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Bendiocarb susceptibility status in *Anopheles gambiae s.l.* larvae from Dogbo district in Couffo department in south-western Benin, West Africa

Nazaire Aïzoun[1]

[1]*Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET), Normal High School of Technical Teaching (ENSET) of Lokossa, National University of Sciences, Technologies, Engineery and Mathematics (UNSTIM) of Abomey, P. O. Box 133 Lokossa Cell: +229 95317939 / +229 69465070 Cotonou, Benin*

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Abstract

The current study was aimed to study the bendiocarb tolerance in *Anopheles gambiae s.l.* larvae from Dogbo district in Couffo department in south-western Benin, West Africa. Larvae and pupae were collected from March to July and August to November 2020 during the rainy season in the locations of Ayomi, Dévé, Honton, Lokogohoué, Madjrè and Totchangni. Larval bioassays were performed on these collected *Anopheles gambiae s.l.* larvae using bendiocarb as larvicide. The results showed that *Anopheles gambiae s.l.* larvae from Dogbo district were susceptible to bendiocarb. However, these *An. gambiae s.l.* populations need to be monitored for insecticide resistance in this area.

* **Corresponding Author:** Nazaire Aïzoun ✉ aizoun.nazaire@yahoo.fr

Introduction

World Health Organization (WHO) recommends a multi-pronged strategy to control and eliminate malaria, which includes vector control interventions, preventive therapies, diagnostic testing, treatment with quality-assured artemisinin based combination therapies (ACTs), and strong malaria surveillance. Effective malaria control and elimination requires strong and well-funded National Malaria Control Programmes (NMCPs), tailored national and regional strategies, extensive applied and operational research, and a close collaboration among partners in the global malaria and development community. Achieving effective scale-up of malaria interventions also requires significant human resources at national, district and community levels, and the regular training of malaria programme staff (WHO, 2013).

Insects, like most eukaryotes, have evolved a complex capacity to transform compounds they encounter in their environments. The development of this ability is important to their survival particularly in chemically unfriendly environments. All insects possess detoxification mechanisms, but the type, nature and capacity differs in different insect species, developmental stages, and the type of the environmental exposure (Yu, 2005). Mosquitoes are of particular interest because of their role as vector of many human diseases including malaria, yellow fever, dengue fever etc. In mosquitoes, like other insect species, the challenge of responding to varieties of xenobiotic assault is compounded by the varieties of breeding ecologies and food sources upon which they rely for their life cycle. The ubiquity of mosquito breeding habitats mean that mosquitoes are found in virtually all environments from arctic to the deserts (Budiansky, 2002). *An. gambiae* in particular is a highly anthropophilic malaria vector distributed widely in sub-Saharan Africa.

This region constitutes 90% of the global malaria burden. Exposure of *An. gambiae* to this array of environmental xenobiotics could undoubtedly select them for tolerance and adaptive responses. Some of these responses could constitute challenges to the insecticide based approaches to malaria management and control initiatives (Strode *et al.*, 2006).

Several previous studies (Mwangagngi *et al.*, 2010; Imbahale *et al.*, 2011; Mala *et al.*, 2011; Animut *et al.*, 2012) have established the impact of several breeding sites ecogeographical, topographical, agricultural, and other environmental indices on *Anopheles* larval diversity, abundance, and dynamics, as well as breeding sites productivity. Also, induction of detoxification enzymes by various environmental xenobiotics in many species of insects has been well documented (David *et al.*, 2013).

Very few researches were published on bendiocarb tolerance in *Anopheles gambiae s.l.* larvae from Couffo department in south-western Benin. Therefore, there is a need to carry out new researches for this purpose.

The goal of this study was to determine bendiocarb tolerance in *Anopheles gambiae s.l.* larvae from Couffo department in south-western Benin.

Materials and methods

Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Couffo. Couffo department is located in the south-western Benin and the study was carried out more precisely in Dogbo district. The choice of the study site took into account the economic activities of populations, their usual protection practices against mosquito bites, and peasant practices to control farming pests. These factors have an impact on resistance development in the local vector mosquitoes. We took them into account to determine bendiocarb tolerance in *Anopheles gambiae s.l.* larvae from Dogbo district. Couffo has a climate with four seasons, two rainy seasons (March to July and August to November) and two dry seasons (November to March and July to August). The temperature ranges from 25 to 30°C with the annual mean rainfall between 900 and 1100 mm.

