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Status and future prospects for molecular diagnosis of Acaricide resistance in *Boophilus microplus*

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ABSTRACT:

Boophilus microplus is an economically significant tick parasite of cattle, responsible for severe negative impacts on animal health and cattle producer profitability in many regions of the world. Our research group has begun the development of molecular-based assays for specific acaricide resistance-associated genes to facilitate the identification of acaricide resistant populations of *B. microplus*. We have devised a PCR-based assay to detect a specific sodium channel gene mutation that is associated with resistance to permethrin. A number of Mexican strains of *B. microplus* have been genotyped by this assay that can be performed on individual ticks at any life stage with results available in a few hours. Additionally, a specific metabolic esterase with permethrin-hydrolyzing activity has been purified and its gene coding region cloned. A mutant allele of this esterase gene has been found, though the mutation seems to provide only a low level of additional permethrin resistance compared to that provided by the wild type allele. Work is continuing to clone specific acetylcholinesterase (AChE) and carboxylesterase genes that appear to be involved in resistance to organophosphates (OP). We are designing a battery of DNA-or ELISA-based assays capable of rapidly genotyping individual ticks for pyrethroid and OP resistance-associated genes.

INTRODUCTION

Boophilus microplus (Canestrini) is an endemic cattle pest in tropical and subtropical regions of the world, causing major economic losses to cattle producers through direct physical effects on the parasitized animal and indirectly through diseases transmitted by the tick's bite. Australia estimates that $100 million AUD are lost annually to *B. microplus* [1]. Economic losses in the United States were sufficient for a *Boophilus sp.* eradication program to be initiated in 1907 and was completed over 50 years later [2]. Coincident with the completion of the eradication program, the U. S. Department of Agriculture established an intensive quarantine program that includes physical inspection and dipping of cattle in acaricide-filled vats at importation stations that only occur along the Texas-Mexico border. Two critical issues impacting the ability of the United States to remain free of *Boophilus* ticks are the management of the tick problem in Mexico and the efficacy of the acaricides formulated for use in the dipping vats at the border import stations. Acaricides, including OPs, synthetic pyrethroids and amitraz, play a major role in controlling *Boophilus* ticks in Mexico and the intensive use of these chemicals has led to the development of resistant tick populations in that country [3]. Acaricide resistant ticks present a hazard to the U. S. cattle industry, particularly if the resistance levels in larvae allow them to survive treatment in the import station and quarantine zone dipping vats. Because of their small size, any surviving larvae will be very difficult to detect by physical inspection of the dipped cattle.

Presently, when *Boophilus* outbreaks occur in the U. S. or when tick inspectors wish to determine the resistance status of ticks found on animals, samples are assayed by larval packet tests [4]. The larval packet

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test provides useful information, however, it does not reveal information about the resistance mechanism, is not sensitive enough to detect resistance in its early stages of development, and takes 4-8 weeks to complete. Oviposition and hatching must occur before these tests can be initiated and samples consisting of only males or nongravid females cannot be analyzed. Other bioassay tests have been proposed for resistance assays, some offering advantages over the larval packet test. The adult immersion test provides results much quicker than the larval packet test. However, the adult immersion test has not been standardized to the extent of the larval packet test and there are many questions that have not been adequately addressed regarding proper sample collection timing, age, numbers, and how to obtain an adequate representative tick sample [4]. Synergist studies associated with bioassays can help determine the involvement of families of metabolic enzymes, such as esterases, mixed function oxidases, and glutathione transferases. However, many of the same problems with larval packet tests apply to synergist studies and the inhibitory activity of synergists is not entirely specific to a single family of metabolic enzymes.

