

Exploiting the modes of action of insecticides and acaricides to manage the development of resistance in arthropod pests in Ghana

K.D. NINSIN

*Animal Health and Food Safety Division, CSIR-Animal Research Institute, P. O. Box AH 20, Achimota, Ghana (Present address: K.D. NINSIN, P. O. Box LG 981, Legon, Ghana)
E-mail: kdninsin@hotmail.com*

ABSTRACT

The importation and use of insecticides and acaricides in agriculture and public health are on the increase in Ghana, while populations of arthropod pests have developed resistance to some active ingredients. However, effective strategies for managing arthropod pest resistance in Ghana are lacking due to the absence of appropriate research for developing effective resistance management strategies. For instance, laboratory studies to evaluate cross-resistance patterns and stability of resistance are not conducted in Ghana. Laboratory studies to determine the mechanisms of resistance to specific active ingredients are also not conducted. In Ghana, resistance research is focused on the detection of esterases in field populations of pests, complemented by resistance ratio which is computed in a wrong manner as susceptibility of test population divided by the recommended field concentration of active ingredient. Until the right research for developing effective strategies to manage resistance are conducted, the modes of action (MoAs) of active ingredients could be used to help manage resistance development. However, in Ghana, insecticide and acaricide recommendation and their use indicate that the knowledge on exploiting MoAs for resistance management is also lacking. For instance, it is recommended that any of the approved insecticides, imidacloprid, thiamethoxam and bifenthrin, for cocoa mirid control could be used when it is time to spray against mirids. The report that the acaricidal products Cypertop[®] (contains mixture of cypermethrin and chlorpyrifos), Vetacid[®] Max (contains mixture of cypermethrin and trichlorfon) and Ectocyp[®] (contains cypermethrin) were alternated to control ticks also shows the lack of knowledge on exploiting MoAs to manage resistance. This review paper discusses how the MoAs of active ingredients of insecticides and acaricides can be exploited to manage resistance development in arthropod pests in Ghana and the scientific basis of the strategy. Resources for determining the MoAs of active ingredients of insecticides and acaricides are also shared.

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Introduction

Arthropod pest resistance to an insecticide or acaricide is a heritable decrease in the susceptibility of an arthropod pest population to an insecticide or acaricide compared with a strain of same species that is susceptible to the

insecticide or acaricide (Ninsin, 2016a). Resistance to insecticides and acaricides remains a global challenge as chemical control continue to be the first-choice control method against arthropod pests in agriculture and public health. Arthropod pest resistance makes useful com-

pounds ineffective, in addition to causing control failures, increase in the incidence of arthropod-borne diseases, increase in pesticide residues in foods and environmental contamination. In the USA, crop losses due to pesticide resistance is estimated to be over \$1.5 billion per annum (Pimentel, 2005). Although the cost of pesticide resistance in the USA is high, it is significantly greater in tropical developing countries because pesticides are not only used to control agricultural pests, they are also vital for the control of arthropod disease vectors (Pimentel, 2005). The recommended field concentrations of pesticides generate high resistance in arthropod pest populations and justify the need to manage resistance development in field populations of pests (Ninsin, 2011a; 2016b). It is, therefore, necessary to incorporate effective resistance management strategies into arthropod pest management programmes that have an insecticide or acaricide control component from the onset.

Although the importation of pesticides into Ghana has been increasing over the years (Kotey, 2007), effective resistance management strategies for preventing resistance development in arthropod pests are nonexistent (Ninsin, 2015). The nonexistence of effective strategies to manage resistance is due to the absence of appropriate research for developing such strategies. For instance, the rotational use of active ingredients of insecticides and acaricides that do not show cross-resistance is an effective countermeasure against arthropod pest resistance development (Saito *et al.*, 1995). However, laboratory studies to evaluate cross-resistance patterns are not being conducted in Ghana. The idea behind the rotation of active ingredients with no cross-resistance between them is that when an active ingredient is withdrawn the susceptibility of resistant arthropod pest will be restored within several generations, allowing the active ingredient to be re-incorporated into pest management programmes (Ninsin & Tanaka, 2005). Laboratory studies to evaluate the stability of resistance are also not conducted in Ghana. An understanding

of the mechanisms by which arthropod pests develop resistance is crucial for designing a successful resistance management programme (Ninsin & Tanaka, 2005).