Fig. 1. Map of Republic of Benin showing Dogbo District.

Mosquito sampling

An. gambiae s.l. larvae were collected from March to July and August to November 2020 during the rainy season in Dogbo district in south-western Benin. The number and kind of breeding sites were recorded in localities of Ayomi, Dévé, Honton, Lokogohoué, Madjrè and Totchangni. Larvae and pupae were collected within both padding and town using the dipping method on several breeding sites. Once, larvae and pupae collected, they were then kept in separated labeled bottles related to the localities surveyed. Larvae collected were then re-distributed evenly in development trays containing tap water. Larvae were provided access to powdered TetraFin® fish food under insectary conditions of 25+/-2°C and 70 to 80% relative humidity at Department of Sciences and Agricultural Techniques located in Dogbo district in south-western Benin. *An. gambiae* Kisumu larvae, a reference susceptible strain was used as a control for the larval bioassays. All larval bioassays were conducted in the Laboratory of Applied Entomology and Vector Control of the Department of Sciences and Agricultural Techniques at 25+/-2°C and 70 to 80% relative humidity.

Preparation of stock solutions or suspensions and test concentrations

Stock solutions and serial dilutions were prepared following the protocol described in WHO guidelines (WHO, 2005). The volume of stock solution was 20

ml of 1%, obtained by weighing 200 mg of bendiocarb and adding 20 ml solvent to it. It was kept in a screw-cap vial, with aluminium foil over the mouth of the vial. Then, it was shacked vigorously to dissolve or disperse the bendiocarb in the solvent. The stock solution was then serially diluted (ten-fold) in ethanol (2 ml solution to 18 ml solvent). Test concentrations were then obtained by adding 0.1–1.0 ml (100–1000 µl) of the appropriate dilution to 100 ml or 200 ml distilled water.

