



# Antixenosis as a mechanism of cocoa resistance to the cocoa mirid, *Sahlbergella singularis* (Hemiptera: Miridae)

[Antixénose comme une mécanisme de résistance du cacaoyer au miride, *Sahlbergella singularis* (Hemiptera: Miridae)]

K. F N'Guessan<sup>1\*</sup>, Ph. Lachenaud<sup>2</sup>, and A. B. Eskes<sup>2</sup>

<sup>1</sup>Centre National de Recherche Agronomique (CNRA), BP 808 Divo, Côte d'Ivoire

<sup>2</sup>CIRAD-CP, TA 80, Avenue Agropolis, 34398, Montpellier Cedex 5, France

\*Corresponding author e-mail: [nguessankf\\_fr@yahoo.fr](mailto:nguessankf_fr@yahoo.fr) ; Tel: (225) 32 76 08 35

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## ABSTRACT

**Objectives:** The objectives of this study were to confirm the existence and heritability of mirid resistance in selected promising cocoa genotypes and to determine whether antixenosis is involved in the mechanism of resistance.

**Methodology and results:** Ten cocoa hybrids obtained from crosses between clones identified as susceptible or promising for resistance to the cocoa mirid, *Sahlbergella singularis*, in previous studies, because they sustained high or low damage in the field, were evaluated using a choice test in 2006 and 2007 for attractiveness to mirid in the laboratory. Nine parental clones among which 3 susceptible and 6 field resistant were also evaluated. The Catongo clone IFC372, susceptible to mirid, was included in the clone test. The choice test was based on comparison of attractiveness of fragments of twigs of the different cocoa genotypes vis-à-vis 4<sup>th</sup> instar mirid nymphs in large Petri dishes (16 cm diameter X 2 cm height). Fives 6 cm-long fragments of twigs with the same diameter and representing five different cocoa genotypes were placed end to end in the Petri dishes. The insects were allowed to feed on the fragments of twigs for 24 hours and the feeding lesions were counted in order to assess attractiveness of the different genotypes. The results showed significant differences ( $P < 0.01$ ) between the 9 parental clones with regard to mirid feeding lesions. Significant differences ( $P < 0.01$ ) were also revealed between the hybrids progenies. During both years, the susceptible parents (IFC5, ICS39 and UF667) were ranked susceptible in the choice test with 8 to 10 lesions. The hybrid obtained from a cross between the susceptible parents (ICS39XIFC5) was also ranked susceptible. The 4 promising parents (T79/501, UPA402, NA32, and PA150) and their hybrids progenies (T79/501XUPA402, PA150XNA32) were less attractive to the mirid nymphs in the laboratory with between 2 to 5 lesions.

**Conclusion and application of the findings:** The results obtained here indicate that antixenosis, based on feeding preference, may partly explain the level of mirid damage observed in the field. The results also indicated that antixenosis can be transmitted from the parents to the progenies. Moreover, the clones T79/501, UPA402, NA32, PA150 and T60/887 have been confirmed in the laboratory to be resistant to *Sahlbergella singularis*.

**Key words:** Cocoa, resistance, antixenosis, *Sahlbergella singularis*.

## RESUME

**Objectif:** L'étude vise à confirmer la résistance à *Sahlbergella singularis* de quelques géotypes prometteurs de cacaoyers et à vérifier le rôle de l'antixénose dans le mécanisme de résistance. L'étude visait également à déterminer la transmission d'un caractère de résistance des parents aux descendances.

