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# ASSESSMENT OF ANTIXENOSIS AND TOLERANCE OF COCOA (*THEOBROMA CACAO L.)* TOWARDS MIRIDS *SAHLBERGELLA SINGULARIS* HAGL. (HOMOPTERA : MIRIDAE)

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**SUMMARY:**
Field tests for antixenosis and tolerance of cocoa tree to damage by mirids were conducted on ten cocoa genotypes in an experimental plot on a research station in Cameroon. *Sahlbergella singularis* nymph was starved overnight and confined on a flush, in a nylon mesh sleeve cage and allowed to feed on the plant for 24 hours. Antixenosis was assessed by counting the number of feeding points on the flush after 24 hours. Assessment of tolerance was based on regular observations on the evolution of feeding damage and on sprouting of attacked twigs. Analyses of results revealed a highly significant effect of the genotype on the number of feeding points, on the ability of cocoa trees to contain extent of damage and on their ability to recover from it. Results also showed that these two last components must be considered as two distinct factors of the genetic resistance to mirid attack.

Keywords: Cocoa mirids, *Sahlbergella singularis*, antixenosis, tolerance, cocoa genotypes

**RESUME :**
Des tests d'antixénose et de tolérance du cacaoyer aux attaques de mirides ont été menés sur 10 génotypes, dans une parcelle expérimentale d'une station de recherche au Cameroun. Une larve de *Sahlbergella singularis*, affamée pendant une nuit, était maintenue sur un jeune rameau de cacaoyer, à l'intérieur d'un manchon de tulle, afin qu'elle si alimente pendant 24 heures. L'antixénose était mesurée par le comptage du nombre de piqûres d'alimentation après 24 heures. La tolérance était estimée par des observations régulières de l'évolution des dégâts dus aux piqûres et de la reprise du bourgeonnement des jeunes rameaux. L'analyse des résultats a montré d'une part un effet hautement significatif du génotype sur le nombre de piqûres et sur les capacités de l'arbre à contenir l'évolution des dégâts et à reprendre une croissance normale après l'attaque, et d'autre part que ces deux dernières composantes doivent être considérées comme deux facteurs distincts de la résistance génétique du cacaoyer aux mirides.

Mots-clés : Mirides du cacaoyer, *Sahlbergella singularis,* antixénose, tolérance, génotypes

## INTRODUCTION

Native to tropical areas of South and Central America, the cocoa tree, *Theobroma cacao* L., was taken from Bahia to West Africa via Sao Tomé and Fernando Po, in the 19$^{th}$ century. In West Africa, cocoa cultivation spread so quickly that production reached about 1,000,000 tonnes in 1980, i.e. around 60 % of the world production (Wood, 1985). In Cameroon, cocoa is nowadays one of the main sources of income of the rural population in the forest zone, where it is cultivated in about 200 000 cocoa farms (Minagri, 2001). However, cocoa production is strongly reduced by damages caused by pests and diseases (Bruneau de Miré, 1965). Cocoa mirids, *Sahlbergella singularis* Hagl. and *Distantiella theobroma* Dist. (Heteroptera: Miridae) are the major pests of cocoa tree in Africa, with 30 to 40 % losses in production in West-Africa (Collingwood, 1977; Lavabre, 1977). Their feeding results in the drying up of flushes and young branches. In addition, the cankers that appear after few days foster the development of parasitic cryptogamic fungi in the tree. Mirids and cryptogams cause damage resulting in premature ageing of cocoa trees and important losses in production (Crowdy, 1947 ; Williams, 1953 ; Lavabre, 1961). In order to control these serious pests, IPM activities are under development in West-Africa, where some research have been notably devoted to measure cocoa tree resistance to mirids in the field, by observing damage (Decazy and Lotodé, 1975; Decazy and Coulibaly, 1981; Sounigo, *et al.*, 1993; Sounigo, *et al.*, 2003). One of the research priorities of the Laboratory of Entomology of IRAD (Institute of Agricultural Research for Development), in Nkolbisson, near Yaoundé, is to gain a better understanding of the mechanisms of genetic resistance to mirids, and to identify cocoa varieties resistant and/or tolerant to these pests. A laboratory micro-test technique (Nguyen-Ban, 1998) has notably been used to assess effect of some genotypes on cocoa attractiveness towards mirids (unpublished results).
The goal of this paper is to present an improved method and the first results of field tests for antixenosis (feeding non-preference) and tolerance of the cocoa tree towards mirids damage.