At our quarantine tick research facility in Mission, Texas, a number of resistant and susceptible *B. microplus* strains have been developed from ticks sampled in Mexico or from outbreaks in Texas. Using larval packet tests in conjunction with synergist studies, likely resistance mechanisms have been identified as target site insensitivity and metabolic enzymes which include esterases and mixed function oxidases (MFO) (Table 1). It would be useful to determine the resistance status of tick samples with a rapid sensitive diagnostic test that could be performed on single ticks and that could also guide the selection of an acaricide which would be both effective and prudent in an acaricide resistance management program. Potential molecular based assays include PCR-based methods of detecting specific resistance-causing nucleotide polymorphisms, reverse transcription-PCR for quantifying transcript levels which could encode enzymes involved in the sequestration or metabolism of pesticides, or ELISA-based methods to quantify pesticide-sequestering or -metabolizing enzymes. Our research group has the goal of developing PCR-based assays for diagnosing resistance in *B. microplus*. The assays are being designed to be completed within a day, determine the specific resistance mechanism and be performed on individual ticks, using whole tick larvae or adult hemolymph or cuticular material. Initial

Table 1. Various Mexican strains of *B. microplus* and resistance mechanisms.

Strain	Resistance		Major Mechanism[a]		
	Pyr	OP	Target	Est	MFO
Gonzales[b]	-	-	-	-	-
Munoz[d]	-	-	-	-	-
Deutsch[d]	-	-	-	-	-
Tuxpan[c]	-	+	+	+	+
San Felipe[b]	+	-	+	-	-
Corrales[b]	+	-	+	-	-
Coatzacoalcos[b]	+	+	-	+	+
San Roman[d]	-	+	+	-	-
Caporal[d]	-	+	+	-	-

[a] Target=insensitive target site; Est=metabolic esterase; MFO=mixed function oxidase
[b] Reference [5]
[c] Reference [6]
[d] Unpublished data from Andrew Y. Li

efforts have focused on developing assays to detect pyrethroid resistance. Progress in this area includes the development of a PCR assay to detect target site resistance to pyrethroids [7]. More recently, focus has shifted to OP resistance, particularly as the OP coumaphos is the acaricide formulated and approved for use in the import station and quarantine zone dipping vats. Work is presently underway to purify acetylcholinesterase (AChE), the target of OP pesticides, and a PCR assay to detect potential target site-mediated OP resistance is anticipated. The status of molecular diagnosis for resistance to the pyrethroids and OPs will be discussed separately.

PYRETHROID RESISTANCE

Target Site-Mediated Resistance

The sodium channel is the target site for pyrethroid pesticides and target site-mediated pyrethroid resistance has been well studied in several insect species, particularly *Drosophila melanogaster* (Meigan) [8] and *Musca domestica* L. [9]. Miller et al. [5] identified target site insensitivity to pyrethroids in two colonized strains of *B. microplus* from Mexico. Both strains were found to possess a nucleotide substitution in the sodium channel gene coding region leading to a Phe → Ile amino acid substitution in the S6 transmembrane

segment of domain III [10]. We designed a PCR assay to detect the presence of this sodium channel gene polymorphism [7] and have used the assay to show that individual larvae with the nucleotide substitution in the homozygous state can survive very high doses of permethrin that kills homozygous wild type sodium channel larvae [11]. Table 2 shows the numbers of surviving or dead ticks from two pyrethroid resistant strains of *B. microplus*, San Felipe (SF) and Coatzacoalcos (Cz), exposed to several doses of permethrin in larval packet tests. These two strains were originally collected from Mexican ranches experiencing control failures with pyrethroid acaricides. Once laboratory strains were established from the field collections, larval packet tests and synergist studies confirmed the pyrethroid resistance [5]. Although other factors besides the sodium channel's nucleotide substitution likely contribute to permethrin resistance in these strains, it is clear the homozygous mutant RR genotype is associated with survival of the permethrin dose while the homozygous wild type SS genotype associates with the dead larvae. The assay protocol basically consists of grinding frozen larvae individually in microcentrifuge tubes with a disposable pellet pestle in 25 µl of buffer (100 mM Tris, pH 8.3; 500 mM KCl), boiling for 3 min and using 1 µl in hotstart PCR reactions with allele specific primers [7]. Each larvae is assayed twice, one reaction diagnostic for the presence of the wild type susceptible (S) sodium channel allele using a primer specific for this allele and a second reaction to detect the mutant pyrethroid resistant (R) allele using a different diagnostic primer specific for the mutant allele. Agarose gel electrophoresis is used to detect the 68 bp diagnostic PCR product and determine the genotype of each larvae as homozygous susceptible (SS), heterozygous (SR) or homozygous resistant (RR). Besides evaluating target site pyrethroid resistance, this PCR assay has been used to verify the genotype of newly established strains brought into the lab for use as a susceptible controls for various research projects and also to verify the resistance status of single ticks sampled from cattle presented for importation.

