ENZYME PROFILE OF THE OIL PALM (*Elaeis guineensis* Jacq.) PEST *Coelaenomenodera lameensis* Berti AND Mariau (*Coleoptera: chryomelidae, hispinae*) ACCORDING TO THE DIFFERENT DEVELOPMENT STAGES

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ABSTRACT

Coelaenomenodera lameensis *Berti and Mariau, (Coleoptera, Chrysomelidae) is the most important oil palm (*Elaeis guineensis *Jacq.) leaf miner pest in Ivory Coast,West Africa. The leaf miner activity of* C. lameensis *was higher in instars 1-3 (L1-L3) larvae. Analysis of the enzymatic profiles indicated that instars 1 and 2 larvae are the most powerful ones in polysaccharides degradation activity, higher gain being obtained with xylan (28 µg of glucose equivalent released per minute (U) per milligramme of protein) and laminarin (18 U mg^{-1}). The most important heterosidasic activities were detected in L3 larvae and to a lesser extent in L1-2. The β-glucoside was hydrolysed at an activity rate higher than 400 U mg^{-1} by L3 larvae. The β-xylosidasic and N-acetyl-glucosaminidasic activities were respectively 39.13 and 35.81 U mg^{-1} for L1-2 and 34.9 and 36.39 U mg^{-1} for L3 larvae. The data suggested that the majority of carbohydrate digestion occurs in the L1-2, and L3 instars. Enzymatic profiles of L4 larvae and adults were characterised by low activities. Finally, the feeding behaviour (foraging activity) and the enzymatic activities were all linked, underlying the digestive capability of each developmental stage of* C. lameensis*. The main digestive enzymes of this pest are identified, the search for inhibitors of these enzymes may be considered.*

Keywords: enzyme profile, *Coelaenomenodera lameensis*, *Elaeis guineensis;* oil palm pest.

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INTRODUCTION

The leaf-miner, *Coelaenomenodera lameensis* Berti and Mariau (*Coleoptera: Chryomelidae, Hispinae*) is a severe pest of oil palm (*Elaeis guineensis* Jacq.) (Berti and Mariau, 1999). During the last 30 years, this leaf miner has become increasingly frequent in new oil palm plantations in West Africa. Both the adult and larval stages cause damage (Morin and Mariau, 1970). Adults generally feed on the underside of the leaves, and most often lay their eggs on the palm leaves. The larva causes a more severe damage by mining tunnels which can reach up to 20 cm long and 2 cm wide (Morin and Mariau, 1970). Larval damage results in desiccation of the fronds and the reduction of photosynthesis. A larval outbreak can defoliate a plantation in approximately six months. Wood *et al.* (1973) reported yield reduction of 40% to 50% in oil palm after the first year of severe

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leaf-miner outbreaks. Regular monitoring of larval and adult densities is recommended to determine optimum timing for insecticide spray application (Mariau and Lescoutre, 2004). Unfortunately, the use of insecticides has brought some indirect environmental impacts. The secondary effect of insecticide applications to the environment has raised concern on sustainable management of the oil palm leaf-miner. Consequently, over the last decades, a considerable effort has been directed toward the implementation of research projects on integrated pest management (IPM) in West Africa. This effort, however, has been directed toward the use of insecticides with less hazardous active ingredients. A key approach of IPM implementation requires the shift to a better understanding of the biology of the pest and its pest-host relationship. Some of this research focused on the insect's biology, morphology and development (Morin and Mariau, 1971), its reproduction and mortality factors (Morin and Mariau, 1971; Mariau and Lecoustre, 2000; 2004). So far, little attention has been paid to the digestive enzymes of the pest. Understanding the relationship between digestive processes of the leaf-miner during different stages might have practical implications for implementation of IPM. For instance, while microorganisms, such as bacteria, fungi, protozoa, are the most efficient cellulose and hemicellulose degraders in nature, it has been established that the degradation of plant material in insects requires the synergic action of many different enzymes (Martin, 1983; Watanabe and Tokuda, 2001; Brune, 2003; Erthal *et al*., 2004). The aim of this study was to determine the enzymatic profile at the different developmental stages of *C. lameensis* and to assess if changes of feeding behaviour in the different developmental stages are linked to the digestive capacities. It may well be possible to conduct research on inhibitors of these enzymes involved in the destruction of palm leaves. Enzyme inhibitors could provide a key role in plant defence against pest by inhibiting major insect digestives enzymes thus hampering the growth of these insects (Mehrabadi *et al.*, 2012). The experiment was conducted using enzymatic analysis. Cellulase and hemicellulase (xylanase, laminarinase, galactomannanase) enzymes were determined because they hydrolyse polysaccharides which are the most abundant plant compounds. In addition, β-glucosidase, α-glucosidase, β-xylosidase were chosen for their critical role in releasing low molecular weight sugars that are important as energy sources for gut microbiota metabolism. Results were discussed with respect to damages caused by the pest at different stages of development.

