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Activity of insecticide detoxification enzymes in *Pectinophora gossypiella* (Saunders): Implications for insect pest management in cotton crops

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ABSTRACT

The pink bollworm *Pectinophora gossypiella* is one of the most important endocarpic pests in cotton. In the management of this pest, the intensive use of synthetic insecticides has led to the reduction of its sensitivity to certain active ingredients. It is in this perspective that the study was conducted to determine the enzymes involved in the metabolism of insecticides in *P. gossypiella*. Insects were collected in five localities (Bouaké, Bouaflé, Vavoua, Sésuéla and Kounahiri) with high pest pressure in the southern cotton zone. The collected insects were ground for the determination of enzymes involved in insecticide metabolism. The analyses showed a significant increase in glutathione-δ-transferase activity in populations of Bouaké compared to the other localities. However, individuals from Séguéla expressed more esterase (alpha and beta) and oxidase activities. Alpha and beta esterase activities were also more expressed in Bouaflé individuals. The high enzymatic activity of esterases and oxidases in individuals from Séguéla and Bouaflé reflects a low sensitivity to insecticides in which esterases and oxidases are involved in the metabolism. On the other hand, in Bouaké, only glutathione-δ-transferases are involved in the detoxification of insecticides.

Keywords: Insect, Individual, Enzymatic, Sensitivity.

INTRODUCTION

Cotton is the main source of ncome for farmers in the central and northern regions of Côte d'Ivoire (Kone et al., 2017). It is also a key of food security and sovereignty thanks to the food crops associated with its cultivation (Konan et al., 2015). However, cotton growing faces difficulties such as pest pressure, which affects production and impacts the economy. The crop losses caused by insects pests are sometimes important and can reduce to nothing the considerable efforts made by the producers (Miranda et al., 2013). The yields losses due to insect pests can be between 30 and 75% in the absence of protection (Soro et al., 2020). In addition, one of the most important pests endocarpic of the cotton crop is the pink bollworm *Pectinophora gossypiella* (Lepidoptera: Gelechiidae), which causes huge losses in quantity and quality of seed cotton.

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(Bonni et al., 2019 ; Shrinivas et al., 2019). To reduce the impact of these cotton insect pests, chemical control remains the primary means of control (Ochou et al., 2012 ; Djihinto et al., 2016). However, the repeated misuse of pyrethroids in cotton cultivation since their appearance in 1977 has caused the selection of resistant individuals in some pests, including the bollworm *Helicoverpa armigera* (Martin et al., 2000; Djihinto et al., 2012). *P. gossypiella* has been found to become increasingly less susceptible to some active ingredients of the pyrethroid family (Doffou, 2013; Ochou, 2019). Furthermore, several studies have shown that *P. gossypiella* is tolerant or even resistant following continuous and intensive use of insecticide against this pest in cotton crops (Kranthi et al., 2002; Mohamady, 2017).

According to Vanoosthuyse et al. (2018), insecticide resistance is classified into two groups including target-related and non-target-related resistance. Non-target resistance encompasses behavioral, physiological, and metabolic mechanisms. In insect pests, metabolic resistance is the common mechanism and attributable to a detoxification system by various enzymes including esterases, mixed function oxidases, and glutathione-δ-transferases (Panini et al., 2016; Pitou et al., 2019). Thus, it is that light that the present study was conducted to determine the activity of enzymes that may be involved in the decreased susceptibility of *P. gossypiella* to insecticides used in the cotton crop in Côte d'Ivoire.

MATERIALS AND METHODS

Study area

P. gossypiella caterpillars were collected in the localities of Bouaflé, Vavoua, Séguela, Kounahiri and Bouaké. These localities are found in the southern cotton production area of Côte d'Ivoire (Figure 1). The enzyme assay was performed at the Entomology Laboratory of the Cotton Research Program in Bouaké.

Insects

The larvae of *P. gossypiella* were collected from infested cotton bolls in the localities mentioned above. The larvae were then placed in boxes containing cotton fibre and transported to the rearing room to obtain the moths.