Table 2. Permethrin challenge assay fate of homozygous wild type (SS) and homozygous mutant (RR) sodium channel genotype larvae.

Strain[a]	Dose[b]	SS		RR	
		Alive	Dead	Alive	Dead
SF	30	0	3	28	8
	15	0	5	25	3
	7.5	0	10	19	1
	3.75	2	20	13	1
	0	3	-	38	-
Cz					
	15	0	9	16	3
	7.5	0	9	16	3
	3.75	1	10	23	2
	1.88	1	5	13	5
	0	19	-	14	-

[a] SF strain LC_{50}=25 % permethrin (95% CL =19-39); Cz strain LC_{50}=0.87 % permethrin (95% CL =0.34-1.4) from [10]

[b] % permethrin

Metabolic Resistance

Riddles et al. [12] described the partial purification of a *B. microplus* enzyme with carboxylesterase-like activity that could hydrolyze permethrin, providing early evidence that metabolic enzymes can be involved in pyrethroid resistance. Miller et al. [5] described synergist studies that identified likely pyrethroid resistance mechanisms associated with several Mexican strains of *B. microplus*. One strain, Cz, was found to possess a moderate level of pyrethroid resistance that could be substantially, though not completely, reduced by the addition of triphenylphosphate (TPP) or piperonyl butoxide (PBO), inhibitors of carboxylesterases and MFOs, respectively. Subsequently, Jamroz et al. [6] noted a specific carboxylesterase, designated CzEst9, with an elevated level of esterase activity only in the Cz strain. Elevated levels of esterase activity toward the model substrates α- and β-naphthyl acetate and permethrin hydrolysis were also found in the Cz strain relative to two permethrin susceptible strains, Gonzales and Tuxpan, and a target site-mediated pyrethroid resistant strain, Corrales. Hernandez et al. [13] identified the coding region of a *B. microplus* esterase, designated clone 13, which possessed multiple copies in the Cz strain compared to three pyrethroid susceptible strains, Gonzales, Ramireno, and Tuxpan, and two target site-mediated pyrethroid resistant strains, San Felipe and Corrales. Work in our laboratory led to the purification of CzEst9 protein [14], verification that the partial amino acid sequence available from CzEst9 was a nearly identical match to the deduced amino acid

sequence of clone 13 (46 of 47 amino acids), and that mRNA which hybridized to a radiolabeled probe from the clone 13 coding region had a 5-fold elevated concentration in the Cz strain compared to two pyrethroid susceptible strains and the target site-mediated pyrethroid resistant strain, San Felipe [15].

A nucleotide polymorphism leading to an Asp → Asn amino acid substitution in the CzEst9 coding region had been postulated to be involved in the resistance mechanism of ticks from the Cz strain [13]. This led us to attempt to detect CzEst9-mediated pyrethroid resistance with a similar strategy as the sodium channel target site-mediated pyrethroid resistance. Although a PCR assay was developed to detect the presence of this polymorphism [15], unlike the sodium channel substitution, a strong correlation of the presence of the CzEst9 polymorphism with pyrethroid resistance was not found [11]. This evidence along with that from earlier studies [6, 13] suggests that the CzEst9-mediated resistance is due to the higher levels of CzEst9 in the Cz strain rather than a specific mutant version of the enzyme. Although elevated levels of CzEst9 message could be quantitated through a reverse transcription-PCR strategy, the level of CzEst9 mRNA would not necessarily be indicative of protein concentration. Thus, work is currently underway to express CzEst9 using a recombinant DNA cloning vector and an ELISA-based assay is anticipated to directly quantitate CzEst9 in individual ticks.

