# Activity of Insecticide Detoxification Enzymes in Cotton Jassids: Agronomic Implications for Cotton Pest Management in Côte d'Ivoire

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## ABSTRACT

Cotton production is one of the main sources of income for the population and contributes to the socio-economic development in the northern and central areas of Côte d'Ivoire. However, cotton crop is subject to large and diversified pest problems, including jassids. Heavy infestations of jassids are likely to induce the stopping of plant development or the shedding of reproductive organs. For the last decade, there has been an increase and persistence of leafhopper attacks throughout the crop cycle despite insecticide treatments. This study aims to determine the biochemical mechanism likely to be involved in insecticide susceptibility of jassids. A biochemical assay of enzyme activities was performed on adult populations of jassids collected from cotton fields in six different localities (Bouaké, Korhogo, Boundiali, Ferké, Ouangolo and Niakara) within the cotton growing area of Côte d'Ivoire. The method of Brogdon et al. (1997) was adopted with minor modifications. Alpha-esterase activity values ranged from 380.334 to 965.775 nmol α-naphtol/min/mg protein for Bouaké and Ouangolo respectively. Beta-esterase activity ranged from 398.640 nmol β-naphtol/min/mg protein for Bouaké to 1,528.885 nmol β-naphtol/min/mg protein for Ouangolo. Oxidase activity ranged from 0.016 nmol P450 EU/mg protein for Ferké to 0.038 nmol P450 EU/mg protein for Ouangolo. Glutathione- δ-transferases varied between 0.554 nmol GSH conj/min/mg protein for Ferké and 1.128 nmol GSH conj/min/mg protein for Niakara.

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*Classification:* For Code: 860999

*Language:* English

London Journals Press

LJP Copyright ID: 925653
Print ISSN: 2631-8490
Online ISSN: 2631-8504

London Journal of Research in Science: Natural and Formal

Volume 19 | Issue 5 | Compilation 1.0

# Activity of Insecticide Detoxification Enzymes in Cotton Jassids: Agronomic Implications for Cotton Pest Management in Côte d'Ivoire

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## ABSTRACT

*Cotton production is one of the main sources of income for the population and contributes to the socio-economic development in the northern and central areas of Côte d'Ivoire. However, cotton crop is subject to large and diversified pest problems, including jassids. Heavy infestations of jassids are likely to induce the stopping of plant development or the shedding of reproductive organs. For the last decade, there has been an increase and persistence of leafhopper attacks throughout the crop cycle despite insecticide treatments. This study aims to determine the biochemical mechanism likely to be involved in insecticide susceptibility of jassids. A biochemical assay of enzyme activities was performed on adult populations of jassids collected from cotton fields in six different localities (Bouaké, Korhogo, Boundiali, Ferké, Ouangolo and Niakara) within the cotton growing area of Côte d'Ivoire. The method of Brogdon et al. (1997) was adopted with minor modifications. Alpha-esterase activity values ranged from 380.334 to 965.775 nmol α-naphtol/min/mg protein for Bouaké and Ouangolo respectively. Beta-esterase activity ranged from 398.640 nmol β-naphtol/min/mg protein for Bouaké to 1,528.885 nmol β-naphtol/min/mg protein for Ouangolo. Oxidase activity ranged from 0.016 nmol P450 EU/mg protein for Ferké to 0.038 nmol P450 EU/mg protein for Ouangolo. Glutathione-δ-transferases varied between 0.554 nmol GSH conj/min/mg protein for Ferké and 1.128 nmol GSH conj/min/mg protein for Niakara. The involvement of α-esterases and β-esterases in controlling the sensitivity level of jassids to active ingredients is thus highlighted. This observation suggests a rational selection of the active ingredients used to control these pests.*

*Keywords:* jassids, insecticides, enzyme activity, protection of cotton, côte d'ivoire.

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## I. INTRODUCTION

Cotton growing is one of the main sources of income for the people living in the northern and central regions of Côte d'Ivoire. Cotton production also contributes to social and economic development of the populations living in these parts of the country (Koffi, 2013). However, this crop is subject to the biological constraints imposed by a large and diversified pest complex (Vaissayre et al., 1995; Ochou & Martin, 2002; Ochou 2011). The yield losses due to pests result in a decrease in income for the cotton producer.