## MATERIAL AND METHODS

### Genotypes

Ten genotypes were tested in July, August and September 2003:

- Two Upper Amazon Forasteros: T 79/501 and IMC 60
- Four Lower Amazon Forasteros: SIC 5, BE 10, Catongo and IFC 5
- Three Trinitarios: Playa Alta 2, ICS 1 and UF 676
- One genotype from French Guyana: GU 255/P.

### Replications

The experimental cocoa plot is divided in four sub-plots in which each genotype is represented by one tree. The density is 4500 trees.ha$^{-1}$, and there are no shade trees.
Eight to 14 observations per genotype were performed, distributed over the four trees of each genotype when they were available.

### Choice of flushes

Approximately thirty terminal buds per genotype were marked with wool strands and their growth was monitored regularly until complete development of the last leaf and the age of the analysed flushes was calculated. The diameter and length of the twigs were also measured in order to estimate the surface available for mirid feeding.
Fifty percent of the labelled flushes were used as control.

### Method of infestation with mirids

The tests were performed with fifth instar nymphs of *S. singularis*, the most common species in the study area, obtained from laboratory rearing.

The larvae were kept in captivity and starved overnight. The next morning, a nymph was transferred and confined on a flush, in a nylon mesh sleeve cage and allowed to feed on the plant for 24 hours, and was then removed.

**Assessment of antixenosis**
Antixenosis was assessed by counting the number of feeding points on the flushes after 24 hours.

**Assessment of tolerance**
Evolution of mirid damage on the twigs
After removal of mirids, the physiological reaction of the flushes/twigs was examined twice a week until 22 days, and assessed using a notation scale, ranging from 0 (healthy twigs) to 4 (dead flushes). For each twig, the mean value was calculated for all scores given at different times of the experiment. This mean index of twig therefore took into account the speed as well as the final degree of degradation.
Ability to recover from damage:
One month after infestation and at the end of the dry season, i.e. 8 to 9 months after infestation, twig ability to recover from damage was estimated by observing sprouting. Each twig was assigned to one of the following classes typified by the symptoms: 1) the branch was sprouting normally, 2) the flush had dried but was sprouting at its base, 3) the twig dried up completely (die-back).
Control treatment
Fifty percent of the marked twigs were mechanically damage with a needle, and were not submitted to feeding by mirids.

**Statistical analysis**
Correlation coefficients (Pearson), variances and covariances were estimated using SAS software (V8). Least Square Means were calculated and all pairwise comparisons were performed by using General Linear Models (GLM).

## RESULTS AND DISCUSSION

**Antixenosis**
The number of feeding points inflicted by mirids was not correlated with the age of the flushes ($r = 0.03$, non significant). There was a weak correlation between the number of feeding points and the estimated surface area of the twig ($r = 0.20$, significant). This low correlation value indicates that differences observed between genotypes in terms of the number of feeding points inflicted do not only result from differences between the surface area of flush , but really reflects levels of antixenosis as well.
In order to measure antixenosis exclusively, the surface area of the flush twig was used as a covariable for the analysis of the number of feeding points. The analysis of covariance revealed a highly significant effect of genotype ($F = 2.94$) and the ranking of values after correction by the use of the covariable, showed significantly different groups of genotypes (Table I). Indeed SIC 5 and CATONGO showed a significantly lower number of feeding points than BE 10, PLAYA ALTA 2, IFC 5 and GU 255/P, and consequently showed a stronger effect of antixenosis.