Further Needs

Although the *B. microplus* sodium channel PCR mutation assay has utility in its present form, a DNA sequencing survey of the sodium channel gene coding region from all available pyrethroid resistant *B. microplus* populations should be made to identify if other resistance-associated mutations exist. If other mutations exist, a multiplex PCR assay could be developed to simultaneously test for all resistance-associated sodium channel mutations. This survey should not be too difficult, as it is likely that only a limited number of amino acids in the sodium channel can be altered to yield both pyrethroid resistance and viable organisms. In addition, guidance from mutation surveys in insects is available [16].

The development of the CzEst9 ELISA-based quantitative assay is needed to detect this metabolic mechanism of pyrethroid resistance. Although it is not known how widespread CzEst9-based resistance might be, the Cz strain was one of the first pyrethroid resistant strains collected from field-established resistant ticks in Mexico [17]. It would be very interesting to know if this mechanism has spread from the southeastern Mexican state of Veracruz where it was collected in 1994 and if this permethrin-hydrolyzing esterase is associated with the moderate OP resistance expressed by this strain.

Although the synergist studies of Miller et al. [5] indicated mixed function oxidases were a component of the pyrethroid resistance mechanism of the Cz strain, little had been reported in the literature concerning these enzymes in *Boophilus* ticks. In conjunction with our data indicating the principal involvement of target site modifications and metabolic esterases with pyrethroid resistance, a lower priority was given to the study of MFOs. However, evidence from insects shows that MFOs can clearly be a major metabolic resistance mechanism [18]. Although not shown to be involved in pesticide resistance, the DNA sequences of three cytochrome P450 genes, designated *CYP41* [19], *CYP4W1* [20], and *CYP319A1* [21], from *B. microplus* have recently been reported and should facilitate further advances in knowledge about MFOs in this species. Our focus on pyrethroid resistance monitoring in Mexican *B. microplus* will remain on the sodium channel and metabolic esterases. Data from synergist studies on new pyrethroid resistant strains, as they are established, will be used to alert us to the emergence of MFOs as a major pyrethroid resistance mechanism requiring the development of molecular-based monitoring assays.

OP RESISTANCE

Target Site-Mediated Resistance

AChE is the target site of OP activity where the pesticide acts as a substrate analogue and inhibits enzymatic function [22]. Seven specific pesticide resistance-associated AChE mutations have been reported in field populations of resistant *D. melanogaster* [23] and *M. domestica* [24] and *in vitro* mutagenized AChE from *Aedes aegypti* [25]. Two of the mutations, Gly227 → Ala and Phe288 → Tyr (numbered based on amino acid sequence of AChE from *Torpedo californica*), occurred in all three species, while Phe78 → Ser was common only to *D. melanogaster* and *A. aegypti*, Ile129 → Val occurred only in *D. melanogaster*, and Val151 → Leu, Gly328

→ Ala, and Gly227 → Val occurred only in *M. domestica*. Various combinations of these mutations lead to different levels of resistance, both in the field and *in vitro*. Two putative *B. microplus* AChE-encoding sequences have been reported [26, 27], however, there is only 33% identity between the deduced amino acid sequences of the two putative AChEs [27]. Although neither putative coding region has been expressed and evaluated for AChE-like activity, both groups reported no evidence for OP resistance-associated mutations and speculated that posttranslational modification or an as yet undiscovered AChE gene was responsible for OP resistance in *B. microplus*. Recently, Baxter and Barker [28] reported finding a second putative AChE gene in Australian populations of *B. microplus*. The deduced amino acid sequence had over 95% identity with the putative AChE reported from Mexican populations of *B. microplus* [27]. However, in Australian ticks, Baxter and Barker found amino acid differences between OP susceptible and OP resistant ticks, in contrast to a lack of differences between the Mexican OP resistant ticks and OP susceptible ticks as reported by Hernandez et al. [27].

