Incipient bifenthrin-resistance in field populations of cocoa mirids, *Distantiella theobroma* (Dist.) and *Sahlbergella singularis* Hagl. (Hemiptera: Miridae)

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ABSTRACT

Bifenthrin is one of the insecticides approved by the Ghana Cocoa Board (COCOBOD) for the control of cocoa mirids. Bifenthrin-resistance levels of *D. theobroma* and *S. singularis* collected from different locations in the Eastern Region (ER), Volta Region (VR) and Central Region (CR) were determined by calculating their resistance ratios (RRs). The highest RRs for *S. singularis* were recorded from New Tafo in the ER and Logba Alikpati in the VR, which displayed 6-fold and 6.2-fold bifenthrin-resistance, respectively. Populations of *S. singularis* from Pankese in the ER and Likpe in the VR also displayed 3.6-fold and 3.9-fold bifenthrin-resistance, respectively. The *D. theobroma* population that displayed the highest bifenthrin-resistance of 3.7-fold was from New Tafo. The results show incipient bifenthrin-resistance in field populations of *D. theobroma* and *S. singularis,* which requires the implementation of effective resistance management strategies to prevent full-blown resistance in the cocoa mirids.

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Introduction

The cocoa mirids, *Distantiella theobroma* (Dist.) (Hemiptera: Miridae) and *Sahlbergella singularis* Hagl. (Hemiptera: Miridae) are serious pests of cocoa (Dudgeon, 1910a; Dudgeon, 1910b). In 2001, the Ghana Cocoa Board (COCOBOD) initiated the Cocoa Diseases and Pest Control (CODAPEC) programme (popularly known as mass-spraying programme) against the cocoa mirids and black pod disease (Adjinah & Opoku, 2010). The CODAPEC programme contributed to raising cocoa output from 380,000 tonnes at its inception in 2001 (Adjinah & Opoku, 2010) to more than 1,000,000 tonnes in the 2010/2011 season (Adu-Acheampong *et al*., 2014). The insecticides that have been approved by COCOBOD for controlling the cocoa mirids in Ghana are imidacloprid 200 g/l, thiamethoxam 240 g/l and bifenthrin 27 g/l (COCOBOD, 2007).

The mirid control failures experienced in 1962 due to the development of insecticide-resistance, following the widespread application of lindane against the cocoa mirids (Dunn, 1963a) prompted determination of the effectiveness of bifenthrin, a pyrethroid insecticide, against *D. theobroma* and *S.*

singularis (Antwi-Agyakwa *et al*., 2016). The results of the study showed that bifenthrin is effective against *D. theobroma* and *S. singularis* and has the same level of control against both species of cocoa mirids (Antwi-Agyakwa *et al*., 2016). That bifenthrin is effective against mirids does not mean the absence of bifenthrin-resistance conferring genes in field populations of *D. theobroma* and *S. singularis*. This is because insecticide-resistance at an early stage of development will not render insecticides ineffective until the frequency of the resistance conferring genes is high in a pest population. Detecting resistance at an early stage of development is, therefore, important to resistance management as it indicates pest control failure due to insecticide resistance. The study was carried out in order to detect bifenthrin-resistance in an early stage of development in field populations of the cocoa mirids, and to allow for the implementation of strategies for preventing full blown resistance in pest populations.

Materials and methods

Collection and maintenance of cocoa mirids

Fourth and fifth instar nymphs of *D. theobroma* and *S. singularis* were collected and used for the study from January 2013 to June 2013. Cocoa farms in five regions in Ghana were visited for mirid collection. However, only mirid populations from New Tafo and Pankese in the Eastern Region, Ahamansu, Logba Alikpati and Likpe in the Volta Region and Twifo Praso in the Central Region of Ghana were used because enough mirid numbers could not be collected in the Western and Ashanti regions during the collection period. The mirids were collected and maintained as described by Antwi-Agyakwa *et al*. (2016) and left overnight at 26 26 ± 2 °C and 78 ± 8 per cent relative humidity to allow weak and injured mirids die, so that only healthy mirids were used for bioassay the next day.

Insecticide

Bifenthrin 27 g/l EC, a commercially available pyrethroid insecticide with the trade name Akate Master®, which is approved by COCOBOD (2007) for mirid control in Ghana, was used. The insecticide is a broad-spectrum contact and stomach poison pyrethroid insecticide that modulates sodium channels by keeping the channels open (IRAC, 2012).

