WORLD COTTON RESEARCH CONFERENCE-3

COTTON PRODUCTION FOR THE NEW MILLENNIUM

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Monitoring insecticide resistance in the bollworm *Helicoverpa armigera* (Hübner) from 1998 to 2002 in Côte d'Ivoire, West Africa

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ABSTRACT

Helicoverpa armigera (Hübner) is the major insect pest of the cotton crop in West Africa. Populations recently developed resistance to pyrethroids via the overproduction of oxidases. To control this pest, a resistance management strategy was applied in the major West African cotton growing countries from 1999 onwards. In Côte d'Ivoire insecticide resistance of H. armigera was monitored in field strains from 1998 to 2002 using insecticide coated vial tests and topical applications of insecticides with third-instar larvae. Vial tests with discriminating doses of cypermethrin were used directly on field-collected larvae at the end of the cotton season. The percentage of resistant larvae varied around a mean of 67% with 6 µg cypermethrin/vial and around 13% with 30 µg/vial in different years and places. Topical applications with various insecticides were used in the laboratory on the first generation of populations collected from cotton (Gossypium hirsutum), tomato (Lycopersicum esculentum) or a strongly infested ornamental flower (Antirrhinum majus). The resistance factors calculated from dose-mortality regressions varied from 5 to 38 with deltamethrin. In the Bouaké area they were always higher for strains collected from cotton at the end of the season. Concerning the pyrethroid alternatives currently used in Côte d'Ivoire, no reduction in susceptibility in the cotton field strains was detected for endosulfan or profenofos showing their potential for use in a resistance management strategy. These results suggest a relative stability of the pyrethroid resistance in H. armigera from 1998 to 2002 and confirm the success of the resistance management strategy.

Introduction

More than two million small-scale farmers cultivate cotton in West Africa, on an average of 1 ha plots. Cotton is one of the most important cash crops in the region and provides more than 50% of the cash income to the agricultural population. It contributes significantly in the struggle against poverty in the countries of the African Cotton Belt. The cotton crop is damaged by a large number of insect species, the most harmful being the cotton bollworm *Helicoverpa armigera* (Hübner). To control the whole cotton pest complex, available and profitable approaches included chemical insecticide applications associated with the use of hairy cultivars and appropriate cultural practices. As a result, this crop received the largest amount of insecticide of all crops cultivated in the area. Since the early eighties, pyrethroids have been extensively used, because they are very efficient against bollworms at low doses and because their toxicity to mammals is low. However, since 1996 high infestations of *H. armigera* have been observed in some areas, suggesting control failure due to the development of insecticide resistance (Vassal *et al.*, 1997; Vaissayre *et al.*, 1998; Martin *et al.*, 2000). In 1998, significant pest infestations extended to several countries in West Africa despite an increase in spaying intensity. This event became highly threatening since a similar resistance observed in India and Pakistan resulted in dramatic losses in cotton production (McCaffery *et al.*, 1988, 1989). Facing this problem, West African countries put together efforts to better understanding the phenomenon, including the study of the resistance mechanism. It appeared that resistance was due to greater degradation of pyrethroids in resistant insects involving oxidases from the cytochrome P450 family (Martin *et al.*, 2002). A network group, named PR-PRAO from the French for "Prevention and Management of Pyrethroid Resistance in *H. armigera*") was implemented, involving CIRAD, IRAC and all the actors involved in cotton protection in West Africa, from research workers to extension and advisory services, to initiate management strategies and monitoring programs to survey the resistance level to the insecticides used.

Endosulfan had been demonstrated to be efficient against *H. armigera* before the introduction of pyrethroids (Ochou and Martin, 2002; Renou and Martin, Annual Reports to CIRAD). There has been no evidence of resistance to endosulfan mediated by the enhanced oxidases seen in pyrethroid-resistant *H. armigera* in West Africa (Martin *et al.*, 2002b). Consequently endosulfan was recommended for use early in the cotton season. This insecticide resistance management (IRM) strategy, restricting the use of pyrethroids, in cotton is described in Ochou *et al.* (1998) and Oucho and Martin (2002). This strategy has been successfully extended across the western African cotton-growing region since the 1999 season.