**Méthodologie et résultats :** Dix hybrides de cacaoyer obtenus à partir de croisements entre clones identifiés comme sensibles ou prometteurs pour la résistance à la miride, *Sahlbergella singularis* dans des études antérieures sur la base de dégâts de mirides au champ, ont été évalués à l'aide d'un test d'antixénose en 2006 et 2007 au laboratoire. Les clones géniteurs, au nombre de neuf, parmi lesquels 3 sensibles et 6 prometteurs ont été également évalués. La variété Catongo IFC372, sensible aux mirides, a été utilisée comme témoin sensible dans le test des géniteurs. Le test d'antixénose était basé sur la comparaison de l'attractivité de fragments de rameaux des géotypes à évaluer vis-à-vis des nymphes de miride de 4<sup>ème</sup> stade. Les insectes se sont alimentés pendant 24 h sur les fragments de rameaux et les lésions provoquées par leurs piqûres ont été dénombrées pour chaque géotype. Les résultats ont révélé des différences significatives ( $P < 0.01$ ) entre les 9 clones géniteurs au regard du nombre de lésions alimentaires des mirides. Des différences significatives ( $P < 0.01$ ) ont également été enregistrées entre les descendances. Au cours de deux années, les géniteurs sensibles (IFC5, ICS39 and UF667) se sont classés sensibles dans le test d'antixénose avec entre 8 et 10 lésions. Les hybrides obtenus par croisement entre géniteurs sensibles (ICS39XIFC5) se sont également classés sensibles. Les 4 géniteurs prometteurs (T79/501, UPA402, NA32, et PA150) et leurs descendances (T79/501XUPA402, PA150XNA32) étaient moins attractifs vis-à-vis des nymphes de mirides avec 2 à 5 lésions.

**Conclusion et application des résultats:** Les résultats ont montré que l'antixénose, basée sur la préférence alimentaire, peut en partie expliquer le niveau de dégâts des mirides observés au champ et que ce caractère de résistance peut être transmis des géniteurs aux descendances. Par ailleurs, les clones T79/501, UPA402, NA32, PA150 et T60/887 ont été confirmés au laboratoire comme résistant aux mirides.

**Mots clés:** Cacao, résistance, antixénose, *Sahlbergella singularis*

## INTRODUCTION

Cocoa is an important source of revenues for producers in Côte d'Ivoire. However, production faces many constraints, including losses due to pests and diseases. The cocoa mirids, *Sahlbergella singularis* (Haglund) and *Distantiella theobromae* (Distant) have always been considered the most important insect pests of cocoa in Côte d'Ivoire. These insects are also serious pests in other cocoa producing countries such as Ghana, Nigeria and Cameroon (Lavabre, 1970, 1977a; Entswitle, 1972).

The biology and behavior of the mirids have been extensively studied (Williams, 1953; Taylor, 1954; Kay, 1961; Gibbs and Pickett, 1966; Braudeau, 1969; Kumar and Ansari, 1974). Mirids feed on every part of the plant except the leaves and the roots. Both adult and immature stages cause damage through punctures made on vegetative parts or fruiting structures. During feeding, saliva is injected into the wound and this has a marked

histolytic effect, probably due to the action of esterases (Williams, 1953). On young shoots, the mechanical damages and the effect of the toxic saliva are sufficient to cause death. On the other hand, on semi-hardened twigs and stems, the mechanical effect is less important. However, subsequent invasion of the wounds by a pathogenic fungus, *Calonectria rigidiuscula* (Berk. & Br.), has been reported (Crowdy, 1947). These attacks result in cankering or bark roughening, destruction of the flower cushions, a severe dieback of twigs and branches, and sometimes partial degradation of the farm. On young pods or cherelles, a high number of feeding punctures may cause distortion during growth or even death of the fruit. Yield losses attributed to mirid alone have been estimated at between 30-40% (Lavabre, 1977a).

In Côte d'Ivoire, cocoa mirids are controlled by combining agronomic practices and insecticide

applications (Lavabre, 1960; Marchart, 1971; Nguyen-Ban, 1971; Decazy, 1979; Decazy and Essono, 1979; Coulibaly *et al.*, 1998). However, many problems are associated with chemical use. In addition, farmers have been reluctant to adopt chemical control because of high costs of chemicals and application equipments. Other problems are related to environmental contamination, adverse effect on non-target organisms and potential residues in cocoa beans. In order to develop alternative control methods, research has been oriented towards the search for resistant cocoa genotypes. Mirid resistance studies in cocoa have been conducted by several researchers including Bruneau de Miré and Lotodé (1974), Decazy and Lotodé (1975), Decazy and

Coulibaly (1981), Nguyen-Ban (1993), Sounigo *et al.* (1993) and N'Guessan *et al.* (2003, 2006). However, these studies have mostly concentrated on assessment of field damage. Recently, a few works have focused on the mechanisms of mirid resistance in cocoa (N'Guesan *et al.*, 2008) in order to investigate possible heritability of resistance characters.

