers was four times greater on the fenitrothion plots than on the chlorpyrifos plots, and this difference was reflected by a decrease in the insect foods eaten by birds after the treatments. Buffalo Weaver and Singing Bush Lark reproduction was monitored on plots. Weavers deserted most colonies in all plots, while large numbers of debilitated fledgling larks were found during plot searches. Effects on weaver desertion probably were indirect through food reduction, but debilitation in lark fledglings was due to direct poisoning. Fledglings showed severe reduction in brain cholinesterase levels; inhibition was comparable to that in adult birds found dead or debilitated immediately after treatments. Brain cholinesterase levels in live birds collected 1 week posttreatment were slightly reduced, but levels were normal after 3 weeks. Reduced reproductive success was the most serious impact identified, as it had the greatest potential for long-term effects on bird populations.



The need for locust control throughout eastern Australia during spring 2010 provided an opportunity to quantify residues of fenitrothion on nymphs of the Australian plague locust, *Chortoicetes terminifera* (Chapter 3). Residues were collected across the different physiological states – live, dead, and debilitated (characterized by ease of capture, erratic hopping, and the inability to remain upright) – of locust nymphs observed following exposure to fenitrothion. The time course of residue depletion for 72 h after spraying was quantified, and residue-per-unit dose values in the present study were compared with previous research. Fenitrothion residue-per-unit dose values in the different physiological states overlapped and ranged from  $6.3 \pm 1.3 \mu\text{g g}^{-1}$  in live *C. terminifera* nymphs, to  $7.8 \pm 1.3 \mu\text{g g}^{-1}$  in debilitated nymphs, and  $16.5 \pm 2.8 \mu\text{g g}^{-1}$  in dead nymphs. Residues of the oxidative derivative of fenitrothion, fenitrooxon, were generally below the limit of quantitation for the analysis ( $0.02 \mu\text{g g}^{-1}$ ), with 2 exceptions – 1 live and 1 debilitated sample returned residues at the limit of quantitation. The results of the present study suggest that sampling of acridids for risk assessment should include mimicking predatory behavior and be over a longer time course (preferably 3–24 h post-spray) than sampling of vegetation (typically 1–2 h post-spray). Current regulatory frameworks may underestimate the risk of pesticides applied for locust or grasshopper control because birds likely feed on prey containing higher residues than accounted for in the risk assessment.

Parallel with the onset of the study of the impact of organophosphates on birds an international consortium, called LUBILOSA, had started to develop a mycoinsecticide based on aerial conidia of the entomopathogenic fungus *Metarhizium acridum*. It was widely felt that such a biopesticide might strongly reduce the need for chemical treatments while obtaining better results, i.e. not causing an impact on predators or parasitoids of locusts and grasshoppers and not causing environmental and human health side-effects. By the end of the 1990s a product was commercialized under the name Green Muscle® (GM) and eventually registered in nine Sahelian countries. How the impact of GM on birds, being the principal predators of locusts and grasshoppers, would compare with chemical insecticides, however, remained largely unknown.

A study at Khelcom, Central Senegal, from September 2008 till June 2009 tested two different dose rates (25 and 50 g conidia ha<sup>-1</sup>) against grasshoppers in a field trial on nine 400-ha plots in three blocks, including controls (Chapter 4). The study area was a deforested sylvo-pastoral reserve, 12.5% cultivated, whereas the remainder was fallow or grassland in succession. Grasshopper and bird densities were calculated from transect counts. Grasshoppers were also assessed by quadrat counts and sweep-net sampling on plots. Energetic requirements of individual bird species were calculated from digestibility-corrected Field Metabolic Rates (FMRs) and for acridivorous species their daily intake of grasshoppers was calculated. Grasshopper densities were very high, with up to 90 ind. m<sup>-2</sup> in September and 30–35 ind. m<sup>-2</sup> in October. Numbers and biomass decreased on treated plots as grasshoppers became infected, and remained significantly different from control plots for three months (until January). Total grasshopper removal during the dry season was 70%, whereas during the rainy season this was < 1%. Birds specifically captured large and medium-bodied grasshoppers, but rarely small-bodied species, whose numbers