Jassids are a major component of sucking insect pests of cotton plants in Côte d'Ivoire. These leafhoppers by their feeding method cause significant damage to plants. For the last decade, a large number of cotton growers have been complaining about the persistence of severe infestations of leafhoppers causing shedding of squares, young bolls, and leading subsequently to significant yield losses. In fact, there has been an

increase in damage to plants caused by jassids throughout the cotton production area of Côte d'Ivoire. Accordingly, cotton leafhopper attacks have become persistent throughout the crop cycle with an increase in the number of plants attacked (Koné et al., 2017).

To ensure effective control of damage caused by jassids, several means and methods are used in the development of integrated management strategies. However, chemical control remains one of the most widely adopted methods by producers. Still, the wide use of chemical formulations have several risks, including the selection of resistance to insecticides used for crop protection. In general, three major resistance mechanisms can be involved in insecticide resistance. An organism's resistance to insecticides may thus be behavioural, physiological or biochemical (Haubruge & Amichot, 1998). It can be characterized by reduced penetration of active ingredients into the cuticle, modification of the target active site in the insect's body and increased activity of detoxification enzymes (Plapp, 1976; Oppenoorth, 1984; Gunning et al., 1996).

Among insect pests, the main metabolic detoxification enzymes are oxidases, esterases and glutathione-δ-transferases. This study aims to determine the mechanism likely to be involved in insecticide susceptibility of jassids.

## II. MATERIALS AND METHODS

### 2.1 Area of study

The biochemical assay of enzyme activities was performed on adult populations of jassids collected from growers' fields. Jassid collection was carried out in the localities of Bouaké, Korhogo, Boundiali, Ferké, Ouangolo and Niakara. The assays were conducted at the Entomology Laboratory of the Cotton Research Program in Bouaké. The study area, as shown in figure 1, indicates that these localities are into the cotton growing area in the savannah region, precisely between 6° - 10°50' N and 4° - 8° W. With regard to rainfall, the cotton growing area was divided into two main regions with respect to the 9$^{th}$ parallel: 1) the Northern region for cotton localities above the 9$^{th}$ parallel, with only one rainy season; 2) the Southern region for cotton localities below the 9$^{th}$ parallel, with two rainy seasons.

### 2.2 Materials

#### 2.2.1 Target insect pest

This study focuses on jassids (*Jacobiella fascialis* Jacobi and *Jacobiasca lybica* Bergevin & Zanon), which are sap sucking insect pests of cotton in Côte d'Ivoire. These are leafhoppers that live on the underside of the cotton leaves. They are small, and are either green, yellow-green or brown. Larvae and adults feed on the secondary veins of the leaves to suck the sap. Severe infestation may lead to the shedding of squares and small bolls. Although this rarely happens, larger bolls may turn soft and spongy and fail to mature (Godfrey et al., 2008; Madar & Katti, 2010; Selvaraj et al., 2011).

#### 2.2.2 Jassid collections

The collection of jassids was operated on cotton plant in farmers' field. The captures have encompassed all stages of development of these pests. The captured insects were put on a young cotton plant within a cage, which was covered with muslin clothes in order to bring them back to laboratory according to the method of Ahmad et al. (1999).

### 2.3 Methods

#### 2.3.1 Preparation of extracts for enzyme assays

The method adopted with minor modifications is that of Brogdon et al. (1997). For each strains, 40 jassids from the cages were individually homogenized in 1.5 ml micro-centrifuge tubes having 200 µl of distilled water. To avoid the degradation of enzymes in insect extracts, the tubes were maintained on ice during the extraction procedure. The homogenates were centrifuged at 14,000 rpm for 2 min at 4°C. Supernatants were collected and kept in wells of chilled micro titration plates. Enzyme assays were

done on supernatants and all assays were replicated.

### 2.3.2 Esterases assay

For each strain, 10 µl of the enzymatic source was deposited in two replicas at the bottom of the wells and 90 µl of 1% triton saline phosphate (PBS) buffer at pH 6.5 was added to each well. This mixture was incubated at room temperature (25°C) for 10 min. Subsequently, 100 µl of a solution containing 600 µl of 0.06 M α-naphthyl acetate (or β-naphthyl acetate), 3 ml of 1% triton saline phosphate buffer at pH 6.5 and 8.4 ml of distilled water were added. The plate was incubated again at 25°C for 30 min. After the incubation, 100 µl of solution containing 0.012 g of fast garnet salt (FGBC) previously dissolved in 12 ml of distilled water were added in the wells. The plate was incubated for 10 min at 25°C with a lid and the absorbance value was recorded at 550 nm.