The developmental status of AChE mutation detection assays in our laboratory is at the early stages. We have used larval packet tests to isolate highly resistant larvae from OP resistant strains of *B. microplus* and highly susceptible larvae from OP susceptible strains and have initiated experiments to clone and sequence the putative AChE coding region which corresponds to that reported by Hernandez et al. [27] from individual larvae. If resistance-associated nucleotide differences are noted, an allele-specific PCR assay will be developed and incorporated with the sodium channel mutation PCR assay to detect both types of target site-mediated acaricide resistance mechanisms. The failure of Hernandez et al. [27] to find resistance-associated differences in their putative AChE coding region may have been because they used pools of unselected larvae instead of individual larvae segregated according to resistance status. Additionally, they used the Tuxpan strain that has a significant metabolic esterase component of its resistance mechanism. We have used the San Roman strain as the source of OP resistant larvae, a strain found to have kinetic parameters indicative of OP-insensitive AChE [29]. We also have purified an enzyme from *B. microplus* that has AChE-like activity and amino acid sequence information should be forthcoming. It will be interesting to see if this enzyme's amino acid sequence matches either of the reported putative *B. microplus* AChEs. Early work in Australian [30] and Mexican [31] strains showed at least two types of AChE activity, one of which was sensitive to OP and the other insensitive. Pruett's study of AChE kinetics in OP resistant strains of *B. microplus* from Mexico showed the OP resistant Caporal and San Roman strains to have reduced AChE activity relative to two susceptible strains [29]. Apparent conformational changes in the target site for OP were reflected by reduced bimolecular reaction constants relative to the susceptible strains. The bimolecular reaction constants were most affected by a slower rate of resistant strain AChE phosphorylation. Interestingly, the AChE from Caporal also appeared to have an increased affinity for substrate and OP relative to San Roman and the two susceptible strains, evidence for two different mechanisms of resistance through an altered target site.

Metabolic-Mediated Resistance

Synergist studies have indicated that metabolic resistance plays an important role in the OP resistance of the Tuxpan strain. Jamroz et al. [6] reported an esterase, designated EST10, with carboxylesterase-like activity that was found in the Tuxpan strain and not in the susceptible Gonzales strain. Figure 1 shows a similar native polyacrylamide gel analysis of the esterase activity of the Gonzales, and the three OP resistant Tuxpan, Caporal, and San Roman strains. The eserine-insensitive qualitative and quantitative differences between the Gonzales and the OP strains suggest metabolic resistance might play a role in the OP resistance of all three strains. Purification of the two esterases noted in Figure 1 and study of their OP sequestration/metabolic capacity would help determine their role in OP resistance. Even though the biochemical evidence indicates that target site OP resistance is a major mechanism in the Caporal and San Roman strains, an ELISA assay to quantify levels of these two metabolic esterases might be useful for OP resistance monitoring.

Further Needs

It is obvious that development of DNA- or ELISA-based resistance diagnostic assays is more advanced for pyrethroid resistance than OP resistance. Initially, only a single OP resistant Mexican tick strain, Tuxpan, was available for study. With the imminent availability of amino acid sequence from purified AChE and the recent DNA sequence information from

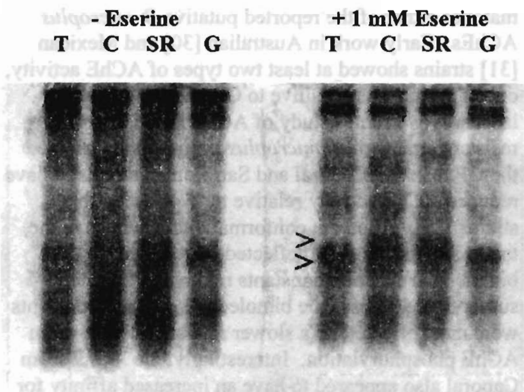

Figure 1. Gel profiles of esterase hydrolytic activity in Mexican strains of *B. microplus*. Protein was extracted from 14 day old, unfed larvae from the OP susceptible Gonzales (G) and the OP resistant Tuxpan (T), Caporal (C), and San Roman (SR) strains, fractionated on 8% polyacrylamide native gels and assayed for the presence of esterase activity using the model substrate α-naphthyl acetate in the presence (+ 1 mM eserine) or absence (-eserine) of the specific AChE activity inhibitor eserine sulfate [6]. The two arrowheads note esterase activities which might be associated with OP resistance either through overexpression (lower arrowhead) or novel esterase (upper arrowhead).