initially increased. These findings were corroborated by field observations and by analysis of regurgitated pellet contents of Montagu's Harriers, i.e. small bodied grasshoppers were only 1.4–1.6% of all grasshoppers taken, whereas they constituted 61–68% of random samples from the field. Densities of acridivorous Palaearctic migratory birds, in particular White Stork, *Ciconia ciconia*, Montagu's Harrier, *Circus pygargus* and Lesser Kestrel, *Falco naumanni*, were very high and unprecedented elsewhere. Their numbers largely exceeded the 1% criterion for international importance.

The Desert Locust upsurge of 2003–2005 offered an opportunity to test the impact of GM also on bird predation of locusts. As the study of Chapter 4 shows, GM-treated grasshoppers attracted predators. This would equally be a complementary advantage in locust control. Therefore avian predation on a population of Desert Locusts in northern Niger aerially sprayed operationally with GM with 107 g viable conidia ha<sup>-1</sup> was assessed (Chapter 5). Populations of adult locusts and birds and vegetation greenness were assessed simultaneously along two transects from 12 days before until 23 days after treatment. Common Kestrels *Falco tinnunculus* and Lanners *F. biarmicus* were the predominant avian predators. Regurgitated pellets and prey remains were collected daily beneath “plucking posts” of kestrels. Locusts started dying five days post-spray and GM had its maximum effect one-two weeks after the spray, with 80% efficacy at day 21. After spraying, bird numbers increased significantly concurrent with decreasing desert locust densities. Locust numbers decreased significantly ( $P < 0.001$ ) with both time since spraying and decreasing greenness. Before spraying, kestrel food remains under plucking posts accounted for  $34.3 \pm 13.4$  prey items day<sup>-1</sup>, of which  $31.0 \pm 11.9$  were adult desert locusts (90.3%), which reduced post-spray to  $21.1 \pm 7.3$  prey items day<sup>-1</sup>, of which  $19.5 \pm 6.7$  were adult desert locusts (92.5%). This reduction was attributable to decreased use of the plucking-posts by the kestrels. After spraying, kestrels took significantly ( $P < 0.05$ ) more larger female (75–80 %) than smaller male (20–25 %) locusts. Avian predation probably enhanced the impact of the GM on the desert locust population, especially by removing large adult females. No direct or indirect adverse side-effects were observed on non-target organisms including invertebrate and vertebrate locust predators such as ants and birds. These substantial ecological advantages should also be considered when choosing between conventional chemical and biopesticide-based locust control.

More than 30 years since the development of *M. acridum* as a selective fungal insecticide for grasshoppers and locusts had started, potential users in Africa still prefer chemical insecticides as is shown by the ongoing outbreaks in the Greater Horn of Africa and beyond. This is partially due to misconceptions about its real costs by not taking into account the externalities associated with chemical insecticides and more importantly by a lack of scouting and early intervention in adjacent countries and a lack of planning to include the lag phase which is inherent to any bioinsecticide. This thesis explains how depending on the different stages of acridid development, birds can play an important role in acridid control and under several conditions even could prevent grasshoppers from reaching Economic Thresholds or Desert Locusts from forming swarms, by combining natural predation with the use of *M. acridum*. The use of chemical insecticides can be

reduced much further to narrow the window of application than currently is the case. The research presented in this thesis also outlines why fenitrothion and chlorpyrifos should be considered for removal from FAO's Pesticide Referee Group assessments because of their negative effects on natural predators of acridids. In all scenarios there is a strong need for habitat restoration and grazing management as these factors would reduce opportunities for acridid development and increase bird numbers. The increasing place of biological locust and grasshopper control in China, both by using biopesticides and by attracting acridivorous birds by improving breeding conditions, is a good example of how to change the chemical oriented views prevailing up to the present in Desert Locust and Senegalese Grasshopper control. If this more comprehensive approach that will include the ecosystem services provided by avian allies will not be prepared in time and the necessary measures not executed, the next major upsurge will again trigger panic reactions resulting in massive use of chemical insecticides and potentially famine.