The mechanisms by which arthropod pests protect themselves from active ingredients used to control them are target-site resistance, metabolic resistance, cuticular penetration resistance and behavioural resistance. The mechanisms of major importance are target-site resistance and the metabolic resistance, which involves three enzyme systems, cytochrome P-450 monooxygenases, esterases and glutathione *S*-transferases. As a preliminary investigation to finding the mechanisms of resistance, the involvement of metabolic enzymes in the resistance of pests to an active ingredient is determined in the laboratory using synergists (Ninsin & Tanaka, 2005). However, synergism studies and other laboratory studies to clarify the mechanisms of resistance to a given active ingredient in a target pest are also not conducted in Ghana.

Resistance research in Ghana is focused on the detection of esterases in populations of field pests such as the cotton aphid, *Aphis gossypii* (Glover) and the diamondback moth (DBM), *Plutella xylostella* (L.) (Owusu, 1997; Obeng-Ofori, Owusu & Kaiwa, 2002 ; Owusu & Yeboah, 2007; Odhiambo *et al.*, 2010; Botw, Eziah & Owusu (2012), complemented by the calculation of resistance ratio (RR) (Odhiambo *et al.*, 2010; Botwe *et al.*, 2012). Ninsin (2016a) has shown that even the method for computing the RR in Ghana, where the susceptibility of a test population is divided by the recommended field concentration of active ingredient is erroneous as it underestimates the resistance levels of test populations.

The nonexistence of effective resistance management strategies in Ghana is exposed by the DBM, which is the second most resistant arthropod pest worldwide (Vasquez, 1995; Nauen, 2012), and the most serious pest of cabbage and other cruciferous crops in Ghana and the rest of the world (Ninsin, 1997; Shelton *et al.*, 1997). For instance, the integrated pest

management (IPM) compatible microbial insecticide, *Bacillus thuringiensis* var. *kurstaki* Berliner (*Btk*), which was used in 1996 to control the DBM (Ninsin, 1997) is rarely used against the pest now because, according to farmers, *Btk* has become ineffective against the DBM. The high residue levels of organophosphate and pyrethroid insecticides detected on cabbage by Odhiambo (2005) had come from farms where high levels of DBM resistance to organophosphates and pyrethroids had been detected (Odhiambo *et al.*, 2010). The resistance to pyrethroids detected in field populations of DBM (Odhiambo *et al.*, 2010) also continues to be present on cabbage farms and causing DBM control failures (Botwe *et al.*, 2012). The need for resistance management strategies that would effectively manage arthropod pest resistance in Ghana is, therefore, urgent.

Until the appropriate locally generated research is conducted for developing effective resistance management strategies in Ghana, the modes of action (MoAs) of insecticides and acaricides used in agriculture and public health could be exploited to prevent or retard the development of resistance in arthropod pests or even regain susceptibility when resistance has already developed. However, in Ghana, knowledge on the exploitation of MoAs to manage resistance development in arthropod pest populations is also lacking. The approved insecticides, imidacloprid (neonicotinoid), thiamethoxam (neonicotinoid) and bifenthrin (pyrethroid) for the control of cocoa mirids, *Distantiella theobroma* (Dist.) and *Sahlbergella singularis* Hagl. (COCOBOD, 2007), and the recommendation that any of the insecticides could be used when it is time to spray against the mirids (COCOBOD, 2008) indicate a lack of knowledge on MoA exploitation for resistance management. Ninsin & Koney (2015) also observed that when Cypertop[®], a product that contains a mixture of cypermethrin (pyrethroid) and chlorpyrifos (organophosphate) could not control ticks on cattle, it was replaced with Vetancid[®] Max, which contains a mixture of

cypermethrin and trichlorfon (organophosphate). When Vetancid[®] Max also failed to control the ticks it was replaced with Ectocyp[®], that contains cypermethrin (Ninsin & Koney, 2015). The observation by Ninsin & Koney (2015) also shows a lack of knowledge on the exploitation of MoAs to manage resistance development in arthropod pests in Ghana.