putative AChE coding regions, progress in the development of an AChE mutation-detecting PCR assay is expected. Purification of carboxylesterases from the OP resistant strains will be necessary to develop diagnostic assays to rapidly detect metabolic OP resistance. This information will be valuable for identifying OP resistant ticks that do not have significant components of target site-mediated resistance.

OTHER ISSUES

With OP and pyrethroid resistance becoming more widespread in Mexico, the use of the formamidine acaricide amitraz is increasing. The target site of formamidines appears to be the octopamine receptor [32], and the DNA from a putative octopamine receptor coding region has been sequenced from amitraz susceptible and resistant Australian strains of *B. microplus* [33]. No sequence differences were noted between the strains. Amitraz resistance is not yet widespread in Mexico and few studies on resistance mechanisms have been published. It would be valuable to determine a diagnostic assay for amitraz resistance before the phenomenon becomes a serious problem. An amitraz resistance diagnostic would likely be a useful tool for a resistance management program to help preserve the efficacy of amitraz. Two amitraz resistant strains have been isolated from Mexico and work is in progress to identify the resistance mechanism. If resistance-associated point mutations in the target site are identified, a PCR diagnostic assay will be developed and can be used to detect the early stages of amitraz resistance.

The issue of MFO-based resistance will likely need to be addressed, as this mechanism contributes to pesticide resistance in a number of arthropod species. Although several *B. microplus* cytochrome P450 coding regions have been reported, the contributions of MFOs to resistance in *B. microplus* has not been well-studied except indirectly though synergist studies with piperonyl butoxide such as those of Miller et al. [7].

In addition to providing the molecular basis for acaricide resistance, these assays are designed to provide useful information for resistance management programs. As such, they must be validated in epidemiological studies of field populations with varying degrees of resistance. Ideally, a correlation between molecular diagnostic assay results and pesticide efficacy can be determined so as to guide the cattle producer in the choice of acaricide. The sensitivity of these molecular assays is such that the early stages of the spread of resistance genes can be detected quickly and control measures adopted, when available, to slow the spread of these genes and lengthen the period of time which a particular pesticide is efficacious against *B. microplus*. Within the U. S., these assays might prove most valuable to tick inspectors with the border quarantine program when outbreaks occur within the U. S. When the full array of molecular tests is available, the initial identification of resistance can occur with the rapid PCR assays, followed by larval packet assays if desired, to allow for the quick selection of the best choice of acaricide for eradication of the outbreak.