The current study was designed to confirm the level of resistance of promising cocoa genotypes and to determine whether antixenosis was involved in the mechanism of resistance. The study also aimed at determining whether, if present, the resistance character is transmissible from the parents to the progenies.

**MATERIALS AND METHODS**

**Study site:** The study was conducted in 2006-2007 at the research station of the National Agricultural Research Center (CNRA) in Divo, Côte d'Ivoire, within the framework of CFC/ICCO/IPGRI project. The methods applied follow the recommended Working Procedures for the CFC/ICCO/IPGRI project (Eskes *et al.*, 2000), with slight modifications described hereafter.

**Plant materials:** The plant materials tested were 9 clones and 10 hybrids. The susceptibility of the clones to *Sahlbergella singularis* has been shown in previous studies (Sounigo *et al.*, 1993). Clones ICS39, FC5, UF667 were found to be susceptible whereas clones P7, UPA402, T60/887, NA32, T79/501, PA150 were identified as promising for resistance to mirids because they sustained low damage in the field. The 10 hybrids were obtained from crosses made between promising

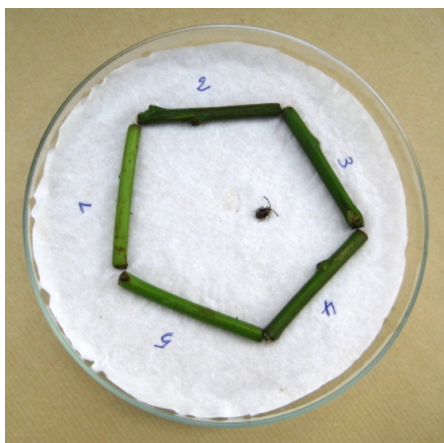
clones (RxR), between susceptible clones (SxS) and between promising and susceptible clones (RxS) (Table 1). The hybrids and the parent clones were evaluated separately for attractiveness to mirids in the laboratory using a choice test. The clone IFC 372, a Catongo was included as a susceptible control in the clone test.

**Experimental layout:** The experimental design was an incomplete block with 9 replicates, 18 blocks and 5 fragments per block for the parental clones and the hybrid progenies. Such a design allows the comparison of each genotype with the others within the same experimental set-up (Cochran and Cox, 1957). Ten series were carried out for both the parents and the hybrid progenies in 2006 and 2007.

**Table 1:** Lists of cacao genotypes evaluated for antixenosis against *Sahlbergella singularis*

Parents		Hybrid progenies	
IFC372	} Susceptible (S)	ICS39 x IFC5	} Susceptible (SxS)
ICS39		T79/501 x IFC5	
IFC5		IFC5 x T79/501	} R x S
UF667		ICS39 x T60/887	
P7	T60/887 x ICS39		
UPA402	UF667 x PA150		
T60/887	PA150 x UF667		
NA32	P7 x T60/887	} Resistant (RxR)	
T79/501	T79/501 x UPA402		
PA150	PA150 x NA32		

Healthy green twigs of young flushes obtained from the field were brought to the laboratory and cut into 6 cm sections. Five fragments of twigs with the same diameter and representing five different cocoa genotypes were placed end-to-end in 18 large Petri dishes (16 cm diameter X 2 cm height), according to the experimental design (Figure 1). One 4<sup>th</sup> instar mirid nymph that was starved for 24 hours was placed in



**Figure 1:** A Petri dish containing fragments of cocoa twigs and a mirid nymph

each Petri dish. The mirid nymphs were collected from the field the day before using them in the experiment. The insects were allowed to feed for 24 hours and the feeding lesions on the fragments of twigs (Figure 2) were counted for each genotype in order to assess attractiveness of the different genotypes. Data were analyzed using the GLM (General Linear Model) procedure of SAS (SAS Institute 1996).