# Acknowledgements

The road leading to this thesis was initially unpaved and in the course of the past 30 years gradually became more accessible as upsurges of Desert Locusts and Senegalese grasshoppers developed and an outbreak of Australian Plague Locusts offered a chance to expand research to another continent. After all, major Desert Locust upsurges or plagues remain rare events, and in the period covered in this thesis there were only three: 1986–1989, 2003–2006 and 2019 till now. The idea of putting the results of this research in the form of a thesis developed over the years, but after a first planning to accomplish it before my 50th, than 60th birthday, eventually it could only be realized before my 70th, after having retired and by gratefully taking advantage of the covid-19 imposed travel restrictions and lockdown...

Having had a professional life spanning five decades (and still continuing...), makes it not easy to draw a line as to whom extend my thanks and whom to omit. Most persons having been instrumental for the execution of the studies covered in Chapters 2 through 5 are either co-authors or have already been mentioned in the acknowledgments of the individual chapters. They include nationals from many different countries who largely contributed to my abilities to work in multi-cultural environments. Here, I will limit myself to those colleagues and friends who have helped me over the years to make it happen, eventually. If I unintentionally have forgotten others having contributed in one way or another to my thesis, please accept my apologies and be assured of my sincere gratitude.

Although covid-19 certainly allowed me to reflect and start writing the missing parts (Chapters 1, 5 and 6) it was Tinka Murk, my promotor, who was the real driving force to keep me on track. For more than 20 years Tinka has been trying to get me finishing this PhD: first at Toxicology, then at Environmental Technology and eventually at Marine Animal Ecology. What a destination for a purely terrestrial subject...! Tinka never stopped believing that one day I would do it, but without her continuous support it would still be in the same rudimentary phase as it was before the first lockdown. Thanks Tinka.

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I started in 1989 in Senegal for FAO in a pilot project to study the impact on birds of chemical grasshopper and locust control together with the late Jim (James O. ) Keith. Jim was a very experienced researcher from Denver Wildlife Research Center and apart from doing good field research he taught me that a candle only burns on one side... Very wise words which often came to my mind (although sometimes a bit too late...) when over the years I had pushed my collaborators once again to the brink of exhaustion during very demanding fieldwork... I feel this is the place to ask them to forgive me, balma'akh as we say in Senegal.

James Everts, had supervised part of my MSc work at Wageningen University, and he also led the environmental study in Senegal of which our bird research was a part and later the Locustox Project before he continued the work on locusts from the FAO headquarters in Rome. We collaborated throughout my work on acridids. Over the years I developed a special friendship with James, although we often disagreed on academic issues, but James was open for arguments. I owe him a special word of thanks.

I also wish to extend my thanks to four other Dutch colleagues: Harold van der Valk, Joost Lahr, Gerrit van de Klashorst and Christiaan Kooyman. With Harold and Joost I collaborated first during the pilot study in 1989 and further for a number of years in the Locustox project. Joost later did his PhD on his aquatic work in the project and built a career at Alterra and recently RIVM, both in The Netherlands. Harold ultimately became a successful international consultant on pesticide legislation. Both became good friends and both have helped me over the years to resolve many questions related to this thesis. Gerrit was my colleague during the period that we worked in the sub-regional Desert Locust office in Dakar and we stayed friends ever since. Together with James, operating from Rome, we developed the QUEST approach and trained national teams throughout the African Desert Locust outbreak area to reduce the environmental footprint of locust control. We also organized together the first international conference on the use of *Metarhizium acridum* in locust control. Gerrit further made the first drafts for a project to obtain funding from the Japanese Embassy in Dakar which later led to the study in Chapter 4 of this thesis. Christiaan was a colleague in Niamey, Niger, in the early 1990s when he was developing Green Muscle® (GM) containing *M. acridum* as a biopesticide, together with his team members of the Lubilosa consortium. Later in Senegal we became colleagues again, when we advised the First Lady who owned a company which produced GM. This came to an abrupt end when Senegal's President was not re-elected for a third term... Christiaan and I did several mutual field studies to the efficacy of GM and the impact of bird predation on acridids. He has also helped me consistently over the years to identify grasshoppers which I had photographed but remained uncertain which species they actually were.