### 2.3.3 Oxidase assay

On a 96 well microplate, 20 µl of enzyme extract were first introduced in two replicas at the bottom of the wells. 80 µl of potassium phosphate buffer ($KHPO_4$) 0.0625 M; pH 7.2 were added to each well. Then, 200 µl of solution composed of 0.012 g of 3,3',5,5'-tetramethyl benzidine (TMBZ) dissolved in 6 ml of methanol and 18 ml of 0.25 M sodium acetate buffer ($NaC_2H_3O_2$); pH 5.0 were added to the wells. Finally, 25 µl of solution composed of 3% hydrogen peroxide ($H_2O_2$) was added in the wells. The set was incubated for 30 min at room temperature (25°C) with a lid. The absorbance value was recorded at 630 nm.

### 2.3.4 Glutathione- δ -transferases assay

In two replicas, 10 µl of enzymatic source was collected and introduced at the bottom of the microplate wells. 200 µl of a solution composed by 0.060 g reduced form glutathione solution (GSH) dissolved in 20 ml of sodium phosphate buffer 0.1 M; pH 6.5 have been added with 0.013 g of 1-chloro-2,4-dinitrobenzene (CDNB) previously and completely dissolved in 1 ml methanol. The absorbance value was recorded in kinetics at 340 nm for 5 min.

### 2.3.5 Data analysis

For data analysis, a transformation of the absorbance value was first performed to obtain activities of each proteins. The statistical analysis of these data was done using software R version 3.4.2. The Kruskal-Wallis non-parametric test was performed to separate the medians.

## III. RESULTS

### 3.1 Activities of alpha esterases of jassid strains

Alpha esterase activity varied across jassids strains as shown in figure 2. The activity values ranged from 380.334 to 965.775 nmol α-naphtol/min/mg protein. The lowest alpha esterase activity was determined for Bouaké strain while the highest activity was determined for Ouangolo strain. Statistical analysis of the activity values of these esterases revealed 3 groups of significant difference ($p < 0.001$). The first group was composed of jassids from Bouaké, Boundiali and Niakara. The second group was composed by Ferké strain. The third group dealt with Korhogo and Ouangolo strains.

### 3.2 Activities of beta esterases of jassid strains

Beta esterase activity values were variable, ranging from 398.640 nmol β-naphtol/min/mg protein (Bouaké) to 1,528.885 nmol β-naphtol/min/mg protein (Ouangolo). Statistical analysis of the activity values of these esterases revealed 4 groups of significant difference ($p < 0.001$). The first group was composed of jassids from Bouaké and Niakara strains. The second group concerned Boundiali strain. The third group was that of Ferké strain. The fourth group dealt with Korhogo and Ouangolo strains. The values of β-esterase activities are illustrated in figure 3.

### 3.3 Activities of oxidases of jassid strains

For oxidases, the activity was low with values ranging from 0.016 nmol P450 EU/mg protein

(Ferké) to 0.038 nmol P450 EU/mg protein (Ouangolo). Statistical analysis of oxidase activity revealed 3 groups of significant difference between strains ($p < 0.05$). The first group dealt with Bouaké, Ouangolo, Boundiali and Niakara jassids strains. The second group concerned Korhogo strain and the third group was the Ferké strain. Figure 4 illustrates oxidase activities by sampling location.

### *3.4 Activities of glutathione- δ -transferases of jassid strains*

Activity values of glutathione-δ-transferases (GST) ranged from 0.554 nmol GSH conj/min/mg protein (Ferké) to 1.128 nmol GSH conj/min/mg protein (Niakara). Statistical analysis of GST activities revealed 3 groups of significant difference ($p < 0.05$). The first group was composed of Bouaké, Ferké, Korhogo and Ouangolo jassids strains. The second group dealt with Niakara strain while the third group was Boundiali strain. Figure 5 shows the activities of glutathione-δ-transferases determined by sampling location.