To enhance on the sustainability of this resistance management strategy, it was essential to survey the pyrethroid resistance levels in each cotton-growing region, because of the migrating habits of *H. armigera* (Nibouche, 1994). In the present investigation we monitored the pyrethroid resistance level in *H. armigera* from 1998 to 2002 in the cotton-growing area of Côte d'Ivoire. Populations were collected in cotton (*Gossypium hirsutum*), in tomato (*Lycopersicum esculentum*) and in a strongly infested ornamental flower (*Antirrhinum majus*). The susceptibility of the pyrethroid alternatives endosulfan and profenofos was also surveyed.

Experimental procedure

Insects

A susceptible *H. armigera* strain (BK77) originally collected in Côte d'Ivoire in 1977 and reared in CIRAD Entomological Laboratory from Montpellier, France was used as a reference strain. Larvae were reared on artificial diet at 25 °C, 75% humidity and at 12h/12h photoperiod in the laboratory as previously described (Couilloud and Giret, 1980). Field samples of different stages of *H. armigera* were collected from 1998 to 2002. Samples were obtained from strongly infested crops in fields from the cotton-growing area. The strains were named according to the nearest large town (BK: Bouaké; SAR: Sarhala; OGL: Ouangolo; NIO: Niofoin; MKN: Mankono; BOU: Boundiali) with the collection date (year/month) and the crop name: *c* for cotton, *t* for tomato, *g* for gumbo and *f* for the ornamental flower. A minimum of 50 larvae were collected in each field and reared in the laboratory of the Centre National de Recherche Agronomique (CNRA) in Bouaké on an artificial diet for one generation at 25 °C. The adults were placed in cages and fed on a 5% honey solution. Their eggs were collected on sterilized gauze and washed with 1% bleach.

Insecticides

The insecticides used were all technical grade materials. Deltamethrin (99%) and endosulfan (99%) were obtained from Aventis CropScience, France. Cypermethrin (93.2%) was obtained from FMC, USA. Profenofos (95%) was provided by Syngenta, Abidjan, Côte d'Ivoire.

Topical application Standard third-instar larvae topical bioassays were used to determine insecticide toxicity. Five serially diluted concentrations were prepared. For each concentration, 10 third-instar larvae (35-45 mg) were treated with 1 µl of solution applied by microapplicator to the dorsal thorax. Each test was replicated three times and included acetone-treated controls. Mortality in the controls was used to correct treatment mortality. Mortality in the controls was less than 10%. After dosage, the test larvae were held individually at 25 °C and 75% humidity. Mortality was assessed 72 h after treatment. Larvae were considered dead if unable to move in a co-ordinated way when prodded with a needle. LD_{50} was determined by using the Finney (1961) method. Transformations and regression lines were automatically calculated by the DL50 1.1 software of CIRAD.

Vial tests Vials were impregnated with technical grade cypermethrin in acetone. Two discriminating doses were chosen: 5 µg/vial which killed 100% of the susceptible larvae from BK77 strain, and 30 µg/vial which killed 100% of the susceptible larvae and 60 to 80% of a resistant population collected in Benin in 1997 (Vaissayre *et al.*, 2002). Ninety larvae were used per test – 30 for each of the two treatments and 30 for the control. The tubes were kept in darkness at ambient temperature. Extension service agents conducted vial tests each October for four years during the strong infestation at the end of the cotton season. Larvae of *H. armigera* measuring 1 to 1.5 cm were collected from farmer cotton fields at least seven days after the last insecticide treatment. Two replications were conducted in different locations. Each larva was placed in a vial without any food. The vials were kept horizontal and protected from heat. Larval mortality was assessed after 24 h. Larva were considered dead if unable to move in a co-ordinated manner. In 1998-2000 they worked in the area of Bouaflé, Bouaké, Boundiali, Ferké or Tortiya. In 2001, vial tests were conducted in twelve areas spread over the Center, West North and North of the country.

Results

Vial tests

The vial test method was directly used in cotton field of farmers to confirm and survey the pyrethroid resistance of *H. armigera* in the whole West African cotton-growing region (Vaissayre *et al.*, 2002). Because of low infestations since the application of the IRM strategy for 1999, vial tests could be used only at the end of the cotton season. The results obtained in Côte d'Ivoire with two discriminating doses of cypermethrin are illustrated in Figure 1. The first discriminating dose (6 µg/vial) showed high percentages of resisting larvae, varying from 40 to 90%. With the second discriminating dose (30 µg/vial) the percentage of resisting larvae varied from 1 to 35%. On average, less than 20% of larvae were resistant to 30 µg/vial of cypermethrin, this threshold corresponding to the minimum causing control failures in fields (Vaissayre *et al.*, 2002). This vial test method applied for the same period in Mali, Burkina Faso, Benin and Togo, showed that *H. armigera* populations from Côte d'Ivoire have the lowest percentages of resistant larvae. Despite he low level of infestation during the survey, these results showed the presence of pyrethroid resistant larvae in all populations tested and high levels of variability between the populations tested.