MATERIAL AND METHODS

Insects

Larvae and adults of *C. lameensis* were sampled at Toumanguié (5°21' N, 3° 23' W) oil palm plantations located at 100 km East of Abidjan (Ivory Coast). Three samples (2 g x 3) of larval instars 3 and 4 (L3-4) and adults (2 g samples equivalent to 313 larvae instar 3; 247 larvae instar 4 (L4) and 297 adults) were collected in three blocks chosen at random. Only 2 g (317 larvae) x 2 larval instar 1-2 (L1-2) was collected because this larval stage was less abundant and their small size requires a greater number of larvae for a weight identical to those of the other stages.

Feeding Activities

The gallery lengths caused by the different larval instars (feeding activities) were determined. Fifty leaflets with galleries were randomly selected; then, each gallery was measured followed by determination of the larval stage found within that gallery.

Enzymatic Extract

Two grammes of whole insects (L1-4 and adult) were homogenised in 10 ml distilled water at 4°C. The enzyme solution was centrifuged at 15 000 rpm for 20 min at 4°C and the supernatant was dialysed in a pig gut membrane (Naturin from Ets Soussana, 92, Orly, France) against distilled water at 4°C for 12 hr. The dialysed solution constituted the enzymatic extract.

Enzyme Assays

Heterodidasic activity. Heterosidasic activities were assayed by measuring the amount of liberated para-nitro phenyl (PNP) following the methodology of Rouland *et al,* (1986). The assay mixture contained 50 µl of substrate (PNP-β-glucoside, PNP-α-glucoside, PNP-β-galactoside, PNP-α-galactoside, PNP-N-acetyl-β-glucosamine, PNP-β-xyloside), 25 µl of Mc Ilvain (1921) buffer at pH 5 and 50 µl enzymatic extract solution. Incubation was carried out at 37°C and stopped by adding 3 ml of Na_2CO_3 (2%). The increase of absorbance was read at 420 nm. One unit (U) was defined as the amount of enzyme that liberated 1 µg of PNP per min at 37°C.

Polysaccharidase activities. Polysaccharidase activities were measured either as the amount of the reducing ends generated during the enzyme reaction determined by the Somogyi-Nelson

method (Somogyi, 1945; Nelson, 1944). This was done by incubating 100 µl of enzymatic solution with 50 µl of Mc Ilvain buffer pH 5 and 100 µl of substrate. After 60 min of incubation at 37°C the enzymatic reaction was stopped by adding 0.5 ml of the Cu alkaline reagent (Somogyi, 1945). Then, the mixture was boiled for 20 min and cooled down in a cold water bath for 15 min. Nelson reagent (0.250 ml) and 4.0 ml of distilled water were sequentially added, and the mixture was left for 10 min at room temperature. The absorbance of the colored solution was measured at 650 nm. All the experiments were replicated three times.

Protein Measurement

The protein assay was performed according to the method of Sedmark and Crossberg (1977) using serum albumin as standard.