In this paper, how the MoAs of active ingredients can be exploited to manage arthropod pest resistance to insecticides and acaricides in agriculture and public health and the scientific basis of the strategy are discussed. Since most of the active ingredients for controlling mites and ticks are insecticides with acaricidal activity, insecticides and acaricides would hereafter be referred to as insecticides. The neonicotinoid insecticides that have a growing use for vegetable pest control in Ghana and applied to control the cocoa mirids would be used to elucidate the exploitation of MoA for resistance management.

Evolution of resistance

Resistance develops in an arthropod pest population through the selection of pre-adapted resistance genes by insecticides (Scott, 1995). As illustrated by van Emden (1974), prior to the exposure to insecticides, the frequency of resistance genes in a population is very low and does not affect the efficacy of insecticides. However, after insecticide application and selection, the frequency of resistance genes increases so that the concentration of insecticide exerting the selection pressure kills fewer individuals in the progeny population than in the parent population (van Emden, 1974). As the insecticide is applied, the pre-adapted resistant individuals in the population are selected through succeeding generations and when the resistant (r)-allele becomes common in the population, the effectiveness of the insecticide is significantly reduced (Metcalf, 1989).

Resistant arthropods protect themselves from the toxicity of insecticides by various mechanisms. Metabolic resistance and target-site resistance are the major resistance

mechanisms. Other mechanisms displayed by arthropod pests are cuticular penetration resistance and behavioural resistance, which is of minor importance. Metabolic resistance occurs when the arthropod's metabolic enzyme systems are enhanced to detoxify or sequester insecticides and render them ineffective. Resistance to the neonicotinoid imidacloprid, a nicotinic acetylcholine receptor (nAChR) agonist (Yamamoto *et al.*, 1995) in the brown planthopper, *Nilaparvata lugens* Stål, has been shown to involve metabolic resistance (Liu *et al.*, 2003).

Metabolic resistance has been implicated in resistance of the whitefly, *Bemisia tabaci* Gennadius to imidacloprid (Nauen, Stumpf & Elbert 2002; Rauch & Nauen, 2003), in resistance of DBM to the neonicotinoid acetamiprid (Ninsin & Tanaka, 2005), and in many other cases of arthropod pest resistance to a variety of insecticides of different chemistries. The enzyme systems, cytochrome P-450 monooxygenases, esterases and glutathione *S*-transferases are involved in metabolic resistance. The resistance to thiamethoxam in the western flower thrips, *Frankliniella occidentalis* (Pergande), where P-450 monooxygenase and esterase were implicated (Gao *et al.*, 2014) demonstrates that more than one enzyme system could be involved in the resistance of a pest to an insecticide.

With regard to target-site resistance mechanism, the specific target that the insecticide attacks for insecticidal activity is genetically modified in ways that do not allow the insecticide to attack the site anymore. As a result, insecticidal activity against the pest is or eliminated. Genetically altered nAChR has been shown to contribute to imidacloprid resistance in the brown planthopper (Liu *et al.*, 2005) and green peach aphid, *Myzus persicae* (Sulzer) (Bass *et al.*, 2011). The reports by Liu *et al.* (2003; 2005) that both metabolic and target-site resistance mechanisms are involved in the resistance of the brown planthopper to imidacloprid also shows that arthropod pests could protect themselves against an insecticide

with both major resistance mechanisms.

When an arthropod pest population develops resistance to an insecticide that has been used for pest control, the resistant pest population could exhibit resistance to other insecticides that have not been used against it, a phenomenon known as cross-resistance. Cross-resistance could exist between insecticides of same chemistry, as demonstrated in the acetamiprid-resistant *B. tabaci*, which showed cross-resistance to imidacloprid, thiamethoxam, thiacloprid and nitenpyram, all being neonicotinoid insecticides (Basit *et al.*, 2011). Cross-resistance could also exist between insecticides of different chemistries, as demonstrated in the phenthoate-resistant DBM and cartap-resistant DBM against acetamiprid (Ninsin, 2004a) and in the acetamiprid-resistant DBM against cartap and phenthoate (Ninsin, 2004b). When insecticides of different chemistries are used against an arthropod pest population without a resistance management strategy, the population could develop resistance to each of the different insecticide chemistries and have an array of resistance mechanisms at the same time, a phenomenon known as multiple-resistance.