Bioassays

During the same period, topical application bioassays were used to follow the annual evolution of the pyrethroid resistance level in field populations. Dose-mortality regression lines were performed using deltamethrin in the first generation of field populations collected from different locations from 1998 to 2001. LD_{50}s varied from 0.30 to 1.05 µg/g indicating a low resistance level (maximum, (RF)=20) (Figure2). To follow the seasonal evolution of the pyrethroid resistance level resistance factor at one location, dose-mortality regression lines were generated for the first generation of all *H. armigera* populations collected each year from various host plants in the Bouaké area. The data showed that the deltamethrin LD_{50}s for *H. armigera* varied from 0.4 to 2 µg/g (Figure 3). Therefore, the resistance factor (RF) for the Bouaké field population varied from 10 to 38 fold compared to the susceptible strain BK77. It was very difficult to find *H. armigera* in vegetable crops because of the sparse, small plots and

the high number of insecticide treatments. The ornamental flower *Anthirrhinum majus* cultivated without any insecticide treatment in small plots near the Cotton Research Station of Bouaké, appeared to be very attractive to *H. armigera* and could be very useful in the future to collect populations throughout the dry season. The highest annual resistance level was observed in populations collected from cotton in October corresponding to the last period of insecticide treatments. The resistance level slightly decreased in the dry season as shown by the resistance level of the populations collected from ornamental flowers. This result was also observed in *H. armigera* populations collected in Benin (Djinto *et al*., unpublished data). They are suggesting a fitness cost of the pyrethroid resistance. Dilution of the resistant insects by susceptible insects in the non-spray season is also possible. *H. armigera* populations collected in October from cotton in Bouaké seemed to be always the most resistant among field populations. This result can be explained by the selection for resistance produced by the high number of insecticide treatments in variety-multiplication plots of the Cotton Research Station of Bouaké. Interestingly, the deltamethrin toxicity of these populations has been surveyed in each year since 1985 (Figure 4). Three plateaus appeared showing schematically the apparition of pyrethroid resistance in *H. armigera* populations from Bouaké: susceptibility (1985-1988), decrease of susceptibility (1989-1995) and resistance (1996-2001). Regarding pyrethroid alternatives, dose-mortality regression lines for endosulfan and profenofos have been produced annually since 1999 for Bouaké populations. Endosulfan LD_{50}s of field populations were at the same level as BK77 (Figure 5). Thus, endosulfan has not show any resistance development in the Bouaké *H. armigera* cotton field population. Profenofos also did not show any resistance in *H. armigera* field populations (Figure 6).

Discussion

Results obtained on larvae with the application of discriminating doses by vial test method confirmed the presence of resistant *H. armigera* in all the field populations collected in Côte d'Ivoire. The larva vial test was not an accurate method. However, results obtained directly in the field was an indicator of the pyrethroid resistance and could be confirm with the bioassay method. Bioassays results showed that the deltamethrin resistance level in *H. armigera* populations could be considered as low in Côte d'Ivoire (in average RF<20) whatever the host plant, the collecting date and the location of the population. The deltamethrin resistance level was generally highest in population collected at the end of cotton treatments and decreased during the dry season. Therefore the pyrethroid resistance appeared globally stable from 1998 to 2002. This result may be an indicator of the success of the resistance management program.

No resistance was still detected for endosulfan and profenofos in field populations indicated the success of these pyrethroid alternatives. Endosulfan cyclodiene was used from the 70's in mixtures with DDT and methyl-parathion. It was replaced by pyrethroids in 1984. But since the development of pyrethroid resistance, endosulfan has been successfully reused alone (Ochou and Martin, 2002a). This success partially originates from the absence of cross-resistance with pyrethroids (Martin *et al*., 2002). Profenofos organophosphorus compound has been used on cotton since 1977 in mixture with pyrethroids to control mites. It was used alone for 2000 as a pyrethroid alternative. No cross-resistance was detected with pyrethroids (Martin *et al*., 2002). Endosulfan and profenofos resistance was showed in *H. armigera* from Pakistan (Ahmad *et al*., 1995) and Australia (Forrester *et al*., 1993; Gunning *et al*., 1995) indicating both a risk of introduction of these *H. armigera* genotypes into West Africa and of the selection of both genotypes if either material is overused.