Statistical Analysis

Gallery lengths (x ± SD) for each larval instar were compared using a one factor (ANOVA), and differences among instars were determined by using Newman-Keuls Test. Specific enzyme activities were compared by using one factor variance analysis (ANOVA) followed by post-hoc Tukey test. Statistical analyses were performed at 5% level for significance. A principal component analysis (PCA) was performed with the software ADE-4 (Thioulouse *et al.*, 1996) to examine the relationships between several biological traits *i.e.* enzyme activities and stage of development. A permutation test was used to appreciate the significance of the groups suggested by the PCA.

RESULTS

Feeding Activity

Visual observation indicated that the first larval instars (L1, L2 and L3) mined galleries whereas this activity was reduced for L4. The average length of L1-2 galleries was 2.5 cm. The lengths of L3 galleries were two times higher (*Table 1*) than those of the earlier instars. In contrast, lengths of L4 and prepupal galleries were not significantly different from L3. These values indicated that the mining activity decrease with the larval development.

Polysaccharidase Activities

All the samples showed galatomannanase activity to various degrees. Adult and L1-2 had the highest activities (superior to 10 U mg^{-1} of proteins). L3 and L4 exhibited lower activities which did not exceed 10 U mg^{-1} of proteins. Amylase activities detected on starch or amylose were the highest in the L1-2 in comparison with the amylase activities detected in the other samples. In the entire sample tested, cellulase activity was less than 10 U mg^{-1} of proteins. The lowest activity was measured in the adults. No difference was observed on carboxymethyl cellulose (CMC) between the L1-2, L3 and L4. Surprisingly on cellulose microcristalline, L4 exhibited an important activity which was two times higher than those obtained in the L1-2 and L3. Although some were detected in the different samples, arabinogalactanase, lichenase, nigeranase and pullulanase had too low activities to be considered as minor enzyme activities (< to 2 U mg^{-1} of proteins) (*Figures 1a* and *1b*).

Heterosidase Activities

The L3 instar presented the highest activities compared to those on the L1-2 and L4 instars. In particular, β-glucosidase activity measured for this instar was outstanding with an activity superior to 400 U mg^{-1}. To a lesser degree, this activity was also important in the L1-2 instars with an activity close to 100 U mg^{-1} of proteins. The β-xylosidase and N-acetylglucosaminidase activities were not significantly different between L1-2 and L3. Adults and L4 exhibited lower activity not exceeding 10 U mg^{-1} of proteins. The β-galactosidase activities were mostly present in the L3 and also, but in a lesser extent, in the L1-2. These activities were less than 10 U mg^{-1} of proteins in the adults and L4. Among the different enzymes tested, α-galactosidase and α-glucosidase presented the lowest activities. These two enzymes were prevailing in the L1-2 and L3 whereas in adults and L4 their activities were less than 2 U mg^{-1} of proteins (*Figure* 2).

Statistical Analysis: PCA and Similarity Dendogram

The first two axes of the PCA (*Figure 3*) explain 71.4% of the total inertia (42.4% on axis 1, 29.0% on

TABLE 1. LENGTH OF GALLERIES BY THE DIFFERENT STAGES OF *C. lameensis* (N=50)

Stage of *C. lameensis*	Average length of the galleries (cm)
L1-2	2.52±0.17 (a)
L3	4.99±0.25 (b)
L4	5.37±0.38 (b)
N	5.70±0.63 (b)

Note: L1-L2: larva of instar 1 and 2; L3: larva instar 3; L4: larva instar 4; N: pupae.
The values followed by the same letters in the same column are not significantly different according to the variances analysis (ANOVA) and post-hoc test of Newman-Keuls (P<0.05).