Bioassay technique for susceptibility test

The bioassay technique described by Antwi-Agyakwa *et al*. (2016), which is a modification of the standard method for laboratory screening of insecticides at Cocoa Research Institute of Ghana (CRIG) (Ackonor & Adu-Acheampong, 2007), was used. Bifenthrin concentrations that gave between 0 – 100 per cent mortalities were used. Petri dishes with base diameter of 9 cm were each lined at the base with Whatman 1 filter paper. One ml insecticide solution of known concentration was evenly spread onto the filter paper in the Petri dish. A fresh piece of chupon measuring 6 cm in length and 0.5 cm in diameter was dipped into the insecticide solution for l min and placed onto the insecticide impregnated filter paper in the Petri dish. For control, only distilled water was used to treat filter paper and chupon. An opening, measuring 3.5 cm × 1.5, cm, was created in the lid of each Petri dish, which served as a window for the introduction of mirids into the Petri dishes.

Five 4^{th} or 5^{th} instar nymphs were introduced into a Petri dish. The opening in the lid of the Petri dish was then plugged with a ball of cotton wool moistened with 1 ml distilled water. The bifenthrin treatments and control were replicated four times and maintained at 26 ±2 °C and 78 ± 8 per cent relative humidity for 24h after which mortality was recorded. Mirids that did not respond or show any sign of movement when prodded with camel's hair brush were considered dead. There was no mirid mortality in the control setup, but when mortality was recorded in the control only data with less than 10 per cent control mortality was used. The data were subjected to probit analysis (Finney, 1971) with a personal computer for LC_{50} and 95 per cent confidence interval (CI). The resistance level of a field population was determined by calculating the resistance ratio (RR), which is the LC_{50} of field population divided by the LC_{50} of reference population. In the absence of a laboratory maintained insecticide-susceptible colony, the field population with the lowest LC_{50}, which indicates the availability of more susceptible individuals compared with other field populations, was used as reference.

Results

Bifenthrin-resistance levels in S. singularis

The bifenthrin-resistance levels of the field populations were determined by comparing LC_{50} values of field populations with Twifo Praso population which recorded the lowest LC_{50} value. The highest RRs were recorded for the New Tafo and Logba Alikpati populations of *S. singularis,* which displayed low bifenthrin-resistance of 6.0-fold and 6.2-fold, respectively (Table 1). Pankese in the Eastern Region and Likpe in the Volta Region displayed very low resistance of 3.6 and 3.9 RR, respectively (Table 1). The RRs recorded for Pankese, Likpe, New Tafo and Logba Alikpati showed incipient bifenthrin resistance in the field populations of *S. singularis*. Although the RRs for Ahamansu in the Volta Region and Twifo Praso populations of *S. singularis* did not indicate resistance, these populations recorded the lowest slopes of 1.24 and 0.91, respectively (Table 1). Regression lines became shallower during selection and steeper as resistance genotypes increased in the populations (Hoskins & Gordon, 1956), and the slopes of Ahamansu and Twifo Praso populations of *S. singularis* showed their genetic potential to develop resistance to bifenthrin with the continuous use of the insecticide.

TABLE 1

Responses of six field populations of Sahlbergella singularis *to bifenthrin after 24 hours treatment*

Population	*LC_{50} (mg/l) (95% CI)*	*Slope (±SE)*	*Resistance ratio* [c]
New Tafo [a]	1.34 (0.947 - 1.89)	1.28 (0.14)	6.0
Pankese	0.795 (0.437 - 1.33)	1.38 (0.24)	3.6
Ahamansu	0.393 (0.124 - 0.815)	1.24 (0.31)	1.8
Logba Alikpati	1.37 (0.234 - 2.99)	1.69 (0.56)	6.2
Likpe	0.861 (0.125 - 1.80)	1.60 (0.52)	3.9
Twifo Praso [a,b]	0.223 (0.027 - 0.569)	0.91 (0.25)	1

[a] Data cited from Antwi-Agyakwa *et al*. (2014)

[b] Reference population

[c] Resistance ratio = LC_{50} of field population divided by LC_{50} of reference population

Bifenthrin-resistance levels in D. theobroma

In the case of *D. theobroma*, enough numbers for bioassay could be collected from only three locations (Table 2) as against six locations for *S. singularis* (Table 1). The Pankese population of *D. theobroma* recorded the lowest LC_{50}, so it was used as the reference population for calculating the RR. The highest RR for *D. theobroma* was recorded in the New Tafo population which displayed very low bifenthrin-resistance of 3.7-fold (Table 2). Logba Alikpati and Pankese populations of *D. theobroma* did not display resistance. However, the regression line for

Pankese population had the shallowest slope, which indicates that the population has the genetic potential to develop resistance to bifenthrin (Hoskins & Gordon, 1956).