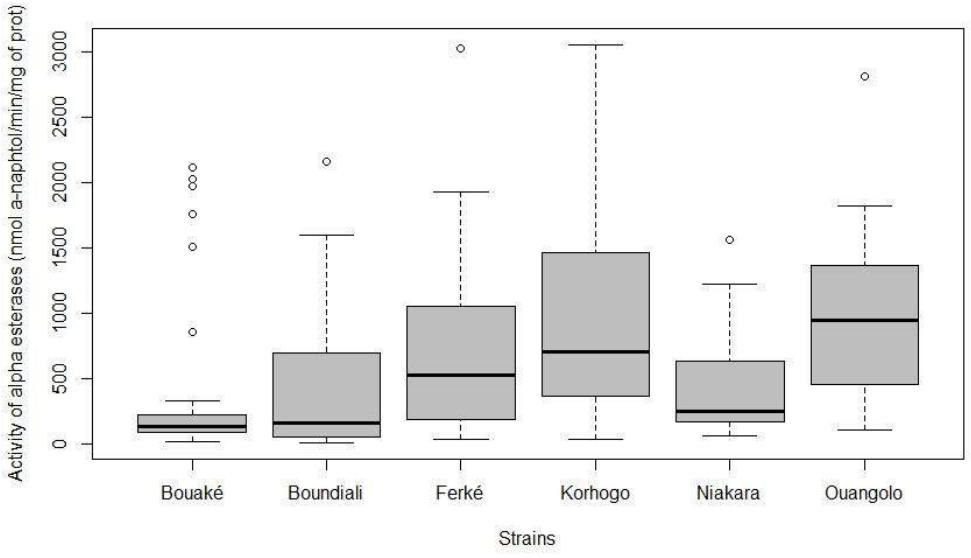


*Figure 2 :* Activity of α of Jasid Strains

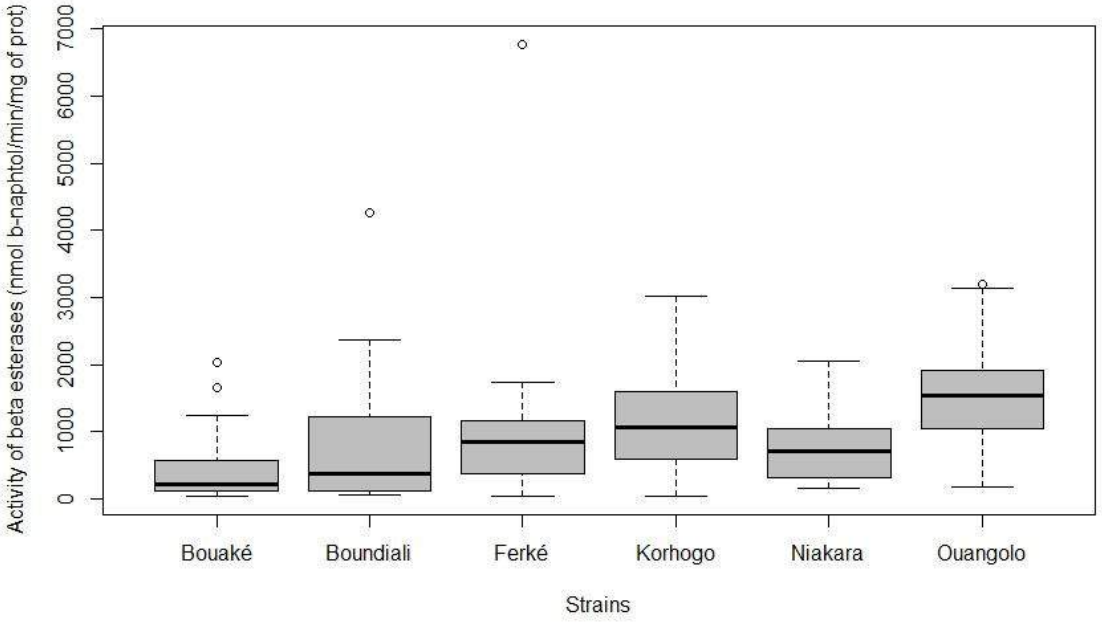


*Figure 3:* Activity of β-Esterases of Jasid Strains

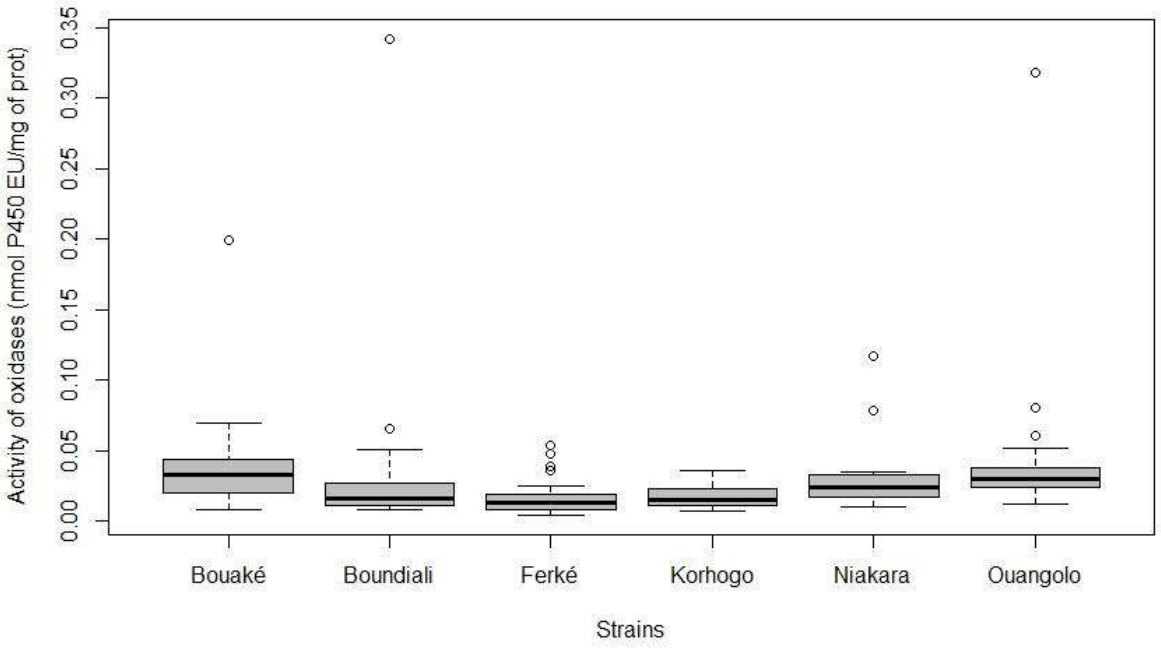


*Figure 4:* Activity of Oxidases of Jassid Strains

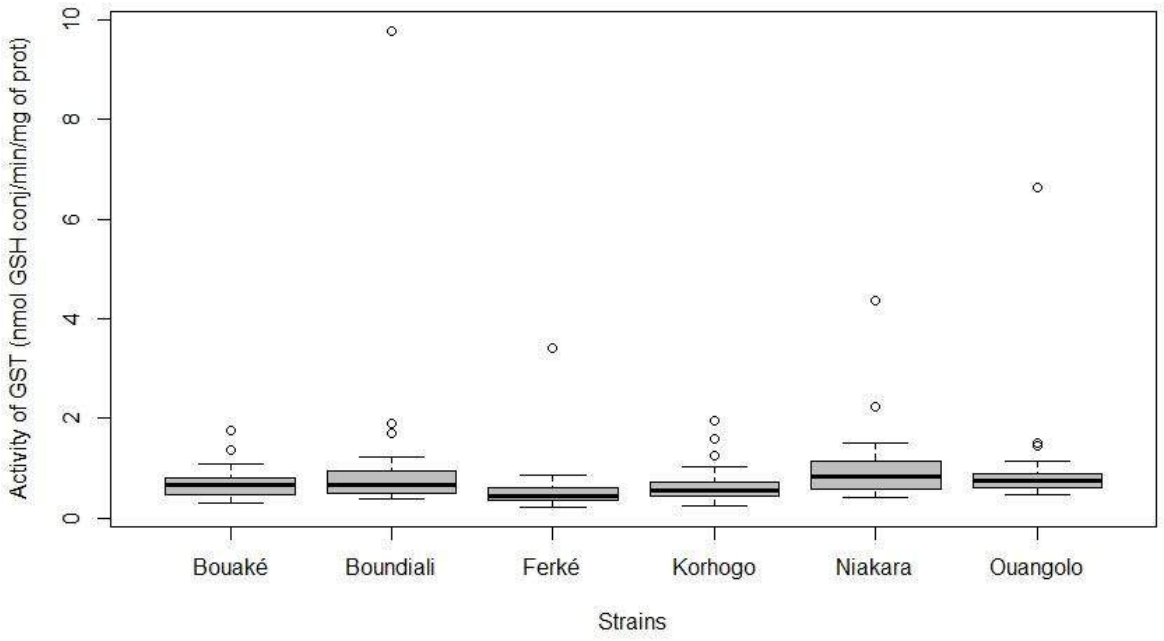


Figure 5: Activity of Glutathione-δ- Transferases of Jassid Strains

## V. DISCUSSION

The dosage of enzyme extracts absorbance from jassids strains from 6 localities has revealed a variability in enzymatic activity according to proteins and localities. For the jassid strains tested, the activity of esterases (alpha and beta) was significantly higher than that of oxidases and glutathione-δ-transferases.

The high activity of esterases compared to the activities of oxidases and glutathione -δ-transferases would thus show a strong involvement of esterases in controlling the sensitivity of jassids to active ingredients used for the protection of cotton in Côte d'Ivoire. The detoxification of active ingredients by esterases is obtained by two main ways. On the one hand, the hydrolysis of the ester bond for carbamates and pyrethroids; and on the other hand, the binding of the pesticide to the active site of esterase for organophosphates (Crow et al., 2007; Holling -worth & Dong, 2008; Liu et al., 2011).