Cotton insecticides are frequently used in vegetable crops during the dry season to control *H. armigera*. This highlights the importance of continuing the monitoring of insecticide resistance and the importance of new molecules such as indoxacarb and spinosad, which are efficient in the control of *H. armigera* (Ochou and Martin, 2002). These are beginning to be used in mosaics with endosulfan and profenofos to limit the risk of selection of resistance to any of the materials.

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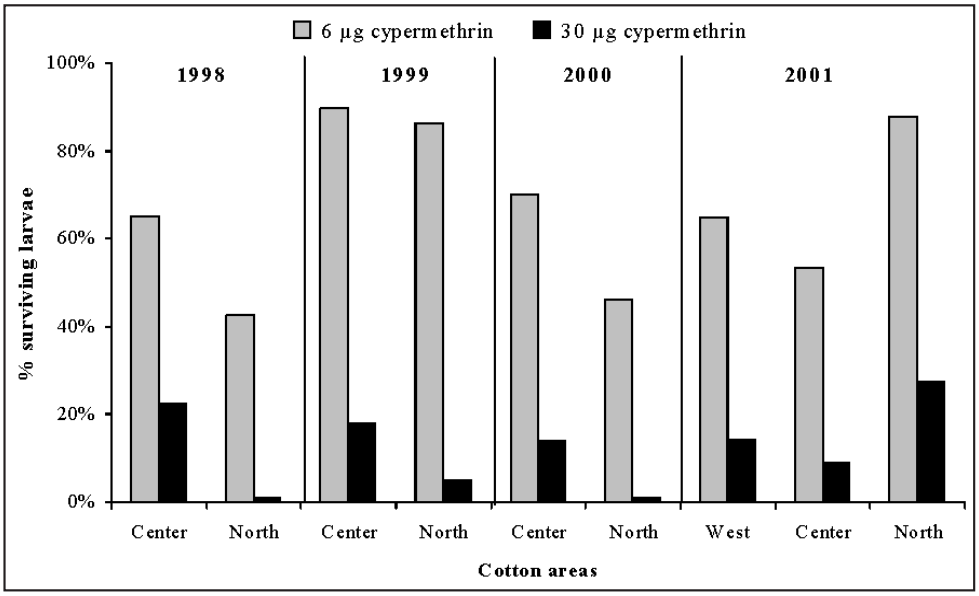

Figure 1. *Vial test with 6 and 30 µg of cypermethrin in third-instar larvae of* H. armigera *populations collected in various cotton area from 1998 to 2001.*