Insecticide mode of action for resistance management

The goal of insecticide-resistance management is to prevent or retard resistance development by making available a high frequency of insecticide susceptible individuals in the pest population (Ninsin, 2016a). This can be achieved through a decrease in insecticide selection pressure, since whether resistance develops or not depends on the presence or absence of insecticides. Thus, selection pressure is critical in managing arthropod pest resistance. In order to influence the selection pressure when insecticidal control is warranted, the moderate use of the insecticide is necessary. The rotational use of insecticides that do not show cross-resistance has been reported as an effective countermeasure against the development of insecticide resistance in pests (Saito *et al.*, 1995). In a rotation, each insecticide is used in moderation, which decrea-

ses the selection pressure. Also, during the rotation, when an insecticide is not being used, the susceptibility of the resistant arthropods will be restored within several generations, allowing the insecticide to be re-incorporated into pest management programmes (Ninsin & Tanaka, 2005).

In certain cases, however, resistance persists over many generations after the withdrawal of selection pressure, as has been observed in imidacloprid-resistant *B. tabaci* of the Q-biotype (Nauen *et al.*, 2002). A stable resistance prevents the successful re-use of an insecticide for pest management (Ninsin & Tanaka, 2005). In order to identify insecticides that can be used in a rotation, it is necessary to determine the cross-resistance patterns, stability of resistance and clarify the underlying resistance mechanisms in the laboratory. However, if such laboratory studies have not yet been conducted, the rotational use of insecticide active ingredients having different MoAs has the potential to manage insecticide-resistance in arthropod pest populations.

Insecticides with same MoA are not suitable to be used in a rotation to manage resistance development in arthropod pests because of a common target-site. If resistance in an arthropod pest population to an insecticide is due to target-site resistance mechanism, the insensitive target-site would confer cross-resistance to other insecticides with the same MoA. This explains why *A. gossypii* which was resistant to imidacloprid as a result of a genetic modification of the nAChR displayed cross-resistance to other neonicotinoid insecticides, acetamiprid, clothianidin and thiacloprid (Koo *et al.*, 2014). Thus, if neonicotinoid insecticides were to be used in a rotation for pest control, neonicotinoid-resistance development in the pest population would be hastened or worsened, if resistance has already developed. The hastening or worsening of resistance in the pest population would be the result of an increased selection pressure due to the summed individual selection pressures from each neonicotinoid insecticide in the rotation on

the neonicotinoid-resistance conferring genes in the target pest population. Using insecticides with the same MoA in a rotation is same as relying solely on a single insecticide for pest control over time, since for both practices there is an increased selection pressure on a single target-site.

When resistance to an insecticide is due to target-site resistance, the insensitive target-site would not be able to resist other insecticides with different MoAs. For this reason, even though the resistance caused by the neonicotinoid-resistance specific modified nAChR produced high resistance to imidacloprid in *A. gossypii*, there was little or no cross-resistance to insecticides of other chemis-tries and sulfoxaflor (Koo *et al.*, 2014). This suggests that when resistance to neonicotinoid insecticide is due to insensitive target-site, the rotation of neonicotinoids with insecticides from different MoA group could prevent or delay resistance development in a pest population through a decrease in the selection pressure from each MoA group. It is understood that with the strategy of exploiting insecticide MoAs to manage resistance development in arthropod pest populations, it is the MoA groups that are rotated, rather than insecticides. For this reason, in a rotation, any of the insecticides in a MoA group that is registered for use against the target pest could be used followed by another registered insecticide from a different MoA group.

Insecticides that should be used in the rotation for arthropod pest control in Ghana must be only those that have been registered by the Environmental Protection Agency (EPA), Ghana, and the Food and Drugs Authority (FDA), Ghana. The EPA compiles the registered pesticides in a register (Table 1). The MoA groups of registered insecticides can be determined from the MoA classification scheme by Insecticide Resistance Action Committee (IRAC) (Table 2). Since insecticides in the rotation would be from different MoA groups, each MoA group of insecticides would act on a different arthropod target-site and select for

insensitive target-site resistance genes that specifically confer resistance to the selecting MoA group only. Thus, in the rotational use of nAChR group insecticides with other insecti-

cides from different MoA group, a decreased use of nAChR group insecticides decreases the selection pressure on the pest population.