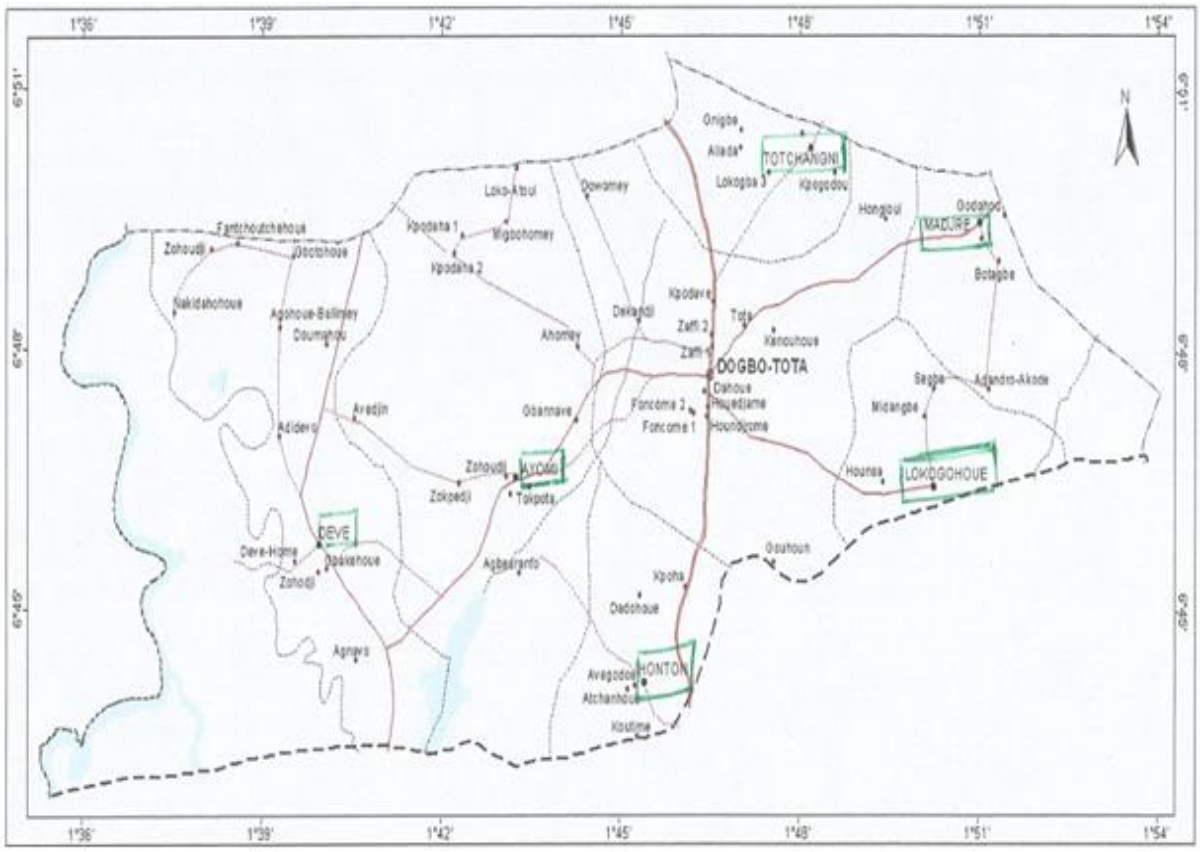

Fig. 2. Map of Dogbo District showing the study area.

Bioassays

Initially, the mosquito larvae were exposed to a wide range of test concentrations of bendiocarb and a control to find out the activity range of the larvicide under test. After determining the mortality of larvae in this wide range of concentrations, a narrower range (of 4-5 concentrations, yielding between 10% and 95% mortality in 24h or 48h) was used to determine LC50 and LC90 values (WHO, 2005). Batches of 25 third or fourth instar larvae were transferred by means of strainers, screen loops or droppers to small disposable test cups or vessels, each containing 100-200 ml of water. Small, unhealthy or damaged larvae were removed and replaced. The depth of the water in the cups or vessels was remained between 5 cm and 10 cm; deeper levels may cause undue mortality. The appropriate volume of dilution was added to 100 ml or 200 ml water in the cups to obtain the desired target dosage, starting with the lowest concentration. Four replicates were set up for each concentration and an equal number of controls were set up simultaneously with tap water, to which 1 ml alcohol was added. Each test was run three times on different days. For long exposures, larval food was added to each test cup, particularly if high mortality was noted in control. The test containers were held at 25-28°C and preferably a photoperiod of 12h light followed by 12h dark (12 L: 12 D).After 24 h exposure, larval mortality was recorded. Moribund larvae were counted and added to dead larvae for calculating percentage mortality. Dead larvae were those that could not be induced to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those incapable of rising to the

surface or not showing the characteristic diving reaction when the water was disturbed. The results were recorded on the result form, where the LC50 and LC90 values, and slope and heterogeneity analysis were also noted.

The form was accommodated three separate tests of six concentrations of bendiocarb, each of four replicates (WHO, 2005).

Statistical analysis

Data from all replicates were pooled for analysis. LC50 and LC90 values were calculated from a log dosage-probit mortality regression line using computer software programs. Bioassays were repeated at least three times, using new solutions or suspensions and different batches of larvae each time. Standard deviation or confidence intervals of the means of LC50 values vere calculated and recorded on a form. A test series was valid if the relative standard deviation (or coefficient of variation) was less than 25% or if confidence limits of LC50 overlap (significant level at $P < 0.05$). Abbott's formula was not used in this study for the correction of mortality rates in test cups because the mortality rates in all controls was always less than 5% (Abbott, 1987). LC50 and LC90 values were estimated using SPSS version 16.0 (SPSS Inc., Chicago, IL). The significance level was set at 5%.

Results and discussion

The analysis of tables 1 and 2 showed that *An. gambiae s.l.* larvae from Dogbo district in couffo department were susceptible to bendiocarb like Kisumu reference strain (Resistance ratios were 1 for RR50 and RR95).

Table 1. Determination of Lethal Concentration LC50 and Resistance ratio RR50.