Technical

For the assay of enzyme activities, insects were crushed in eppendorfs tubes (1.5ml) using a plunger and centrifuged using a refrigerated centrifuge. Standard quality Thermo NUNC 96-well flat-bottom microplates, pipettes, tips, reagent reservoirs, magnetic stirrer, precision balance, pH meter, boys pipette, serological pipettes, falcon tubes (15ml) and an ice-layer were used in the preparation of the enzyme assay reaction mixture. A Multiskan GO microplate spectrophotometer was used for the kinetic readings.

Chemical

The chemical material for the oxidase assay consisted of potassium phosphate buffer (KHPO4); 3,3',5,5' tetramethyl benzidine (TMBZ); methanol; sodium acetate buffer (NaC2H3O2) and hydrogen peroxide (H2O2). The assay of esterase activity (alpha and beta) was performed with triton phosphate saline (PBS) buffer, alpha-naphthyl acetate for esterase, beta-naphthyl acetate esterase beta and fast garnett salt (FGBC). For glutathione-δ-transferase activity, the reagents used were glutathione in reduced form (GSH), sodium phosphate buffer, 1-chloro-2,4-dinitrobenzene (CDNB) and methanol. The total protein assay was determined mainly using Coomassie Plus Protein Assay Reagent (CPPAR) solution**.**

Enzyme extract

The entire extraction process was performed on ice to avoid rapid degradation of enzymes at room temperature. The newly emerged moths were individually put into 1.5

ml eppendorfs tubes containing 100 µl of distilled water and crushed with plastic pestles. Then 100 µl of distilled water was used to rinse the plastic pestles to have a final volume of 200 µl of grindings. The tubes containing the grindings were centrifuged at 14,000 rpm for 2 minutes at 4°C using a refrigerated centrifuge. The supernatants were transferred to new tubes for enzyme assay according to the method of William and Janet (1997) with a modification to the volume of extract to be deposited in the microplates.

Glutathione-δ-transferases assay

At the bottom of the microplate wells, 10 µl of enzyme extract was plated in two replicates by adding 200 µl of a previously prepared solution. This solution contained 0.060 g of reduced glutathione (GSH) dissolved in 20 ml of sodium phosphate buffer (0.1 M; pH 6.5) to which was added 0.013 g of 1-chloro-2,4-dinitrobenzene (CDNB) well crushed with the spatula and completely dissolved in 1 ml of methanol. Optical density reading was performed kinetically at 340 nm for 5 min against the blank containing all solutions except enzyme extracts.

Oxidase assay

In two replicates, 20 µl of distilled water and standards were introduced into the first two columns of the microplate. In the other wells 20 µl of enzymatic extract were deposited. Then, in the 96 wells of the microplate, were added 80 µl potassium phosphate buffer ($KHPO_4$) (0.0625 M at pH 7.2) and 200 µl of solution containing 0.012 g of 3,3',5,5'-tetramethyl benzidine (TMBZ) dissolved in 6 ml of methanol (the TMBZ well crushed with the spatula) and 18 ml of sodium acetate buffer ($NaC_2H_3O_2$) (0.25 M at pH 5.0). Finally, 25 µl of 3% hydrogen peroxide (H_2O_2) solution was added to all wells for incubation at 30 min prior to spectrophotometer reading at 630 nm.

Alpha and beta assay

In the microplate wells, 10 µl of extract from each individual was plated in two replicates and 90 µl of 1% triton phosphate saline (PBS) buffer at pH 6.5 was added to each well. This mixture was incubated at 25°C for 10 min before adding 100 µl of solution containing 600 l of 0.06 M alpha-naphthyl acetate (or beta-naphthyl acetate), 3 ml of 1% triton phosphate saline buffer at pH 6.5 and 8.4 ml of distilled water. The plate was incubated again for 30 min at 25°C. Then, 100 µl of solution containing 0.012 g of fast garnett salt (FGBC) previously dissolved in 12 ml of distilled water were placed in the wells. Finally, the microplate was kept at 25°C for 10 min before proceeding to the spectrophotometer reading at 550 nm.