The results obtained indicate that on average the activity of β-esterases was 1.45 times higher than that of α-esterases. This trend is similar to that of Karunaratne (1994) who showed that on average the activity of β-esterases was 3 times higher than that of α-esterases. Also, Paton et al (2000) showed that mosquitoes from the species *Culex pipiens quinquefasciatus*, selected individually, the activities of β-esterases were 2 to 30 times higher than those of α-esterases.

It also appears that the lowest activities of α-esterases and β-esterases were found in Bouaké while the highest activities were recorded for jassids from Korhogo and Ouangolo. This could mean that jassids in the locality of Bouaké are more sensitive to active ingredients for which α-esterases and β-esterases are involved in enzymatic detoxification. On the other hand, compared to the Bouaké strain, the jassids of the localities of Korhogo and Ouangolo are less sensitive to these active ingredients.

In contrast to these results, an earlier study on the cotton jassid *Amrasca biguttula biguttula* in India showed the involvement of oxidases in the detoxification of insecticides used in cotton cultivation (Sagar et al., 2013).

The strong involvement of esterases in the insecticide resistance mechanism in cotton jassids suggests a rational selection of the active ingredients used to control these pests. Esterases are a set of polyvalent enzymes capable of metabolizing a variety of exogenous and endogenous substrates. In this sense, some authors showed that when esterases are involved in the detoxification of insecticidal molecules, they can degrade a wide range of active ingredients including pyrethroids, organo phosphates and carbamates (Hollingworth & Dong, 2008; Liu et al., 2011; Muthusamy et al., 2014).

## VI. CONCLUSION

The measurement of the enzymatic activity of detoxification proteins has revealed a higher activity of esterases compared to those of oxidases and glutathione-δ-transferases. The involvement of α-esterases and β-esterases in the mechanism of loss of sensitivity in jassids to active ingredients is thus demonstrated. Jassids of the Bouaké strain showed the lowest esterase activities compared to those of Korhogo and Ouangolo. The Bouaké strain would therefore be more sensitive to active ingredients with ester bonds than any other jassid strain. The results obtained could be used as a basis for routine monitoring of the expression of enzymatic activities and therefore for monitoring the evolution of the level of sensitivity of jassids to insecticides in addition to toxicological assays. Moreover, these results enable to make a decision regarding the choice of active ingredients to be used to control the damage caused by jassids.

## ACKNOWLEDGMENTS

This research was supported by INTERCOTON (interprofessional association of cotton) and FIRCA (interprofessional fund for research and agricultural advice) of Côte d'Ivoire.

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