Strains	LC50 (mg/l)	RR50
Kisumu	0.0111	_
Dogbo	0.0111	1

The analysis of table 3 showed that there were more breeding sites made of marshes in Totchangni location than breeding sites made of streams in Lokogohoué. Very few breeding sites made of ditches were found in Ayomi location. Resistance management strategies are mainly based on the rational use of the compounds already available, especially in public health because the number of insecticides is very limited. Carbamates (CX) and Organophosphates (OP) are the main alternatives for indoor residual spraying or larval treatments against mosquitoes in case of pyrethroid resistance. So, insecticide resistance is an impediment in the control of pests and vectors of human diseases and has emerged because of heavy insecticide treatments. Different resistance mechanisms (mostly target mutation or increased detoxication) have been selected in insects depending on the insecticide used (Aïzoun *et al.*, 2013a; 2013b; 2013c; 2014a; 2014b; 2014c; 2014d; 2014e, 2014f; 2014g; 2014h; 2014i; 2014j).

An. gambiae s.l. larvae from Dogbo district in couffo department were susceptible to bendiocarb as Kisumu reference strain. So, the larval treatments are important as when all larvae and pupae of a *Anopheles gambiae* population were controlled there was no adult in malaria transmission.

Table 2. Determination of Lethal Concentration LC95 and Resistance ratio RR95.

Strains	LC95(mg/l)	RR95
Kisumu	0.0443	_
Dogbo	0.0443	1

With the rapid extension of pyrethroid resistance in the main malaria vectors from Africa and the various resistance mechanisms involved (metabolic resistance and *kdr*), it is important to envisage resistance management strategies now. Thus, the knowledge of the *ace-1R* effects on phenotypes of *An. gambiae* will

help us to strengthen the basic and operational researches on the development of strategies that will use organophosphates or carbamates as alternatives against pyrethroids-resistant malaria vectors in the field. The combination of insecticides using rotations, mosaics or mixtures is a possible way to overcome insecticide resistance within malaria vectors not only for indoor residual sprayings (Hemingway *et al.*, 1997; Penilla *et al.*, 2006) but also for impregnated nets (Hougard *et al.*, 2002).

Table 3. Recording of number and kind of breeding sites in locations surveyed.

Locations	Number of breeding sites	Kind of breeding sites
Ayomi	4	ditches
Dévé	8	brick pits
Honton	9	pools
Lokogohoué	13	streams
Madjrè	7	puddles of water
Totchangni	16	marshes

This is all the more interesting that in some case insecticides can have synergistic interactions particularly against resistant mosquitoes (Corbel *et al.*, 2004). However, combinations can sometimes have antagonistic effect as it was demonstrated using carbamate and pyrethroid for *C. pipiens* with *ace-1R* mutation (Corbel *et al.*, 2004). Different breeding sites were found in Dogbo district such as**:** ditches, brick pits, pools, streams, puddles of water, marshes etc. For that, in additional of the larval treatments with larvicides which are effective against larvae, it would also be important to practice physical control by destroying breeding sites.

The use of insecticides for crop protection may likely explain the level of Carbamates (CX) and Organophosphates (OP) resistance observed in some of our rural areas. So, it is clear that closer collaboration between resistance experts in agriculture and public health is needed.

Fig. 3. Collection of *An. gambiae s.l.* larvae in a breeding site in Totchangni location.

Public health agencies can definitely benefit from the extensive experience gained by the agricultural sector in promoting integrated pest-management principles as well as disseminating simple and pragmatic guidelines for insecticide resistance management.

Conclusion

Given the importance of the vector control against malaria disease, there is an urgent need of field and laboratory surveys of insecticide resistance. *Anopheles gambiae s.l.* larvae from Dogbo district were susceptible to bendiocarb. However, these *An. gambiae s.l.* populations need to be monitored for insecticide resistance in this area.

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Author contribution

The author designed the study, supervised the experiment, analyzed and interpreted the data, contributed to the mapping and drafted the manuscript.

Conflict of interest

I declare that there is no conflict of interest regarding the publication of this article.

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References

Abbott WS. 1987. A method of computing the effectiveness of an insecticide**.** Journal of the American Mosquito Control Association **3(2),** 302-303.

Aïzoun N, Aïkpon R, Akogbéto M. 2014a. Evidence of increasing L1014F *kdr* mutation frequency in *Anopheles gambiae s.l.* pyrethroid resistance following a nationwide distribution of LLINs by the Beninese National Malaria Control Programme. Asian Pacific Journal of Tropical Biomedicine **4(3),** 239-243.