**Tolerance**
Evolution of damage
For each genotype, the mean index of degradation of twigs mechanically damaged with a needle (control) was consistently very low (approximately 1) and always differed very strongly from those of twigs damaged by mirids. This indicates that this assessment specifically measures the reaction of cocoa to mirid attacks and not to general stress.

The mean degradation index failed to show any correlation with the age of the flush (r = 0.17, non significant), or with the twig estimated surface area (r = -0.03). On the other hand, a significant but rather low (r = 0.48) correlation was shown between the mean index of degradation of twigs and the number of feeding points. This rather low correlation value indicates that the differences observed between genotypes for the evolution of damage do not simply result from the different numbers of feeding points they received. It shows that the ability to contain the extent of damage must be considered as a component of the genetic resistance to mirids, independently from the antixenosis. As an illustration, the scatter plot (Figure 1) shows a higher mean degradation index for SIC 5 and CATONGO than for IMC 60 and UF 676, despite a higher mean number of feeding points for the last two genotypes. In addition, T 79/501 showed a slightly lower mean index of degradation than SIC 5 and CATONGO but a higher mean number of feeding points.

Table I: Values and ranking of the tested genotypes for the number of feeding points corrected by the use of the estimated flush-twig surface area as a covariable
Tableau I : Valeurs et classement des génotypes pour le nombre de piqûres corrigé par l'estimation de la surface du jeune rameau comme covariable

| Genotype | Mean number of feeding points | |
|---|---|---|
| IFC 5 | 14,48 | a * |
| Gu 255/P | 14,09 | a b |
| Be10 | 12,92 | a b c |
| Playa alta 2 | 12,23 | a b c |
| T 79/501 | 11,77 | a b c d |
| ICS 1 | 11,17 | b c d |
| IMC 60 | 10,63 | c d |
| UF 676 | 9,93 | c d |
| Catongo | 9,04 | d |
| SIC 5 | 9,01 | d |

* Means followed by a common letter are not significantly different at the 5% level according to pairwise comparisons (t test).

When the number of feeding points was used as a covariable, the analysis revealed a highly significant "genotype" effect (F = 3.57 and 2.72 with and without the use of covariable, respectively) for the mean index of degradation.
The use of the covariable did not change the ranking for the three genotypes with the lowest mean degradation index: UF 676, IMC 60 and T 79/501 (Table II). In both cases, UF 676 and IMC 60 showed a significantly lower value than most of the other tested genotypes. These two genotypes were also among the ones with the lowest numbers of feeding points. In both analyses, the four genotypes with the highest mean degradation index were the same: IFC 5, BE 10, ICS 1 and PLAYA ALTA 2. The use of the covariable resulted in ICS 1 showing the highest value instead of IFC 5, because ICS 1 had a rather high average mean number of bites.

Figure 1: Scatter plot of the degradation mean index in relation with the mean number of feeding points
Figure 1 : Indice moyen de dégradation en fonction du nombre moyen de piqûres

