



## Insecticide residues in cotton soils of Burkina Faso and effects of insecticides on fluctuating asymmetry in honey bees (*Apis mellifera* Linnaeus)

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### ABSTRACT

Four insecticides (acetamiprid, cypermethrin, endosulfan and profenofos) are used quarterly in the cotton-growing areas of Burkina Faso, West Africa. These insecticides were investigated in soils collected from traditionally cultivated and new cotton areas. Also, the effects of insecticide exposure on the developmental instability of honey bees, *Apis mellifera*, were explored. In soil samples collected three months after insecticide treatments, endosulfan and profenofos concentrations varied in the range of 10–30  $\mu\text{g kg}^{-1}$  in the traditionally cultivated zones and 10–80  $\mu\text{g kg}^{-1}$  in the new cotton zones, indicating a pollution of agricultural lands. However, only profenofos concentrations were significantly higher in the new cotton zone than the traditionally cultivated zones. In addition, the index of fluctuating asymmetry, FA1, in the length of second tarsus ( $L_{HW}$ ) was increased for bees when exposed to pesticide treated cotton fields for 82 d, and their FA levels were significantly higher than those in the control colony in an orchard. The other studied traits of bees exposed to insecticides were not significantly different from controls. Our results indicate that FA may be considered as a biomarker reflecting the stress induced by insecticide treatments. However, the relationship between FA and stressors needs further investigations.

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### 1. Introduction

Cotton (*Gossypium hirsutum* L.) is an important cash crop of Burkina Faso, a nation in West Africa. Burkina Faso's total cotton production for 2006–2007 was 263 000 tons (DAGRIS, 2008), which corresponds to 44.7% and 1.04% of African and world cotton production, respectively. This cotton-growing fields totalled 26 135 ha in 2006 and 15 293 ha in 2007. To fertilise the cotton fields and to fight against agricultural pests in the cotton-growing areas of Burkina Faso, approximately one hundred active ingredients were used (Toé, 2003). Around 75% of these active ingredients were insecticides, acaricides or nematicides, such as organophosphorus compounds (profenofos, triazophos), pyrethroids (cypermethrin, deltamethrin), organochlorines (endosulfan) or neonicotinoids (acetamiprid) (Orou Guidou, 1998). The amount of pesticides applied in 2006–2007 was approximately 7.5  $\text{kg ha}^{-1}$  (Savadogo, pers. com.). These compounds are toxic to human (OMS, 2002) and were used in the cotton-growing areas of Burkina

Faso to fight against pests, and often without considering ratification procedures (Abiola, 2000).

A few studies have been conducted in Africa to understand the behaviour of pesticides, particularly in soils. In Ghana, Ntow et al. (2007) observed a rapid dissipation of endosulfan with a half-life ( $t_{1/2}$ ) of 6 d in a sandy loam soil (pH 6.5). In the peasant and experimental stations of Burkina Faso, Savadogo et al. (2006) measured residual concentrations of endosulfan ranging from 1 to 22  $\mu\text{g kg}^{-1}$  in cotton soils. In addition, Tapsoba and Bonzi-Coulibaly (2006) discovered high concentrations of endosulfan ranging from 0.05 to 3.80  $\mu\text{g l}^{-1}$  in water of the cotton zones in Burkina Faso during the rainy season of 2005. To estimate the risks of pollution by the insecticides used on cotton crops, their behaviour in soil, water, and biomass must be analysed. The objectives of the present study were to estimate: (i) the persistence of pesticides in the cotton soils of Burkina Faso; and (ii) the effect of pesticide exposure on a non-target organism, *Apis mellifera*. *A. mellifera* was selected because bees gather nectar from cotton flowers treated with insecticides, and this species is easily available. Pesticides can lead to the pollution of soil, water and air; several studies have also shown that pesticides can cause developmental instability in non-target insects (Hardersen and Frampton, 1999; Chang et al., 2007a,

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2007b). The assessment of developmental instability in organisms provides a sensitive tool that can be used before a “traditional” life history parameter, such as survival or fertility, and therefore potentially allows appropriate remedial action (Clarke, 1993). The developmental instability can be reflected by an increase in the levels of fluctuating asymmetry (FA) in some morphological characters in the studied organisms.

Fluctuating asymmetry (FA) is a pattern of developmental instability caused by environmental or genetic perturbations during development (Zakharov, 1992). Some authors defined FA as the random non-directional deviation from perfect symmetry of a symmetrical bilateral trait (Palmer and Strobeck, 1986; Møller and Swaddle, 1997). The FA level of bilateral traits of an organism is proposed as a monitoring tool to assess the impacts of environmental stresses (Clarke and McKenzie, 1992; Markow, 1995; Lens et al., 2002). Most studies report an increase in FA levels in insect populations subjected to environmental stresses such as temperature variations, a lack of food resources or chemical pollution. Jentsch et al. (2003) found a significant increase in FA levels in the locust populations of *Chorthippus parallelus* exposed to agrochemical pollution via plant fertilisation (including Mg-, N-, P-, K-compounds). Similarly, Chang et al. (2007b) observed that the FA level of the wing length and the first tibia length of a damselfly (Odonata, Coenagriidae) increases with insecticide (triazophos) treatments. Ribeiro et al. (2007) also observed a significant relationship between the FA level of Weevil, *Sitophilus zeamais* and pyrethroid insecticide exposure. In this study, the residual insecticide concentrations were analysed in soil samples from the traditionally cultivated and the new cotton zones of Burkina Faso to identify the zone that exhibited higher concentrations of insecticide residues. Also, the impacts of pesticide treatment on developmental instability on natural populations of a non-target organism, *A. mellifera*, were investigated. Moreover, the results of this study will provide new data on the evolution and, to some extent, on the impact of insecticides on soils and biomass in the context of western Africa cotton practices, especially in Burkina Faso.