TABLE 1

Extract from the Environmental Protection Agency, Ghana compiled pesticides register^a

| Environmental Protection Agency, Accra: Revised Register of Pesticides as at 31 st December 2014 under Part II of the Environmental Protection Agency Act, 1994 (Act 490) | | | | | | |
|--|-------------------|----------------------------------|---|--------------|---|------------------------------------|
| (A) Fully Registered Pesticides (FRE) | | | | | | |
| (A1) Insecticides | | | | | | |
| No. | Trade name | Registration No. / Date of issue | Concentration of active ingredient | Hazard class | Crops/Uses | Local distributor |
| 1. | Aceta Star EC | FRE/12100/00494G August 2012 | Bifenthrin (30 g/l) +Acetamiprid (16 g/l) | II | Insecticide for the control of capsids in cocoa | Makhteshim Agan West Africa, Accra |
| 2. | Actellic 300CS | FRE/1406/00811G December 2014 | Pyrimiphos-methyl (300 g/l) | III | Insecticide for public health purposes | Calli Ghana Co. Ltd, Tema |
| 3. | Agro-thoate 40 EC | FRE/1310/00602G June 2013 | Dimethoate (400 g/l) | II | Insecticide for the control of insect pests in vegetables | Reiss & Co. Ghana Ltd, Accra |
| 4. | Akape 20 SC | FRE/1202/00520G November 2012 | Imidacloprid (200 g/l) | II | Insecticide for the control of insects pest in vegetables | Agrimat Limited, Accra |

^aComplete pesticide register available from the Environmental Protection Agency, Ghana

Insensitive nAChR genes would either not accumulate (resistance is prevented) or slowly accumulate (resistance is retarded) in the pest population. The same would apply to the other insecticides with different MoA in the rotation and their respective target-site resistance conferring genes. Whilst an insecticide is not in use during the rotation, the susceptibility of resistant arthropods to the insecticide increases (Ninsin & Tanaka, 2005). Thus, when insecticides with different MoAs are rotated to control arthropod pest populations, the likelihood that resistance genes for a specific

MoA group would accumulate in the pest population to levels that would cause control failures is reduced or eliminated.

Although it is expected that there would be no cross-resistance between insecticides from different MoA groups because of different target-sites, there is a possibility of cross-resistance. When cross-resistance between insecticides in the same MoA group is due to other resistance mechanisms, the cross-resistance could extend to other insecticides with different MoA. It has been shown that acetamiprid-resistant *B. tabaci*, which showed

TABLE 2

Extract from the Insecticide Resistance Action Committee (IRAC) MoA Classification Scheme^a

| IRAC MoA Classification version 7.3, February 2014 | | |
|--|--|---|
| Main group/primary site of action | Chemical subgroup or exemplifying active | Active ingredients |
| 4 Nicotinic acetylcholine receptor (nAChR) agonists Nerve action {Strong evidence that action at one or more of this class of protein is responsible for insecticidal effects} | 4A Neonicotinoids | Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid, Thiamethoxam |
| | 4B Nicotine | Nicotine |
| | 4C Sulfoxaflor | Sulfoxaflor |
| | 4C Butenolides | Flupyradifurone |
| 5 Nicotinic acetylcholine receptor (nAChR) allosteric activators Nerve action {Strong evidence that action at one or more of this class of protein is responsible for insecticidal effects} | Spinosyns | Spinetoram, Spinosad |
| 6 Chloride channel activators Nerve and muscle action {Strong evidence that action at one or more of this class of protein is responsible for insecticidal effects} | Avermectins, Milbemycins | Abamectin, Emamectin benzoate, Lepimectin, Milbemectin |

^a Full IRAC MoA classification scheme (IRAC, 2014) and e-tools available at www.iraac-online.org

cross-resistance to other neonicotinoids, imidacloprid, thiamethoxam, thiacloprid and nitenpyram also displayed cross-resistance to bifenthrin, a pyrethroid insecticide (Basit *et al.*, 2011). It is, therefore, important that more than two MoA groups are used in a rotation to improve the possibility that insecticides that do not show cross-resistance are available in the rotation. Therefore, the more MoA groups used in a rotation the better.

Insecticide concentrations lower than the recommended field concentrations eliminate susceptible (*ss*)-individuals in field populations of pests, leaving behind resistant (*rs/rr*)-individuals that generate high resistance in pest populations over successive generations (Ninsin, 2011a; 2016b). The recommended field concentrations of insecticides would, therefore, generate very high resistance in arthropod pest populations (Ninsin, 2011a;

2016b). For this reason, in the rotation, insecticides with the same MoA must be applied during one generation of the target arthropod pest. Subjecting a generation of the pest to selection pressure from insecticides with the same MoA would prevent or retard the accumulation of *rr*-individuals in the target pest population.