Aïzoun N, Aïkpon R, Gnanguenon V, Azondekon R, Oké-Agbo F, Padonou GG, Akogbéto M. 2014b. Dynamics of insecticide resistance and effect of synergists piperonyl butoxide (PBO), S.S.S-tributylphosphorotrithioate (DEF) and ethacrynic acid (ETAA or EA) on permethrin, deltamethrin and dichlorodiphenyltrichloroethane (DDT) resistance in two *Anopheles gambiae s.l.* populations from southern Benin, West Africa. Journal of Parasitology and Vector Biology **6(1), 1**-10. https://doi.org/10.5897/JPVB2013.0137

Aïzoun N, Aïkpon R, Gnanguenon V, Oussou O, Agossa F, Padonou GG, Akogbéto M. 2013c. Status of organophosphate and carbamate resistance in *Anopheles gambiae sensu lato* from the south and north Benin, West Africa. Parasits and Vectors **6,** 274. https://doi.org/10.1186/1756-3305-6-274

Aïzoun N, Aïkpon R, Padonou GG, Oussou O, Oké-Agbo F, Gnanguenon V, Ossè R, Akogbéto M. 2013b. Mixed-function oxidases and esterases associated with permethrin, deltamethrin and bendiocarb resistance in *Anopheles gambiae s.l.* in the south-north transect Benin, West Africa. Parasits and Vectors **6,** 223. https://doi.org/10.1186/1756-3305-6-223

Aïzoun N, Azondekon R, Aïkpon R, Anagonou R, Gnanguenon V, Akogbéto M. 2014c. Dynamics of insecticide resistance and exploring biochemical mechanisms involved in pyrethroids and dichlorodiphenyltrichloroethane (DDT) cross-resistance in *Anopheles gambiae s.l.* populations from Benin, West Africa. Journal of Cell and Animal Biology **8(3),** 41-50. https://doi.org/10.5897/JCAB2014.0406

Aïzoun N, Azondekon R, Akogbéto M. 2014d. Exploring Glutathione *S*-transferases involved

in dichlorodiphenyltrichloroethane (DDT) and permethrin cross-resistance in *Anopheles gambiae s.l.* populations in the south-north transect Benin, West Africa. International Journal of Current Microbiology and Applied Sciences **3(9),** 392-403.

Aïzoun N, Azondekon R, Akogbéto M. 2014e. The L1014F *kdr* mutation in *Anopheles gambiae s.l.* lambdacyhalothrin resistant populations from Kandi district in northen Benin, West Africa. International Journal of Current. Research in Biosciences and Plant Biology **1(4),** 9-14.

Aïzoun N, Azondekon R, Akogbéto M. 2014g. Status of deltamethrin resistance in three *Anopheles gambiae sensu lato* populations from main ecological settings in Benin, West Africa. International Journal of Current Microbiology and Applied Sciences **3(9),** 58-64.

Aïzoun N, Azondekon R, Akogbéto M. 2014h. Propoxur resistance in *Anopheles gambiae s.l.* populations from N'dali district in northern Benin, West Africa. International Journal of Current Microbiology and Applied Sciences **3(9),** 224-229.

Aïzoun N, Azondekon R, Akogbéto M. 2014i. Cyfluthrin resistance in *Anopheles gambiae s.l.* populations from the cotton growing area of Hlassoé in Glazoué district in the central part of Benin, West Africa. International Journal of Current Microbiology and Applied Sciences **3(10)**, 899-904.

Aïzoun N, Azondekon R, Akogbéto M. 2014j. Susceptibility status to malathion in *Anopheles gambiae s.l.* populations from Toffo district in southern Benin, West Africa. International Journal of Current Microbiology and Applied Sciences **3(10),** 893-898.