Data analysis

Enzyme activities were calculated on Excel version 2013. The Shapiro-Wilk test and the Levene test were performed to check the normality of the data distribution and the equality of variances at the 5% threshold, respectively. Because normality and variances were not verified, a Kruskal-Wallis test (nonparametric ANOVA) was performed on the enzyme activities. In addition, Dunnett's T3 test was performed in case of significant difference between the means of the enzyme activities. Analyses were performed using IBM SPSS statistics 22

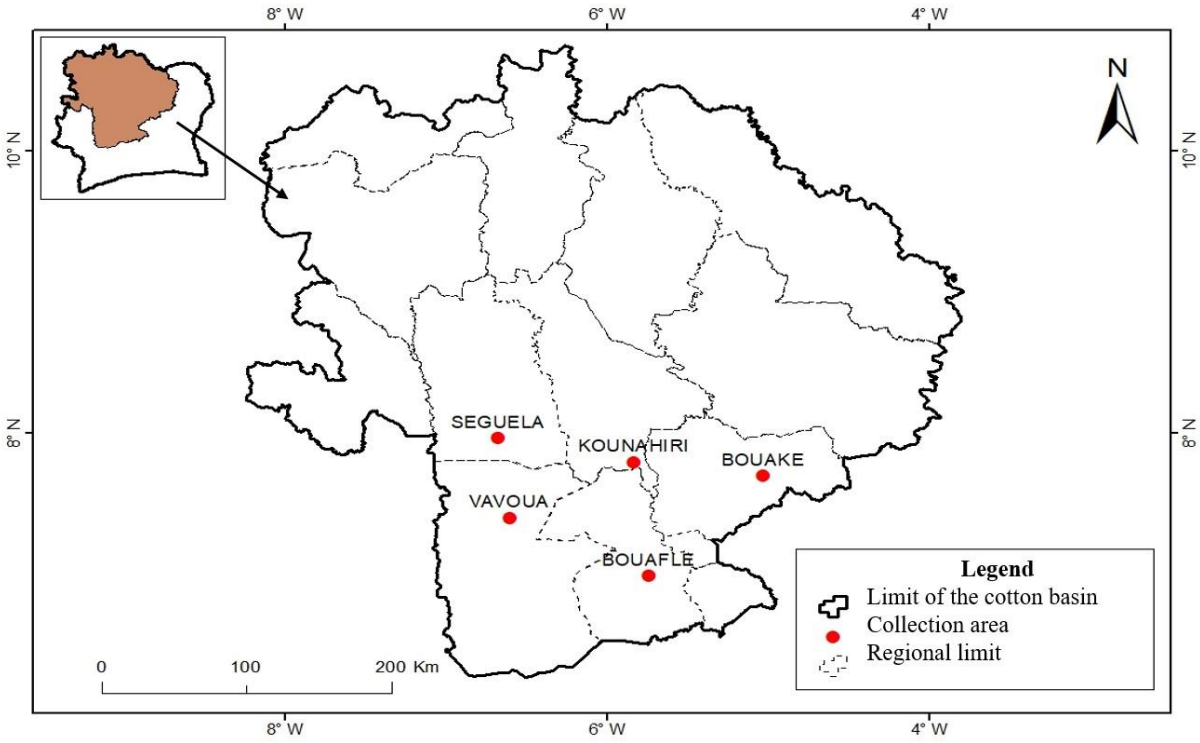

Figure 1 : Area of study showing samples of *Pectinophora gossypiella*.