The late Dr Clive Elliott, with whom I have closely collaborated from 1993 till his retirement in 2006. both on issues related to granivorous birds and Desert Locusts. We shared a common passion for birds and this helped me to obtain funding through FAO for the study

of bird predation in Niger, leading to Chapter 5 of this thesis. Over the years Clive and I had many discussions on the use of mass trapping devices to reduce *Quelea quelea* damage to crops and submitted several proposals for funding. Unfortunately donors were reluctant to fund such projects, involving the destruction of birds, even if it would reduce damage to crops and in particular reduce the use of avicides.

Many colleagues at FAO helped me over the years, be it with administrative matters, security clearance or help in the field. I'd like to thank two colleagues in particular: the late Joyce Magor for the many discussions and help in finding data and Helena Eriksson for discussions, collaboration during field work and for her friendship.

The precautionary principle triggered by Covid-19 reduced the official ceremony of the defence in the Aula to an online event. Therefore I'd like to thank in particular my two paranymphs Almut Schlaich and Gerrit van de Klashorst for their share in the preparations and their moral support. It is thanks to them that you have a copy of the thesis in your hands (or on your screen...) as they organized the invitations, printing and distribution. Thanks Almut and Gerrit.

Dick Visser professionally redrew the figures and prepared my thesis for the printer. I feel particularly lucky that Dick accepted my request to do the entire trajectory from files in Word to the final print, as I know he is a very sought after graphic designer. Really appreciated Dick, thanks.

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This thesis, by the sheer length of time it took to accomplish, came at great personal costs. Sarah, my eldest daughter, I know that I was too often away during a critical phase of your life. Staying weeks or even months in the field, out of reach in the deserts of Niger, Mauritania or the Mahgreb. I cannot give you back the time we missed together, therefore I have dedicated this thesis to you, your children (my grandchildren) and siblings (my other children). To Marième, whom I met during the *Metarhizium* conference in 2007, I wish to express my thanks for your support while I was in central Senegal, Australia and Canada working on research which lead to several chapters in this thesis. I even missed the birth of our daughter Aïcha by 30 minutes, being unable to cover the 250 km from my study area in Khelcom in time. I regret that our marriage, which I used to call the best result of the conference, did not withstand the test of time.



# Curriculum Vitae

Willem Cornelis (Wim) Mullié was born on 17 March 1952 in Middelburg, The Netherlands. After finishing his preparatory scientific education (Hogere Burgerschool-A) in 1971 he started working at the Public Works Department (Provinciale Waterstaat) of the Province of Zeeland, starting as an administrative civil-servant in the section of dikes and waterways. Thanks to the efforts of his farsighted superior, the late Cor (C.A.L.) Kotvis, who realized that his heart was outside, and not within, the confinement of four office walls, he was able to move to the environmental section where he became responsible for the field coordination of provincial responses to avian disease (botulism and avian cholera). He was also responsible for comparative industrial and environmental photography culminating in his photographic contributions to the exposition and book "*Waken en Bewaren*" (1981) on the history of 100 year Provinciale Waterstaat. Because his initial secondary education lacked mathematics, physics and chemistry, preventing him to qualify for either a position or an academic study in biology, he attended secondary evening education while working part-time and obtained his Atheneum-B diploma. In 1983 he left Provinciale Waterstaat and started a study Population Biology at the then Landbouwhogeschool (which became Landbouwniversiteit in 1986 and Wageningen University & Research in 1998) in Wageningen, The Netherlands, and in 1989 he obtained his MSc ("Ingenieur") diploma *cum laude*. During his studies he also executed ornithological research in Egypt, together with colleagues from The Netherlands, the USA and Egypt. This led to a multitude of publications among which the book "*The Birds of Egypt*" (Oxford University Press, 1989) and in 1986 he was recipient of the prestigious Herman Klomp Award for a study on the decline of birds of prey in Egypt which he had executed together with his Dutch colleague and friend Peter Meininger. Immediately after obtaining his MSc degree he was asked by his supervisor to participate in a multidisciplinary study on the environmental side-effects of locust- and grasshopper control in Senegal, under a contract with the Food and Agriculture Organization of the United Nations (FAO). His contribution to this study is also Chapter 2 of this thesis. Upon his return to The Netherlands he was contracted for a desk study to the uses of ionizing radiation at the Netherlands' Institute for Public Health and the Environment (RIVM) before being employed by the Netherlands' Ministry of Foreign Affairs (DGIS) as a lecturer and researcher in Ecology and Ecotoxicology at the Département de Formation en Protection des Végétaux (DPFV) at the Agrhymet Regional Centre in Niamey, Niger (1991–1995). Here he trained 125 professionals of nine Sahelian countries, many of whom later became high ranking personalities within their respective governments or followed further academic careers. In 1995 he returned to Senegal to join his Dutch colleagues from the 1989 pilot study in the follow-up FAO Locustox Project to study environmental, human health and livestock side-effects of acridid control. This proved to be a very productive period, lasting until 2004, in which he first lead the section on vertebrate toxicology, and after the departure of his Dutch