**Figure 2:** A fragment of cocoa twig with mirid feeding lesions and a mirid nymph

## RESULTS AND DISCUSSION

Significant ( $P < 0.01$ ) differences were observed between the 9 parental clones in 2006 and 2007 with regard to the number of mirid feeding lesions. Clones PA150, T79/501, NA32, T60/887 and UPA402 were the least attractive in the laboratory (Table 2). These clones sustained between 2 and 5 lesions compared to the most attractive ones and the susceptible control that sustained between 8 and 10 lesions. The number of feeding lesions also varied significantly among the hybrids progenies during the two years. The hybrids PA150XNA32, T79/501XUPA402, and P7XT60/887 were among the least attractive with numbers of lesions between 2 and 3, compared to the most attractive hybrids having 8 or 9 lesions (Table 3). These least attractive hybrid progenies were obtained from crosses between promising parents.

The results showed that the parents known to be susceptible were also ranked susceptible in the choice test, indicating that antixenosis or feeding preference may be involved in the damage caused in the field. Moreover, hybrids obtained from crosses between susceptible parents ranked susceptible in the test. The

hybrids obtained from crosses between promising clones sustained the lowest number of feeding lesions, suggesting that the antixenosis trait was transmitted from the parents to the progenies (Table 3). Overall the results suggested that antixenosis is involved in the mechanism of resistance of the genotypes tested and that this character is transmissible from the parents to the progenies.

The development and use of resistant varieties is an important and effective tool for pest control. Three mechanisms of plant defense to insect damage have been described (Painter, 1951; Kogan, 1982; Smith, 1989). Although the causes of antixenosis have not been investigated in this study, previous works on other crops have attributed antixenosis to the presence of morphological factors such as hairs and trichomes, the thickness of plant tissues, and biochemical factors such as the presence of allelochemicals that adversely alter the insect behavior, resulting in the insect moving away and selecting an alternate susceptible host plant (Painter, 1951; Kogan, 1982; Smith, 1989).

**Table 2:** Numbers of feeding lesions of *S. singularis* nymphs on fragments of twigs of 10 cacao clones.

2006		2007	
Parent clones	Feeding lesions	Parent clones	Feeding lesions
IFC372	9.9 a	IFC372	10.28 a
ICS39	8.6 b	IFC5	8.88 ab
FC5	7.8 b	ICS39	8.36 b
UF667	7.5 b	UF667	7.44 bc
P7	6.0 c	P7	6.14 c
UPA402	4.7 d	T60/887	4.47 d
T60/887	4.2 d	T79/501	4.39 d
NA32	4.2 d	UPA402	4.26 d
T79/501	4.1 d	NA32	3.90 de
PA150	2.8 e	PA150	2.68 e

Means followed by the same letters along the columns are not significantly different ( $P>0.5$ , Waller Duncan K ratio T test).

**Table 3:** Numbers of feeding lesions of *S. singularis* nymphs on fragments of twigs of 10 cacao hybrids.

2006		2007	
Progenies	Feeding lesions	Progenies	Feeding lesions
ICS39XIFC5	8.4 a	ICS39XIFC5	9.87 a
T79/501XIFC5	4.6 b	IFC5XT79/501	4.53 b
IFC5XT79/501	4.6 b	ICS39XT60/887	3.96 bc
ICS39XT60/887	3.9 bc	UF667XPA150	3.92 bc
T60/887XICS39	3.7 bcd	T79/501XIFC5	3.68 bc
UF667XPA150	3.7 bcd	PA150XUF667	3.55 bc
PA150XUF667	3.4 cde	P7XT60/887	3.05 cd
P7XT60/887	3.3 cde	T60/887XICS39	2.87 cd
T79/501XUPA402	2.7 de	T79/501XUPA402	2.57 cd
PA150XNA32	2.4 e	PA150XNA32	1.76 d

Means followed by the same letters along the columns are not significantly different ( $P>0.5$ , Waller Duncan K ratio T test).