## 2. Materials and methods

### 2.1. Study area

The study area, located in Burkina Faso, has a typical Sudanese-type tropical climate with annual precipitation ranging between 600 and 900 mm and has a single maximum rainfall in August. The mean annual temperature varies from 28 to 30 °C. Climatic and geomorphologic factors promote cotton cultivation in two types of agricultural soils: lixisol and vertisol (FAO, 1998). Two cotton zones, subjected to the same cultural techniques, can be distinguished: (1) traditionally cultivated zones (since 1980) on the Western part of Ouagadougou (12°20'N, 1°40'W); and (2) new cotton zones (since 2004) mainly in the Eastern part (Fig. 1).

### 2.2. Sampling sites and insecticide treatments

Soils were collected from eight locations (seven peasant sites and one experimental station, Boni) distributed in the traditionally cultivated and new cotton zones of Burkina Faso (Fig. 1). These two historically different cotton zones were selected to identify which one has higher insecticidal concentrations in the soil. One cotton plot for sampling was selected from each location, except from Boni and Zanawa. Three cotton plots from Boni (Boni, Boni farm 1 and Boni farm 3), and two plots from Zanawa were monitored (Zanawa 1 and Zanawa 2). Overall, 11 cotton plots were selected for the study. For each plot, seven insecticidal treatments were applied from July 2006 to September 2006 using six commercial for-

mulated products (Fanga 500 EC, Rocky C 386 EC, CAPT 88 EC, Caïman 500 EC, FURY P 212 EC and Endocoton 500 EC) from the cotton bloom. The main physical-chemical properties and agronomic dose of each active ingredient (acetamiprid, cypermethrin, endosulfan and profenofos) applied to the cotton field, and their corresponding concentrations in the surface soil (0–20 cm) for both cotton soils are given in Table 1.

### 2.3. Soil sampling

Samples of the lixisol and vertisol surface soils (0–20 cm) were collected from the 11 cotton plots in June 2006 before insecticide application ( $T_0$ ) and again 3 months ( $T_3$  months) after insecticidal treatments. For each cotton plot, three samples were collected from the top, the hillside and the bottom slope. In addition, five fallow counterparts were collected near five cotton plots: Pama, Boni, Zanawa 1, Tiébélé and Dossi. In total, 76 soil samples, weighing approximately 2 kg each, were obtained, and the physical-chemical characteristics of both cotton soils were analysed in the Laboratory of Soil, Water and Plants in the Institute of the Environment and Agricultural Researches (INERA) in Burkina Faso.

Physico-chemical properties of soils were determined for 38 soil samples: 23 lixisols and 15 vertisols. The soil texture was clayey sandy loam for lixisol ( $61.7 \pm 14.8\%$  sand,  $28.6 \pm 12.1\%$  silt, and  $10.0 \pm 4.2\%$  clay) and loamy clay for vertisol ( $17.8 \pm 2.7\%$  sand,  $47.8 \pm 4.5\%$  silt,  $34.4 \pm 7.0\%$  clay). Organic carbon content was  $0.6 \pm 0.2\%$  with pH of  $5.9 \pm 0.3$  for lixisol and  $1.8 \pm 1.4\%$  with pH of  $6.1 \pm 0.4$  for vertisol. Lixisol and vertisol have respective bulk densities of  $1.7 \text{ g cm}^{-3}$  and  $1.6 \text{ g cm}^{-3}$ .

### 2.4. Pesticide analysis in cotton soils

For each soil sample, 1 kg subsample was frozen and another 1 kg subsample was air-dried at  $28 \pm 4^\circ\text{C}$  and sieved with a 2 mm mesh. These two different storage methods were used to evaluate the effect of the storage method on the concentrations of insecticide residues in the soils. About 100 g of dried soil and 100 g of frozen soil were subsampled from each main soil sample and approximately 152 samples were sent to France for pesticide analyses. Acetamiprid, cypermethrin, endosulfan and profenofos were extracted from the soil and analysed at the Pasteur Institute (Lille, France). The pesticides from the dried soil samples were extracted using the Accelerated Solvent Extraction (ASE) method with 50 mL of solvent (acetone: dichloromethane), and the pesticides from the frozen soil samples were extracted using the Soxtec method with 60 mL of acetone: dichloromethane, 1:1 (v/v) solvent mixture. The extracted pesticide residues were analysed using a Gas Chromatograph coupled with a Mass Spectrometer (GC-MS), following the XP X 33-012 method and using the NF EN ISO/CEI 17 025 European norm. The detection limit was  $0.01 \text{ mg kg}^{-1}$  for  $\alpha$ -endosulfan,  $\beta$ -endosulfan and profenofos, and the detection limit was  $0.05 \text{ mg kg}^{-1}$  for acetamiprid and cypermethrin.

For all the treatments, the average concentrations of each pesticide were analysed using the Mann-Whitney U-test (XLSTAT) with a 95% confidence interval.

### 2.5. Honey bee sampling and insecticide treatments

Before starting our study, the bee hives were located in an orchard for more than one year in Po area ( $11^\circ 17'N$ ,  $1^\circ 15'W$ ) (Fig. 1). A part of this hive was placed in the cotton plots in Po (site No. 4, Fig. 1), and divided into three smaller hives ( $T_1$ – $T_3$ ) randomly arranged and separated by 8 m. On August 31st, these hives were stationed in the cotton field treated with insecticides and remained there until 21 November 2006. This exposure period of insecticide treatments (approximately 3 months) is of the same order as that

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