colleagues the general section biology, which also included aquatic and terrestrial ecotoxicology. Eventually, after departure of the Chief Technical Advisor, who was his former supervisor at WUR, he was appointed to this position. Immediately after ending his contract, he was rehired by FAO until 2007, now as a consultant, to execute field research and train colleagues of National locust control organizations throughout Western, Northern and Central Africa during the Desert Locust upsurge of 2003–2006. He was also appointed as head of the temporary sub-regional Desert Locust office in Dakar. One of the studies, executed during this contract together with one of his co-promotors of this thesis, became Chapter 5. The last activity under his contract was the organization, together with FAO colleagues, of an International Conference in 2007 on the use of biopesticides in acridid control. The results of this conference eventually paved the way for FAO and the Pesticide Referee Group (2014) to recommend the entomopathogenic fungus *Metarhizium acridum* as the pesticide of choice -whenver possible- in locust- and grasshopper control. After the end of his contract with FAO he established himself as a private international consultant in environmental toxicology, biological research and scientific photography. He executed a study to the efficacy of *M. acridum* on grasshoppers for a private company, and wrote an extensive chapter on birds, locusts and grasshoppers in the book "*Living on the edge*" which book was awarded "*Best Bird Book of the Year*" and received the "*Luc Hoffmann Medal*". He was subsequently appointed as Chief Technical Advisor at the Senegalese Ministry of Agriculture to execute a study from 2009 till 2011 to the efficacy of low dosages and the environmental impact of the use of *M. acridum* on birds and honey bees. A part of the results of the first phase of this project became Chapter 4 in this thesis. Concurrently with the ongoing research in Senegal, in 2010 he executed a study, together with one of his co-promotors of this thesis and a colleague of the Australian Plague Locust Commission, to the behaviour of birds in reaction to organophosphate sprays and to residues in locust nymphs. The second part of this study is Chapter 3 of this thesis. From 2012 till 2017 he lead a field team of Mauritanian scientists and lab technicians to establish environmental baseline levels of micro-contaminants and biomarkers in the marine and coastal environment, following expected developments in the offshore oil and gas industry. During one of the field missions in 2013 he discovered a stranded Omura's Whale, the first ever recorded in the Atlantic Ocean. From 2017 till 2019 he obtained a contract as Programme Manager with the German Gesellschaft für Internationale Zusammenarbeit (GIZ) and was delegated as an Integrated Expert at a Civil Society Organization in Ghana to accompany citizens in the assessment of the environmental impact of oil and gas exploitation offshore. This has led to the adaptation of a biomarker for use in citizen science projects and changes in the Oil Spill Contingency Plan of Ghana's EPA. In 2019-2020 he was awarded a contract by BirdLife International to develop a regional strategy for four West-African countries for monitoring of the environmental impact of ongoing developments in offshore oil and gas exploitation. From 1998 till now, in parallel to his paid activities, he executed together with his colleague and friend Jan Veen from The Netherlands, a long-term study to the breeding and feeding ecology of seabirds in West-Africa. This study, the longest of its kind in West-Africa, is still ongoing