Discussion

The resistance levels displayed by *D. theobroma* from New Tafo and *S. singularis* from New Tafo, Pankese, Logba Alikpati and Likpe showed that bifenthrin-resistance is in its early stage of development. The reference *D. theobroma* and *S. singularis* used for calculating the RRs of the field populations were the lowest LC_{50} values recorded for each mirid species. Considering that the field populations are sprayed with insecticides, the reference populations may have some resistant alleles in their gene pool which may make them less susceptible than laboratory susceptible strains. With both reference populations having the shallowest slopes, which suggest heterogeneous response to bifenthrin due to presence of some resistance genes, it is possible that the reference populations used to calculate the RR of the field populations would be less susceptible than a laboratory susceptible strain. Thus, the RRs of the field populations obtained in this study could have been higher if it had been compared to a laboratory susceptible strain, a situation that would have made all the field populations of *D. theobroma* and *S. singularis* exhibit incipient bifenthrin-resistance.

TABLE 2

Responses of three field populations of Distantiella theobroma *to bifenthrin after 24 hours treatment*

Population	*LC_{50} (mg/l) (95% CI)*	*Slope (±SE)*	*Resistance ratio* [c]
New Tafo [a]	2.43 (0.994 - 4.70)	1.45 (0.35)	3.7
Logba Alikpati	0.96 (0.328 - 1.92)	1.46 (0.35)	1.5
Pankese [a,b]	0.65 (0.295 - 1.14)	1.25 (0.24)	1

[a] Data cited from Antwi-Agyakwa *et al*. (2014)

[b] Reference population

[c] Resistance ratio = LC_{50} of field population divided by LC_{50} of reference population

COCOBOD recommends the use of any of the approved insecticides, i.e. imidacloprid 200 g/l, thiamethoxam 240 g/l or bifenthrin 27 g/l, to control the cocoa mirids (COCOBOD, 2008). In addition to insecticide applications under the Ghana government's CODAPEC programme, Antwi-Agyakwa *et al.* (2015) observed that farmers in the regions where nymphs were collected most of the time sprayed bifenthrin and imidacloprid to control mirids. However, the levels of bifenthrin-resistance detected in the field populations of *D. theobroma* and *S. singularis* indicated that bifenthrin selection pressure is low on cocoa farms. A contributory factor to the incipient bifenthrin-resistance detected is cross-resistance from imidacloprid which, as noted by Antwi-Agyakwa *et al.* (2015), is also used on the farms where mirid collections were made. Prabhaker *et al*. (1997) reported that a 75-fold imidacloprid-resistant silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring, displayed a 7-fold cross-resistance to bifenthrin.

The detection of bifenthrin-resistance in its early stage of development in *D. theobroma* and *S. singularis* requires the implementation of strategies that would retard the progress and spread of the resistance on cocoa farms and prevent the failures of mirid control. When lindane-resistance was not detected in the early stage of development, it resulted in a full-blown resistance in *D. theobroma* and mirid control failures at Pankese in 1962 (Dunn, 1963a). The lindane-resistance in *D. theobroma* showed cross-resistance to cyclodiene insecticides (Dunn, 1963b) and the spread to other areas in Eastern and Ashanti regions in 1963 (Telford, 1964). Similarl observations were made in Nigeria, after the detection of resistance in *S. singularis* to the organochlorine insecticides in 1962, the area of the resistant *S. singularis* spread from 60 sq. miles to over 700 sq. miles between 1962 and 1968 (Youdeowei, 1975). The resistance in *S. singularis* was further exacerbated as the search for alternative insecticides to use against the resistant mirids was not immediately successful (Youdeowei, 1975). These cases of spread of resistant *D. theobroma* in Ghana and *S. singularis* in Nigeria called for the urgent need for resistant management strategies to be implemented on cocoa farms to safeguard cocoa output.

Ninsin & Adu-Acheampong (2014) have recommended measures for preventing resistance in *D. theobroma* and *S. singularis* to the COCOBOD approved insecticides. The search for other efficacious insecticides of different chemistries that do not show cross-resistance to bifenthrin and the other COCOBOD approved insecticides, imidacloprid and thiamethoxam, is now urgent (Ninsin & Adu-Acheampong, 2014). A programme to determine the intensity and spread of bifenthrin-resistance has to be pursued. Monitoring to detect changes in the susceptibility of *D. theobroma* and *S. singularis* to imidacloprid and thiamethoxam is also urgently needed.

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