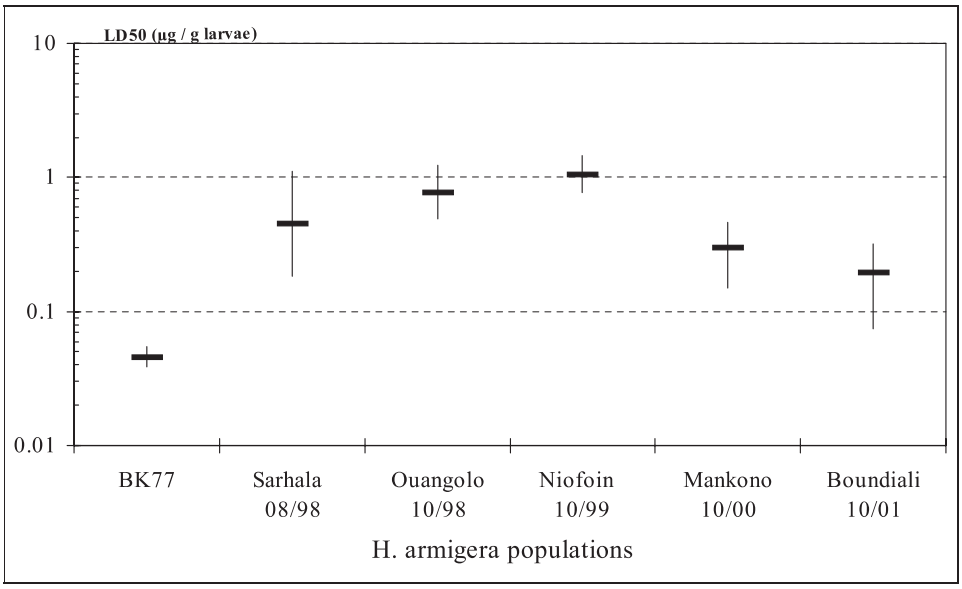

Figure 2. *LD_{50} of deltamethrin in* H. armigera *populations collected in various cotton areas from 1998 to 2001.*

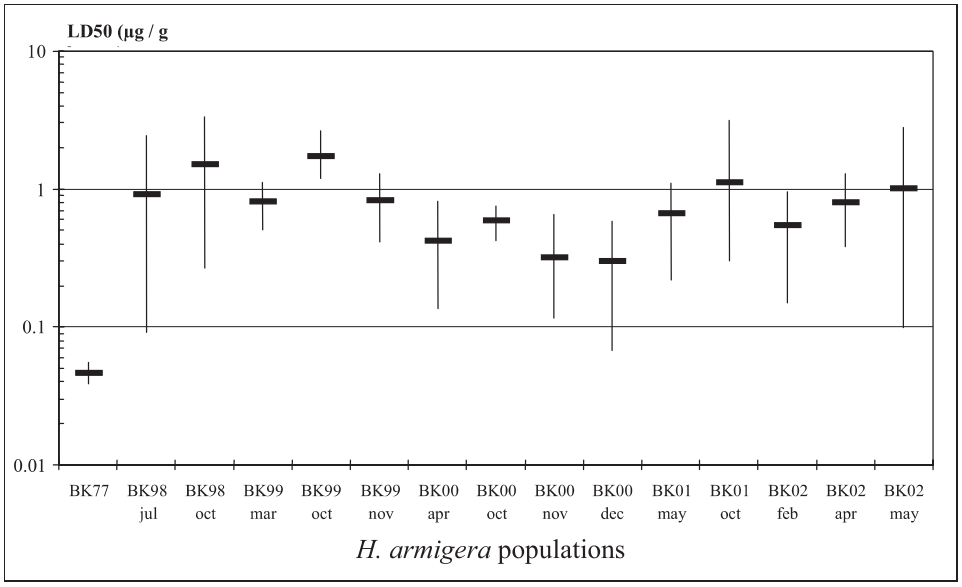

Figure 3. *LD_{50} of deltamethrin in* H. armigera *populations collected in Bouaké area from 1998 to 2002 at various period in the year compared to LD_{50} of the susceptible strain (BK77).*

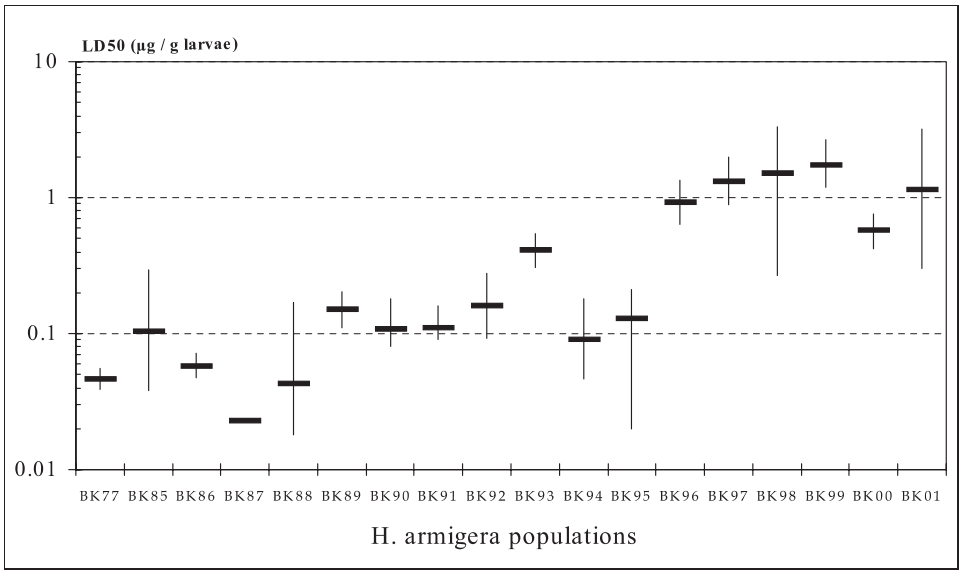

Figure 4. *LD_{50} of deltamethrin (with 95% confidence intervals) in susceptible strain BK77 and annual Bouaké cotton field populations of* H. armigera *collected in October from 1985 (BK85) to 2001 (BK01).*