Aïzoun N, Gnanguenon V, Azondekon R, Anagonou R, Aïkpon R, Akogbéto M. 2014f. Status of organophosphate and carbamate resistance in *Anopheles gambiae sensu lato* from the Sudano Guinean area in the central part of Benin, West Africa. Journal of Cell and Animal Biology **8(4)**, 61-68. https://doi.org/10.5897/JCAB2013.0400

Aïzoun N, Ossè R, Azondekon R, Alia R, Oussou O, Gnanguenon V, Aikpon R, Padonou GG, Akogbéto M. 2013a. Comparison of the standard WHO susceptibility tests and the CDC bottle bioassay for the determination of insecticide susceptibility in malaria vectors and their correlation with biochemical and molecular biology assays in Benin, West Africa. Parasits and Vectors **6**, 147. https://doi.org/10.1186/1756-3305-6-147

Animut A, Gebre-micheal T, Balkew M, Lindtjorn B. 2012. Abundance and dynamics of Anopheline larvae in a highland malarious area of south-central Ethiopia. Parasits and vectors **5,**117. https://doi.org/10.1186/1756-3305-5-117

Budiansky S. 2002. Creatures of our own making. Science **298,** 80-86.

Corbel V, Raymond M, Chandre F, Darriet F, Hougard JM. 2004. Efficacy of insecticide mixtures against larvae of *Culex quinquefasciatus* (Say) (Diptera: Culicidae) resistant to pyrethroids and carbamates. Pest Management Science **60,** 375-380. https://doi.org/10.1002/ps.809

David JP, Ismail AM, Chander-Proust A, Paine MJ. 2013. Role of cytochrome P450s in insecticide resistance: Impact on the control of mosquito-borne diseases and use of insecticides on earth. Philosophical Transactions of the Royal Society B **368**, 2012.0429. https://doi.org/10.1098/rstb.2012.0429

Hemingway J, Penilla RP, Rodriguez AD, James BM, Edge W, Rogers H, Rodriguez MH. 1997. Resistance management strategies in malaria vector mosquito control. A large-scale Weld trial in Southern Mexico. Pesticide Science **51,** 375-382. https://doi.org/10.1002/(SICI)10969063(199711)51:3%3C375::AID-PS636%3E3.0.CO;2-K

Hougard JM, Fontenille D, Chandre F, Darriet F, Carnevale P, Guillet P. 2002. Combating malaria vectors in Africa: current directions of research. Trends in Parasitology **18,** 283-286. https://doi.org/10.1016/S1471-4922(02)02291-2

Imbahale SS, Paajimans KP, Mukabana WR, Van LR, Githeko AK, Takken W. 2011. A longitudinal study on Anopheles mosquito larval abundance in distinct geographical and environmental settings in western Kenya. Malaria Journal **10**, 81. https://doi.org/10.1186/1475-2875-10-81

Mala AO, Irungu LW, Shililu JI, Muturi EJ, Mbogo CC, Njagi JK, Githure JI. 2011. Dry season ecology of *Anopgeles gambiae* complex mosquitoes at larval habitats in two traditionally semi-arid villages in Baringo, Kenya. Parasits and vectors **4**, 25. https://doi.org/10.1186/1756-3305-4-25

Mwangagngi JM, Shililu J, Muturi EJ, Muriu S, Jacob B, Kabiru EW, Mbogo CM, Githure J, Novak RJ. 2010. Anopheles larval abundance and diversity in three rice agrovillage complexes mwea irrigation scheme, central Kenya. Malaria Journal 9, 228. https://doi.org/10.1186/1475-2875-9-228

Penilla RP, Rodriguez AD, Hemingway J, Torres JL, Solis F, Rodriguez MH. 2006. Changes in glutathione S-transferase activity in DDT resistant natural Mexican populations of *Anopheles albimanus* under different insecticide resistance management strategies. Pesticide Biochemistry and Physiology **86,** 63-71. https://doi.org/10.1016/j.pestbp.2006.01.006

Strode C, Steen K, Ortelli F, Ranson H. 2006. Differential expression of detoxification genes in the different life stages of the malaria vector *Anopheles gambiae*. Insect Molecular Biology **15(4),** 523-530. https://doi.org/10.1111/j.1365-2583.2006.00667.x

WHO. 2013. World Malaria Report. Geneva, World Health Organization.

WHO. 2005. "Guidelines for laboratory and field testing of mosquito larvicides," World Health Organization Communicable Disease Control, Prevention and Eradication WHO Pesticide Evaluation Scheme. WHO/CDS/WHOPES/GCDPP/2005.13.

Yu SJ. 2005. Detoxification mechanisms in insects. Encyclopedia of entomology **4,** 687-699.