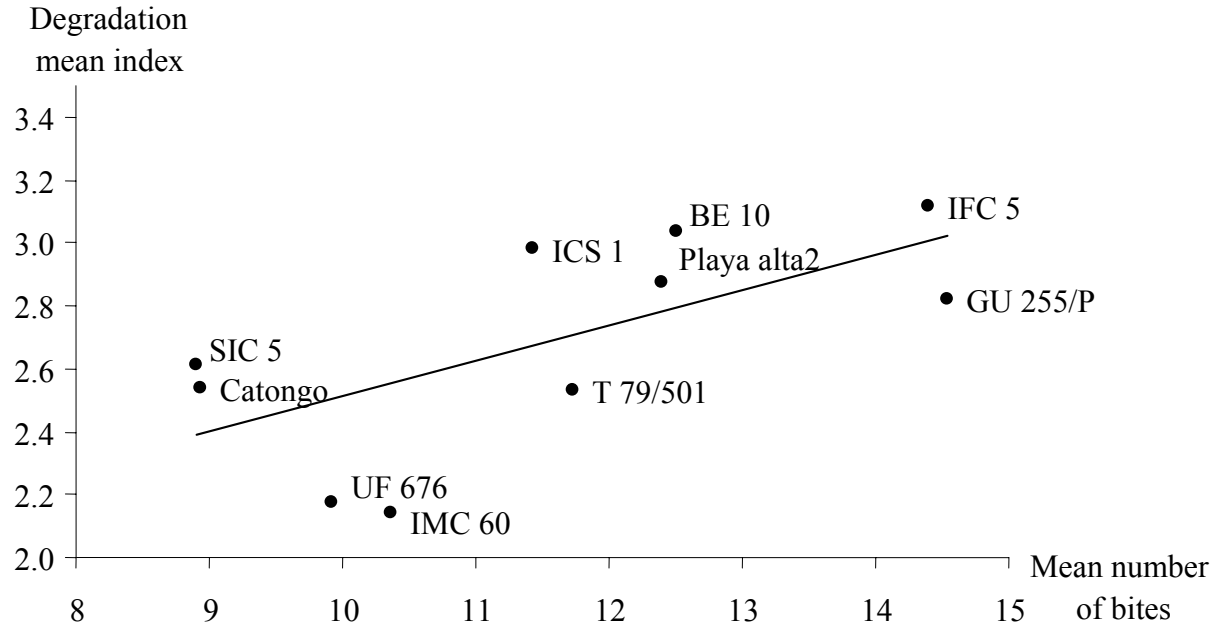



Table II: Values and ranking of genotypes for the mean degradation index
Tableau II : Valeurs et classement des génotypes pour l'indice moyen de dégradation

| Genotype | Mean degradation index | | Genotype | Mean degradation index (with the number of feeding points as a covariable) | |
|---|---|---|---|---|---|
| IFC 5 | 3.11 | a * | ICS 1 | 2.97 | a |
| BE 10 | 3.03 | a b | BE 10 | 2.96 | a b |
| ICS 1 | 2.98 | a b | IFC 5 | 2.90 | a b |
| Playa alta 2 | 2.87 | a b | Playa alta 2 | 2.80 | a b |
| GU 255/P | 2.82 | a b | SIC 5 | 2.78 | a b |
| SIC 5 | 2.61 | a b c | Catongo | 2.71 | a b |
| Catongo | 2.54 | b c | GU 255/P | 2.60 | a b c |
| T 79/501 | 2.53 | b c | T 79/501 | 2.51 | b c |
| UF 676 | 2.18 | c | UF 676 | 2.28 | c |
| IMC 60 | 2.14 | c | IMC 60 | 2.21 | c |

* Means followed by a common letter are not significantly different at the 5% level according to pairwise comparisons (t test).