Regarding the cocoa tree, when different genotypes are exposed to mirids, in the form of fragments of twigs in the laboratory, the level of preference for these genotypes is expressed by the differences in the number of feeding lesions. In the field, this can be observed as differences in the level of dieback in the canopy, as a result of the damage caused by feeding. In the present study, the parental clones PA150, T79/501, NA32, T60/887 and UPA402 and the hybrids progenies PA150XNA32, T79/501XUPA402, and P7XT60/887 sustained low numbers of lesions compared to the other clones and hybrids. This indicates that these clones show some level of antixenosis.

Antixenosis was earlier identified to be one of the components of cocoa resistance to *S. singularis* (N'Guessan et al., 2004, 2008; Anikwe et al., 2009). Indeed, these researchers found the clone PA150 to be

antixenotic against *S. singularis*. On the other hand, the clone UF 676 which sustained high number of lesion in this study was found to sustain low number of lesions in the study carried out by Anikwe et al. (2009). This is probably because the clone UF676 was more preferred by *S. singularis* in comparison with the other clones used in our study as opposed to the clones used by Anikwe et al. (2009). Nevertheless, the results obtained in this study confirmed in part those of Sounigo et al. (1993) who found the parental clones to be promising for mirid resistance, based on low field damages in clone trials.

Antixenosis resistance has been revealed in many other crops for various insect species. The general method for assessing antixenosis in the laboratory is the choice test (Smith, 1989). However, in some cases, using excised plant parts reduces the attractiveness, deterrence or repellence of a particular antixenotic

genotype. This has been observed in aphids on *Medicago truncatula* (Klingler et al., 2005). However, in the study by Coleson and Miller (2005) where the excised plant parts were placed on water-moistened Whatman filter paper no. 2 in Petri dishes, antixenosis was revealed in cultivars of taro, *Colocasia esculenta* (L.). In our study the clones that exhibited little canker on the trunk and branches as a result of low number of lesions (Sounigo et al., 1993), showed low numbers of lesions in the choice test in the laboratory. This indicates that the choice test, based on attractiveness of fragments of twigs can be used to screen for resistance to cocoa capsids. Indeed, interesting results have been obtained by several scientists with regard to mirid resistance in cocoa (Nguyen-Ban, 1993; N'Guessan et al., 2008; Anikwe et al., 2009). However, in the field antixenosis can be reinforced by other factors. Studies by Lavabre (1977b) indicated that clone UPA134 was less attacked by mirids in field compared to neighbors, as result of color of the young leaves. Indeed, the color of the leaves of plants has an antixenotic effect on insects. Smith (1989) stated that antixenosis resistance in crop cultivars has been achieved by genetically altering the color of plant foliage. Moreover, it has been shown that some cucurbit cultivars with silver colored leaves reflect more blue and ultraviolet wavelengths of light than normal cultivars, and are resistant to aphids (Smith, 1989). Similarly, it has been shown that the red leaf color trait

in cotton is a heritable character that causes antixenotic reactions in adult boll weevils, *Anthonomus grandis grandis* Boneman (Smith, 1989).

Limited works have been conducted to investigate the cause of antixenosis in cocoa. The study conducted by Debost *et al.* (1988) showed that chemicals such as flavan-3-ols are involved in cocoa resistance to black pod disease. These chemicals may well be involved in cocoa resistance to mirids. Indeed, the work carried out by Cros *et al.* (1996) on young cocoa plants showed that two phenolic compounds, quercétine (flavonol 4) and kampférol (flavonol 7) may be involved in mirid resistance in cocoa. Nguyen-Ban (1993) stated that water content of the shoots may play an important role in the choice of food by cocoa mirids, but indicated that other factors including hairs on the shoots, colour of flushes and flavonols contents are involved in the food preference or non-preference by mirids.

Nevertheless, further studies need to be conducted to elucidate the cause of antixenosis in cocoa. Globally, the use of resistant varieties is an important component of integrated pest management and can easily be combined with other control methods. In this instance, the results obtained here are important. The hybrids that showed some antixenotic resistance, as well as their resistant parents, could be recommended for cultivation if they have good agronomic characteristics, or be integrated in a breeding programme to incorporate the resistance genes into suitable cultivars.

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