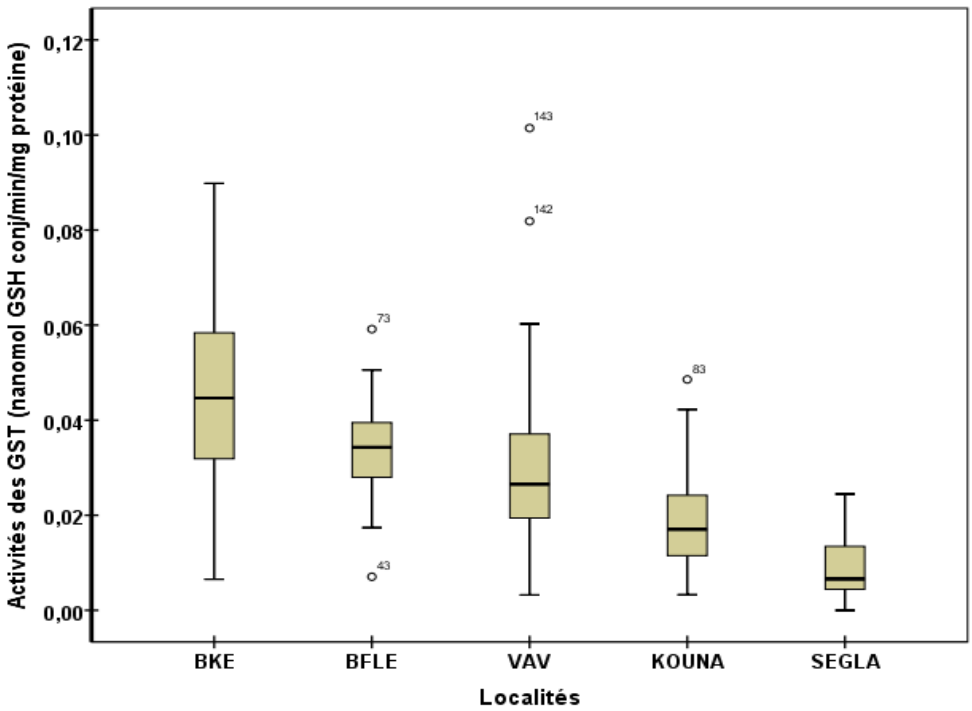

Figure 2: Activity of glutathion-δ-transférases.

RESULTS

Activities of glutathione-δ-transferases

The enzymatic activity of glutathione-δ-transferases (GSTs) varied among localities (p=0.00). These localities were classified into four groups based on insect-produced GST enzymatic activities. In decreasing order, the first were individuals from Bouaké (BKE), the second individuals from Bouaflé (BFLE) and Vavoua (VAV) localities, the third individuals from Kounahiri (KOUNA) and individuals from Seguela (SEGLA) locality for the fourth group

Activities of oxidase

Figure 3 shows a variation in oxidase activities of *P. gossypiella* among localities with values ranging from 0.02 to 0.06 nmol P450 EU/mg protein. Statistical analysis of oxidase activities revealed 4 groups with significant differences between localities (p = 0.00). The first group consisted of individuals from Seguela (0.05 nmol P450 EU/mg protein). The second group consisted of individuals from the localities of Vavoua and Bouaflé. The third and fourth groups consisted of individuals from Kounahiri and Bouaké respectively**.**

Activities of alpha-esterases

Alpha-esterase enzyme activity varied between locations (p = 0.00). Statistical analysis of enzyme activities showed a significant difference (p = 0.00) between localities. In addition, individuals from the Séguéla locality showed a higher alpha esterase activity compared to individuals from other localities. On the other hand, those from Bouaké and Vavoua showed low esterase activity compared to individuals from Bouaflé and Kounahiri.

Activities of beta esterases

Statistical analysis of enzyme activities showed a significant difference (p = 0.00) between localities. In addition, individuals from Bouaflé showed a higher beta esterase activity than individuals from other localities. On the other hand, those from Vavoua showed a low beta esterase activity compared to individuals from Bouaké, Kounahiri and Séguela.