Ability to recover from damage

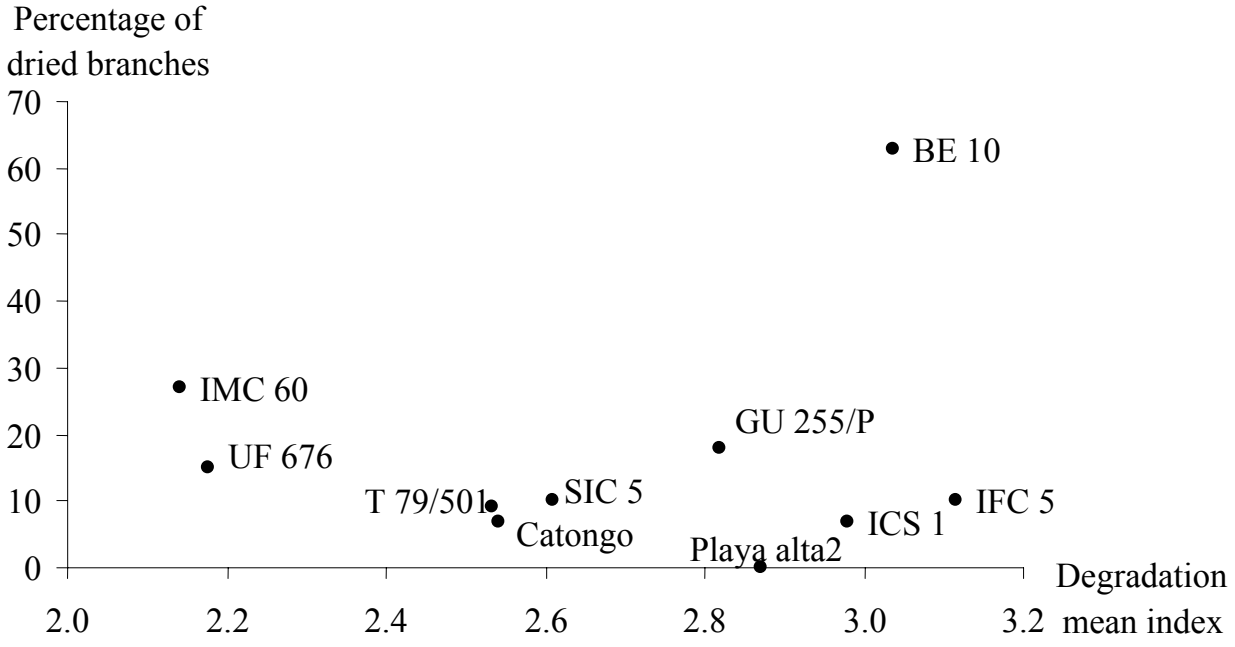
The correlation between the mean degradation index and the percentage of dried branches without sprouting was not significant (r = 0.11), showing that tolerance of the genotypes does not result exclusively from the ability to contain damage.

As could be expected, a strong significant negative correlation was found between the percentage of normal sprouting twigs per genotype after one month and the degradation mean index (r = - 0.81, significant). Conversely, there was no significant correlation between the percentage of basal sprouting after one month and the degradation mean index (r = 0.45).

Figure 2 is a scatter plot showing the percentages of dried branches without sprouting after one month in relation to the mean degradation indexes. Some genotypes, such as PLAYA ALTA 2, ICS 1 and IFC 5, had a low level of dried branches without sprouting despite a high mean degradation index. These genotypes had a high percentage of sprouting at the base of the dried flushes, as shown in Figure 3. On the other hand, IMC 60 had a relatively high percentage of dried branches without sprouting, despite a low mean degradation index. Figure 3 shows a low percentage of sprouting at the base of the dried flushes for this genotype.

Figure 2: Scatter plot showing the % of dried branches without sprouting after one month in relation with the mean degradation index

Figure 2 : Pourcentages des rameaux desséchés sans repousse un mois après piqûres en fonction de l'indice moyen de dégradation




A similar relationship is observed between mean degradation index and percentage of dried branches without sprouting after the dry season, which was expected because of the strong positive and significant correlation (r = 0.80) between the percentages of dried branches without sprouting after one month and after the dry season. But both Figures 3 and 4 show differences between this variable when measured at the two different periods for some of the genotypes. This is the case for T 79/501 and CATONGO, which showed a much higher value after the dry season than after one month. Two different situations are observed for these

two genotypes. In the case of CATONGO, the increase in dried branches without sprouting results from the drying of the branches which showed a normal sprouting after one month, while, in the case of T 79/501, this increase results from the drying of new flushes issued from the base of the dried flushes. Other genotypes, such as ICS 1, UF 676, and IFC 5 did not show any increase in the % of dried branches without sprouting. Here again, a difference appears between ICS 1 and IFC 5 on one hand, and UF 676 on the other. Indeed, the same percentages of the three classes of branches are observed after the two periods in the case of ICS 1 and IFC 5, while the percentage of normal sprouting has decreased and the percentage of sprouting at the base of the dried flushes has increased in the case of UF 676. The lowest level of tolerance is observed for BE 10, which had a low level of normal sprouting, agreeing with its high mean degradation index, combined with a low percentage of sprouting at the base of the dried flushes.