Although some insecticidal products have a mixture of active ingredients, the use of insecticide mixtures for arthropod pest control is not encouraged since mixtures promote the development of multiple-resistance in pest populations. However, if it is necessary that mixtures are used, the active ingredients making up the mixture should show no cross-resistance. In the absence of information on cross-resistance, the active ingredients for the mixture should also be from different MoA groups. The mixture should then be incorporated in the rotation such that the use of each MoA group in the rotation is reduced so that the overall selection pressure from any one MoA group is decreased.

The insecticide recommendation for the control of cocoa mirids, which is an example of insecticide recommendations in Ghana, is briefly discussed. The approved insecticides, imidacloprid, thiamethoxam and bifenthrin (COCOBOD, 2007) belong to only two MoA groups, the nAChR agonists group and the sodium channel modulators group (IRAC, 2014). It is recommended that any of the insecticides could be sprayed when it was time for mirid control (COCOBOD, 2008). The recommendation could lead to the spraying of only one approved insecticide which would increase the selection pressure of the focused insecticide, risking resistance development in the mirids to one MoA group. The recommendation could also result in the rotation of imidacloprid and thiamethoxam and increase the selection pressure from the nAChR agonist group on neonicotinoid-resistance conferring genes in mirids.

That the approved insecticides are from only two MoA groups and cross-resistance has been

established between the neonicotinoids and bifenthrin (Prabhaker *et al.*, 1997; Basit *et al.*, 2011; Basit *et al.*, 2013) increase the risk of resistance development to all three approved insecticides whenever any of the insecticides is sprayed for mirid control (Ninsin & Adu-Acheampong, 2014). Acetamiprid, another neonicotinoid insecticide, has been registered by EPA (2014) as a single active ingredient insecticide and as a component active ingredient of insecticide mixture (acetamiprid+bifenthrin) for the control of cocoa mirids. This registration would, however, continue to intensify the selection pressure of neonicotinoids and bifenthrin on mirids and increase the resistance risk to nAChR agonist and sodium channel modulator groups of insecticides. It is important to exploit insecticide MoAs to help prevent or retard the development of resistance in cocoa mirids. This could be achieved by identifying additional effective active ingredients from different MoA groups and recommend a robust rotation strategy for the insecticides.

Conclusion

Incorporating an insecticide resistance management strategy into insecticidal control programmes against arthropod pests from the start is essential to prevent or retard the development of resistance in agriculture and public health. It has been explained in this paper that when resistance has not been characterized and so the cross-resistance patterns and underlying mechanisms are not known, the rotational use of insecticides with different MoAs helps manage resistance development through a decrease in the selection pressure on genes that confer target-site resistance to each MoA group. It has been recommended that due to the risk of cross-resistance between insecticides from different MoA groups, insecticides from more than two MoA groups should be rotated. In order to make it easy for users to know the MoA of registered insecticides in Ghana, it is recommended that the EPA and FDA indicate the MoAs of registered insecticides. It is also recommended that the EPA and FDA make insecticides that they

register for arthropod pest control in Ghana freely available in their offices across the country and on their internet websites for users to freely download.

The potential for managing resistance development with the rotational use of insecticides from different MoA groups does not mean it is not necessary to conduct important laboratory studies such as cross-resistance patterns, stability of resistance and determine the underlying resistance mechanisms. As indicated earlier, resistance due to other mechanisms other than target-site resistance could cause cross-resistance to exist between insecticides from different MoA groups which, if present, would undermine the exploitation of insecticide MoAs for resistance management. A full characterization of resistance is, therefore, essential to manage resistance development. Since laboratory colonies for resistance research are lacking in Ghana, it is important, as a first step, to establish susceptible and resistant strains of arthropod pests that have the genetic ability to develop resistance to insecticides in the laboratory. Ninsin (2011b) has shown how such laboratory susceptible and resistant strains could be established. The laboratory colonies could then be used to characterize the resistance of arthropod pests in Ghana for the design of comprehensive resistance management programmes.

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