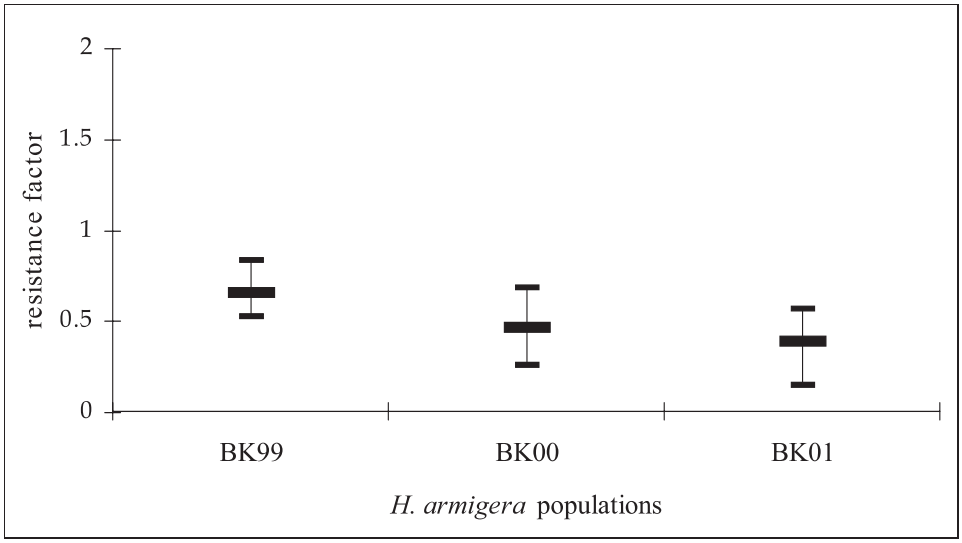

Figure 5. *Resistance factor (RF) for endosulfan in three field populations collected from cotton in Bouaké in 1999 (BK99), 2000 (BK00) and 2001 (BK01).*

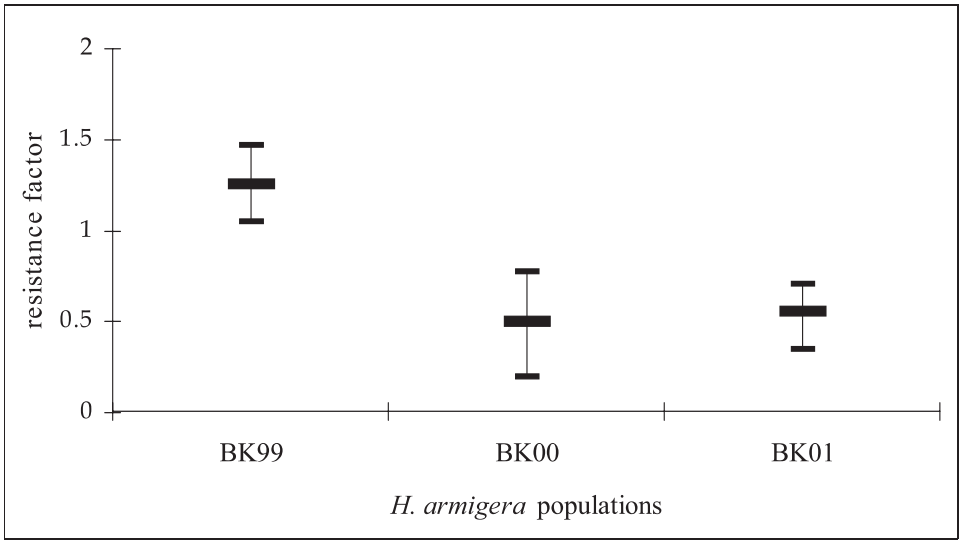

Figure 6. *Resistance factor for profenofos in three field populations collected from cotton in Bouaké in 1999 (BK99), 2000 (BK00) and 2001 (BK01).*