Figure 3: Pie chart showing the percentages of the three categories of sprouting observed on each genotype (black = normal terminal sprouting, grey = sprouting at the base only, and white = dried flushing without sprouting (die-back))
Figure 3 : Graphiques circulaires montrant le pourcentage des trois types de repousse observes pour chaque génotype (noir = repousse terminale normale, gris = repousse à la base du rameau seulement, et blanc = dessèchement total du rameau sans repousse)

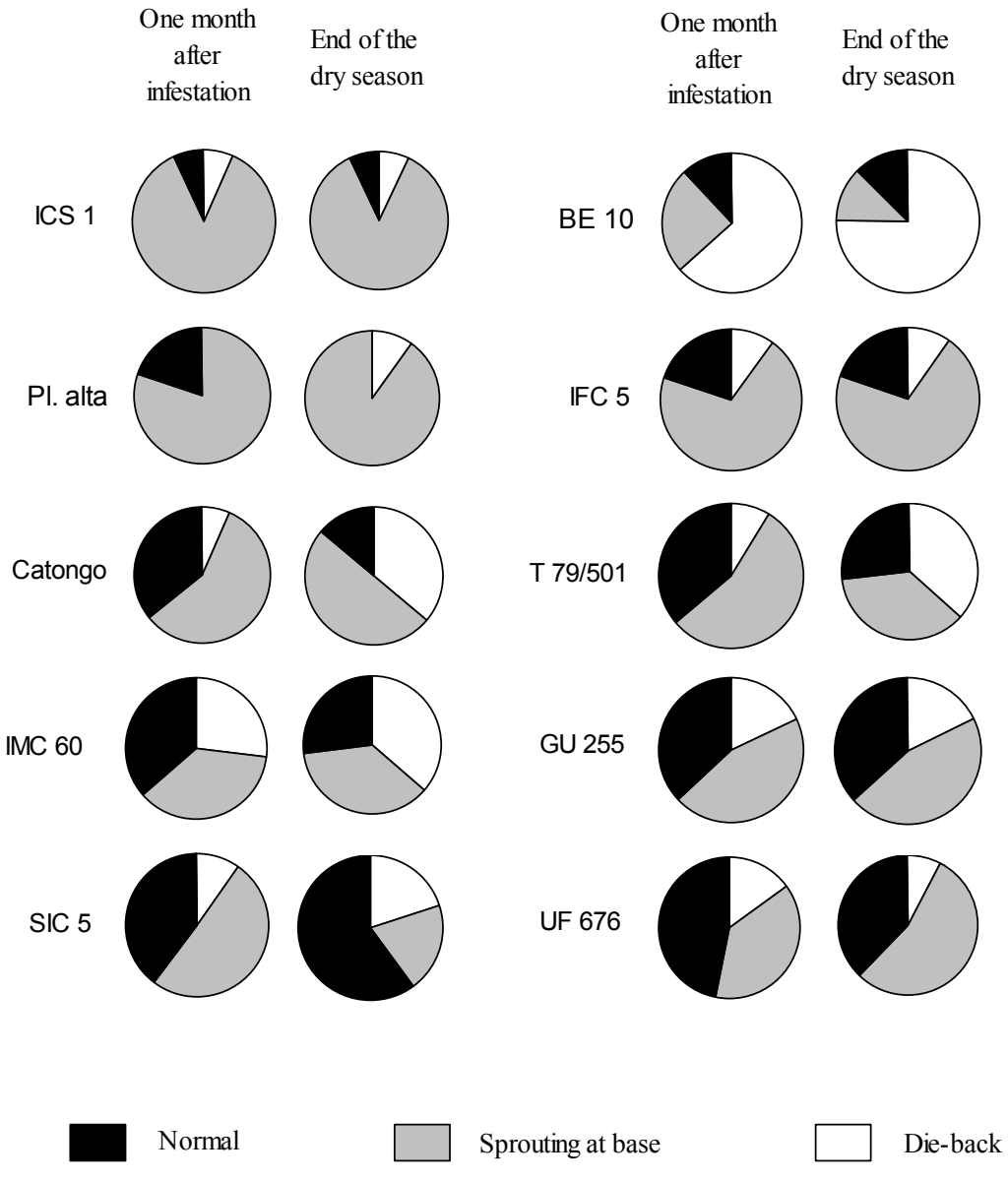
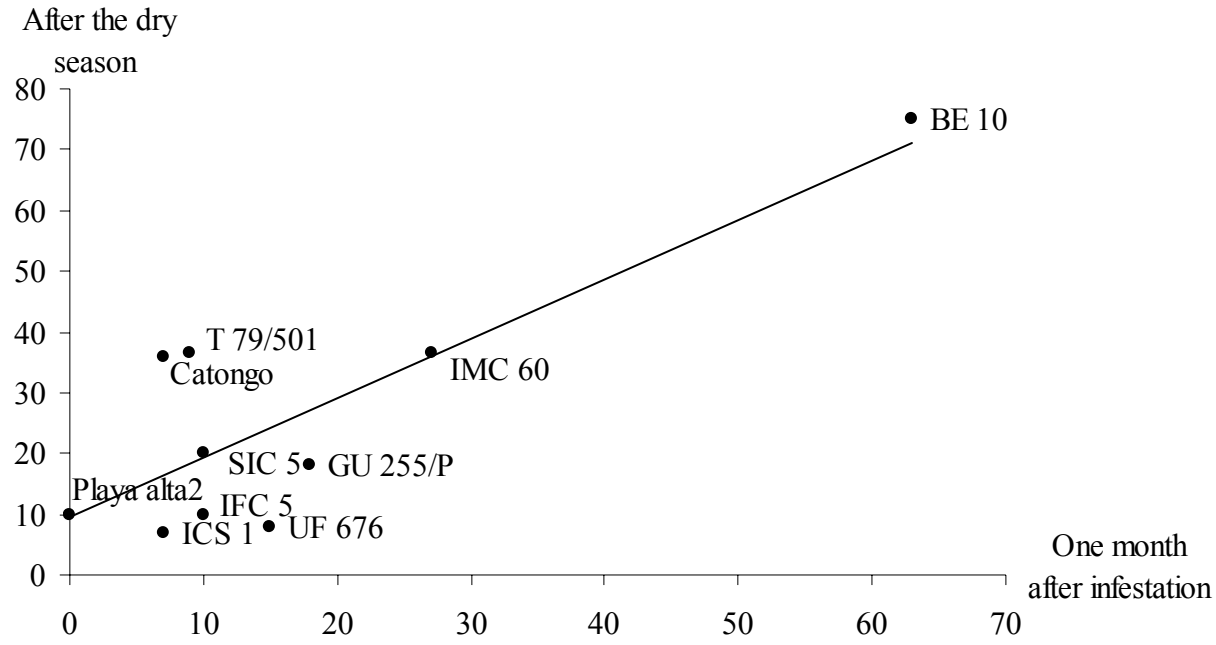


Figure 4: Scatter plot of the percentage of dried branches after the dry season in relation to the percentage of dried branches one month after infestation
Figure 4 : Pourcentage de rameaux desséchés après la saison sèche en fonction du pourcentage de rameaux desséchés un mois après l'attaque.