and leading to new publications. He is Scientific Advisor of both the CERES-Locustox environmental laboratory (Senegal) and the African Aquatic Conservation Fund (USA/Senegal) and has been, or still is, a member of several toxicological and ornithological professional societies and was until 2019 an Eurotox Registered Toxicologist. He has travelled to, or worked in, more than 50 countries or territories in five continents and is an avid photographer and naturalist.



# List of publications

## Locust and grasshopper control and acridivorous birds related publications

- Mullié W.C., Cheke R.A., Young S., Ibrahim A.B. & Murk A.J. 2020. Increased and sex-selective avian predation of Desert Locusts *Schistocerca gregaria* treated with *Metarhizium acridum*. PLoS ONE (2021) 16(1): e0244733. <https://doi.org/10.1371/journal.pone.0244733>
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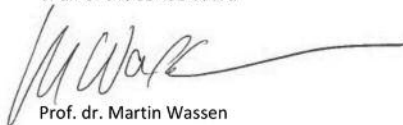
***Willem Cornelis Mullié***

born on 17 March 1952 in Middelburg, The Netherlands

has successfully fulfilled all requirements of the  
educational PhD programme of SENSE.

Wageningen, 24 February 2021

Chair of the SENSE board



Prof. dr. Martin Wassen

The SENSE Director



Prof. Philipp Pattberg

*The SENSE Research School has been accredited by the Royal Netherlands Academy of Arts and Sciences (KNAW)*



K O N I N K L I J K E N E D E R L A N D S E  
A K A D E M I E V A N W E T E N S C H A P P E N



The SENSE Research School declares that Willem Cornelis Mullié has successfully fulfilled all requirements of the educational PhD programme of SENSE with a work load of 57.9 EC, including the following activities:

Selection on SENSE requirements

- o Research in context activity: 'Contributed to the conception and writing of the FAO brochure 'Fighting the locusts...safely' (2005)
- o Contributing to several granted research proposals

Selection of Other PhD and Advanced MSc Courses

- o Open standards for the practice of conservation, Conservation Measures Partnership/MAVA/FIBA Foundation, Senegal (2012)

Selection of External training at a foreign research institute

- o Analysing data collected during 2010 field study in Australia, Environment Canada/National Wildlife Research Centre/Carleton University, Ottawa, Canada (2011)

Selection of Management and Didactic Skills Training

- o Member Scientific Council Regional Centre for Ecotoxicological Research and Food Security in the Sahel, Dakar, Senegal (2010-now).
- o Supervising MSc student with thesis entitled 'Breeding and feeding ecology of Caspian Terns in Senegal' (2012)
- o Supervising MSc student with thesis entitled 'Feeding ecology of Caspian Terns, Royal Terns and Slender-billed Gulls in Senegal based on data loggers' (2014)
- o Supervising MSc student with thesis entitled 'A novel method of assessing chick condition in the Caspian Tern in Senegal' (2014)

Oral Presentations

- o '*Synergy between bird predation and locust control with Metarhizium*'. ANCAP/SETAC Africa Branch-International Conference on Pesticide Use in Developing Countries. Environmental fate, effects and public health implications, 16-20 October 2006, Arusha, Tanzania
- o '*Does bird predation enhance the impact of Green Muscle for grasshopper control - Experiences from cleared woodland in Central Senegal*'. Orthopterists' Society, 10th International Congress of Orthopterology, 21-25 June 2009, Antalya, Turkey

SENSE coordinator PhD education

Dr. ir. Peter Vermeulen

## COLOFON

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United States Department of Agriculture: Chapter 1

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