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REFERENCES

[1] Angus, B. M. 1996, Int. J. Parasitol., 26, 1341-1355.
[2] Graham, O. H., and Hourrigan, J. L. 1977, J. Med. Entomol., 13, 629-658.
[3] Fragoso, S. H., Soberanes, C. N., Ortiz, E. M., Santamaria, V. M., and Ortiz, N. A. 1995, Proceedings, III Seminario Internacional de Parasitologic Animal, pp. 45-57, October 11-13 1995, Acapulco, Guerrero, Mexico.
[4] FAO, 1984, Ticks and tick-borne disease control. A practical field manual. Vol. 1, FAO, Rome.
[5] Miller, R. J., Davey, R. B., and George, J. E. 1999, J. Med. Entomol., 36, 533-538.
[6] Jamroz, R. C., Guerrero, F. D., Pruett, J. H., Oehler, D. D., and Miller, R. J. 2000, J. Insect Physiol., 46, 685-695.
[7] Guerrero, F. D., Davey, R. B., and Miller, R. J. 2001, J. Med. Entomol., 38, 44-50.
[8] Pittendrigh, B., Reenan, R., ffrench-Constant, R. H., and Ganetzky, B. 1997, Mol. Gen. Genet., 256, 602-610.
[9] Williamson, M. S., Martinez-Torres, D., Hick, C. A., and Devonshire, A. L. 1996, Mol. Gen. Genet., 252, 51-60.
[10] He, H., Chen, A. C., Davey, R. B., Ivie, G. W., and George, J. E. 1999, Biochem. Biophysiol. Res. Com., 261, 558-561.
[11] Guerrero, F. D., Li, A. Y., and Hernandez, R. 2002, J. Med. Entomol., 39, 770-776.
[12] Riddles, P. W., Davey, P. A., and Nolan, J. 1983, Pest. Biochem. Physiol., 20, 133-140.
[13] Hernandez, R., He, H., Chen, A. C., Waghela, S. D., Ivie, G. W., and George, J. E. 2000, Insect Biochem. Mol. Biol., 30, 969-977.
[14] Pruett, J. H., Guerrero, F. D., and Hernandez, R. 2002, J. Econ. Entomol. 2002, J. Econ. Entomol., 95, 1001-1007.
[15] Hernandez, R., Guerrero, F. D., George, J. E., and Wagner, G. G. 2002, Insect Biochem. Mol. Biol., 32, 1009-1016.
[16] ffrench-Constant, R. H. 1999, Insect Biochem. Mol. Biol., 29, 397-403.
[17] Ortiz, E. M., Santamaria, V. M., Ortiz, N. A., Soberanes, C. N., Osorio, M. J., Franco, B. R., Martinez, I. F., Quezada, D. R., and Fragoso, S. H. 1995, Proceedings, III Seminario Internacional de Parasitologic Animal, pp. 58-66, October 11-13 1995, Acapulco, Guerrero, Mexico.
[18] Scott, J. G. 1999, Insect Biochem. Mol. Biol., 29, 757-777.
[19] Crampton, A. L., Baxter, G. D., and Barker, S. C. 1999, Insect Biochem. Mol. Biol., 29, 829-834.
[20] Crampton, A. L., Baxter, G. D., and Barker, S. C. 1999, Insect Biochem. Mol. Biol., 29, 377-384.
[21] He, H., Chen, A. C., Davey, R. B., and Ivie, G. W. 2002, Insect Biochem. Mol. Biol., 32, 303-309.
[22] O'Brien, R. D. 1967, Insecticides Action and Metabolism, Academic Press, New York.
[23] Mutero, A., Pralavorio, M., Bride, J.-M., and Fournier, D. 1994, Proc. Natl. Acad. Sci., USA, 91, 5922-5926.
[24] Walsh, S. B., Dolden, T. A., Moores, G. D., Kristensen, M., Lewis, T., Devonshire, A. L., and Williamson, M. S. 2001, Biochem. J., 359, 175-181.
[25] Vaughan, A., Rocheleau, T., and ffrench-Constant, R. 1997, Exper. Parasitol., 87, 237-244.
[26] Baxter, G. D., and Barker, S. C. 1998, Insect Biochem. Mol. Biol., 28, 581-589.
[27] Hernandez, R., He, H., Chen, A. C., Ivie, G. W., and George, J. E. 1999, J. Med. Entomol., 36, 764-770.
[28] Baxter, G. D., and Barker, S. C. 2002, Insect Biochem. Mol. Biol., 32, 815-820.
[29] Pruett, J. H. 2002, J. Econ. Entomol., In press.
[30] Nolan, J., Schnitzerling, H. J., and Schuntner, C. A. 1972, Pest. Biochem. Physiol., 2, 85-94.
[31] Wright, F. C., and Ahrens, E. H. 1988, J. Med. Entomol., 25, 234-239.
[32] Evans, P. D., and Gee, J. D. 1980, Nature, 287, 60-62.
[33] Baxter, G. D., and Barker, S. C. 1999, Insect Biochem. Mol. Biol., 29, 461-467.