**CONCLUSION**

In this paper, we have described a relatively user-friendly method to assess the resistance of cocoa genotypes to mirids. This method requires a limited number of mirids, since one single nymph can be used for several assays. In addition, this method can be applied under field conditions, the observations are not time-consuming, are easily repeatable, and do not require much handling that could affect the nymphs of mirids.
This assessment method also allowed us to confirm the existence of three distinct components of the reaction of cocoa to mirids: antixenosis, ability to contain damage and ability to recover from damage.
Genetic differences were observed for these different components of the resistance to mirid attack despite the rather low number of cocoa genotypes assessed. These differences allowed us to identify some promising genotypes for different components of resistance to mirids: CATONGO and SIC 5 for antixenosis, IMC 60 and UF 676 for ability to contain the evolution of damage, and PLAYA ALTA 2, ICS 1 and UF 676 for ability to recover from damage. Unfortunately, we failed to identify a single genotype accumulating favourable alleles for the three components of resistance, but this might be possible by screening a large number of them. In the absence of such genotypes, breeding programs will need to consider making crosses between promising genotypes for the three components in order to create progenies and progenitors accumulating favourable alleles for all components.

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**REFERENCES**

Bruneau de Miré P., 1965 - Les recherches sur l'écologie des mirides du cacaoyer (*Sahlbergella singularis* Hagl. et *Distantiella theobromae* Dist.) en République Fédérale du Cameroun. In : IFCC (Ed.), Rapport d'activité, Centre de Recherche du Cameroun, Cameroon.

Collingwood C. A., 1977 - African Mirids. In : Lavabre, E.M. (Ed.), Les Mirides du Cacaoyer, pp. 71-83, G-P. Maisonneuve et Larose, Paris, France.

Crowdy S. H., 1947 - Observations on the pathogenicity of *Calonectria rigidiuscula* (Berk. & Br.) Sacc. on *Theobroma cacao* L. The Annals of Applied Biology 34, 1, 45-59.

Decazy B., Lotodé R., 1975 - Comportement de familles hybrides de cacaoyers soumis aux attaques de *Helopeltis* Sign. Café Cacao Thé 19, 4, 303-306.

Decazy B., Coulibaly N., 1981 - Comportement de cultivars de cacaoyers à l'égard de quelques insectes déprédateurs : possibilité d'une sélection précoce des cacaoyers tolérants. In: J. de Laforest (Ed.), Proceeding of the 8th International Cocoa Research Conference, 18-23 Oct. 1981, Cartagena, Colombia, pp. 685-688. Stephen Austin & Sons. Hertford.

Lavabre E. M., 1961 - Protection des cultures de caféiers, cacaoyers et autres plantes pérennes tropicales. IFCC, Paris, 268pp.

Lavabre E. M., 1977 - Les Mirides du Cacaoyer. G.-P. Maisonneuve et Larose, Paris, 366pp

Minagri 2001 - Programmes Cacao et Café Robusta. In : Ateliers de concertation avec les opérateurs économiques sur les politiques agricoles. Douala, 27 February.-2 March, Douala, Cameroon.

Sounigo O., N'Goran J., Coulibaly N., Clément D., Lachenaud P., 1994 - Evaluation de clones de cacaoyers pour la productivité, la résistance aux Mirides et la résistance à la pourriture des cabosses. In : J. de Laforest (Ed.), Proceeding of the 11th Cocoa Research Conference, Yamoussoukro, Côte d'Ivoire, pp 375-381. Stephen Austin & Sons. Hertford.

Sounigo O., Coulibaly N., Brun L., N'Goran J., Cilas C., Eskes A.B., 2003 - Evaluation of resistance of *Theobroma cacao* L. to mirids in Côte d'Ivoire : results of comparative progeny trials. Crop Protection. 22, 615-621.

William G., 1953 - Field Observations on the Cacao Mirids, *Sahlbergella singularis* Hagl. and *Distantiella theobroma* (Dist.), in the Gold Coast, I. Mirid Damage. Bull. Entomol. Res. 44, 101-119.

Wood G.A.R., 1985 - History and development. In: Wood G.A.R., Lass, R.A. (Eds.), Cocoa. 4th Edition. Longman Scientific and Technical, New York, pp. 1-10