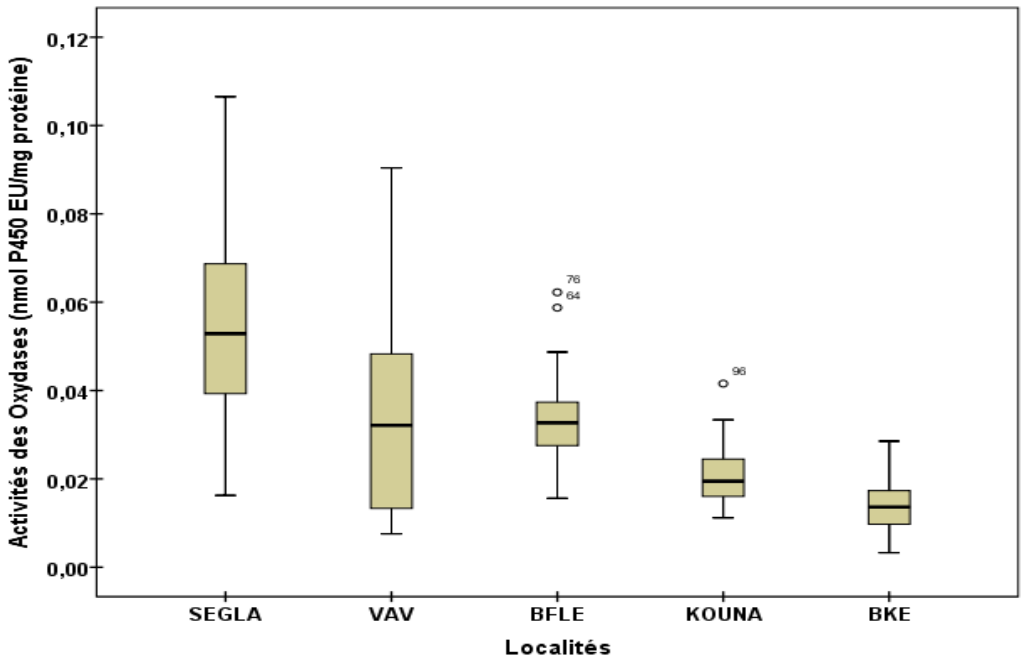

Figure 3 : Activity of oxydases.

DISCUSSION

The enzyme assay showed a difference in the variation of enzyme activity between localities. Indeed, the activity of glutathione-δ-transferases (GST) was higher in individuals from Bouaké but low in the population of Seguela. This would indicate that individuals from Bouaké are less sensitive to the active ingredients for which GSTs are involved than individuals from Seguela. The results corroborate with those of Pitou et al. (2019). These authors showed that GST activity of jassids was significant in Bouaké individuals only in some localities. On the other hand, individuals from Séguéla, Vavoua and Bouaflé showed a high oxidase activity than individuals from Kounahiri and Bouaké. Oxidases would therefore play an important role in the metabolism of several insecticides of different families used in cotton sanitary protection in these localities. Panini et al. (2016) showed that oxidases were critically important in the metabolism of various insecticides including pyrethroids, organophosphates, carbamates and neonicotinoids.

Alpha and beta esterase activity was more expressed in individuals from Séguéla, Bouaflé and Kounahiri localities compared to individuals from Vavoua and Bouaké localities. In addition, beta esterase activity was more pronounced than alpha esterase activity. This trend was observed in the work of Pitou et al. (2019) on jassids. This present results would explain the strong involvement of esterases in the detoxification of xenobiotics (pyrethroids and organophosphates) in insects. Previous studies conducted on plot-collected individuals of Pectinophora gossypiella showed a positive correlation between high esterase activities and the development of resistance to organophosphates and pyrethroids (Mohamady, 2017). Furthermore, insecticide detoxification reported in more than several insect pests was characterized mainly by increased esterase activities (Cao et al., 2008).

Considering all the enzymatic activities, it appears that individuals from Bouaflé and Séguéla are less sensitive to certain active ingredients than individuals from other localities. This could reflect the strong presence of *P. gossypiella* in these localities. However, Ochou et al (2018) have shown in their studies the strong presence of endocarpic organisms (*P. gossypiella* and *T. leucotreta*) in the southern zone of the cotton basin, precisely in certain localities such as Bouaflé and Séguéla.

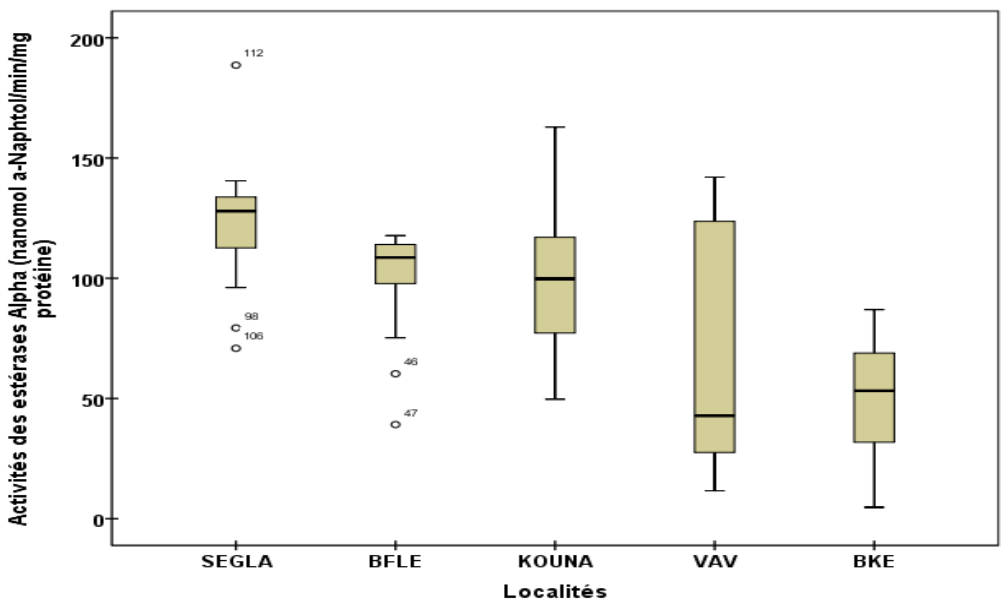

Figure 4 : Activity of alpha esterases.

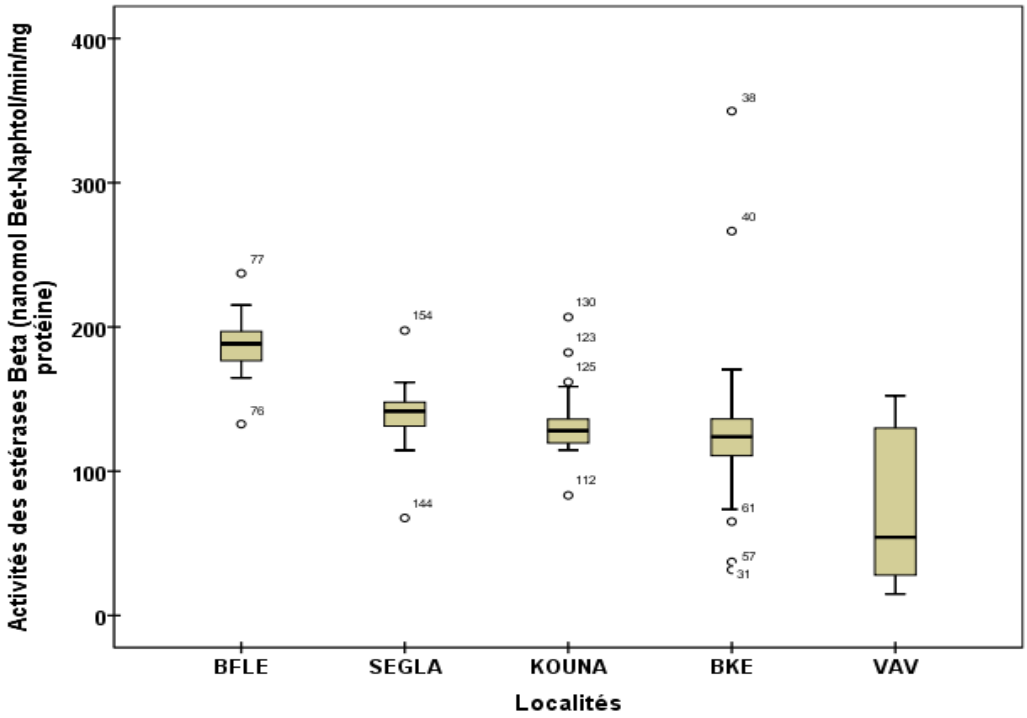

Figure 5 : Activity of beta esterases.

Conclusion

Individuals from Séguéla, Bouaflé and Kounahiri localities are less sensitive to insecticides whose esterases are involved in metabolism, while those from Bouaké are more sensitive. On the other hand, individuals from Bouaké have a low sensitivity to active ingredients whose glutathione-δ-transferases can block the insecticidal action and more sensitive to products degradable by oxidases.
It is therefore important to use these results in control programs against *P. gossypiella* to evaluate the efficacy of insecticides in order to detect the appearance of loss of sensitivity or even resistance to insecticides.

COMPETING OF INTERESTS

The authors declare that they have no competing interests concerning this article.

AUTHORS' CONTRIBUTIONS

This work was carried out with the collaboration of all the authors**.** The corresponding author of the article KDK conducted the work, analyzed the data and drafted the manuscript. KDKr, MK and KKNB contributed to the interpretation of the data and the critical revision of the content of the article. OGO, initiator of the research activity, read and corrected the manuscript. The final manuscript was approved by all authors.

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