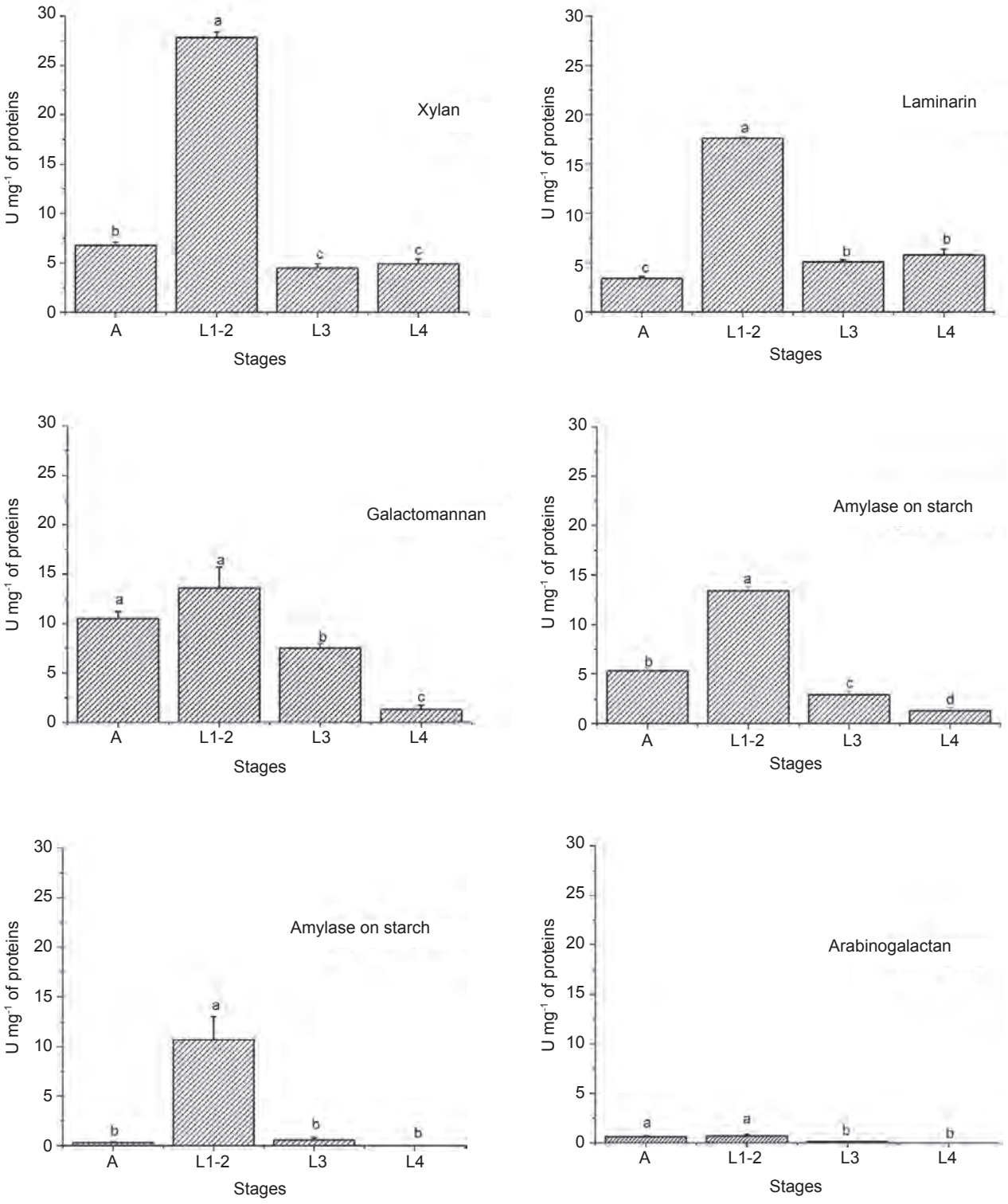

Figure 1a. Polysaccharidase activities of C. lameensis *at different stages (xylan, laminarin, galactomannan, starch, arabinogalactan) (A: adults; L1-2: larvae instars1-2; L3: larvae instar 3; L4: larvae instar 4). Specific activity values are expressed as microgrammes glucose equivalent per minute per milligramme protein.*

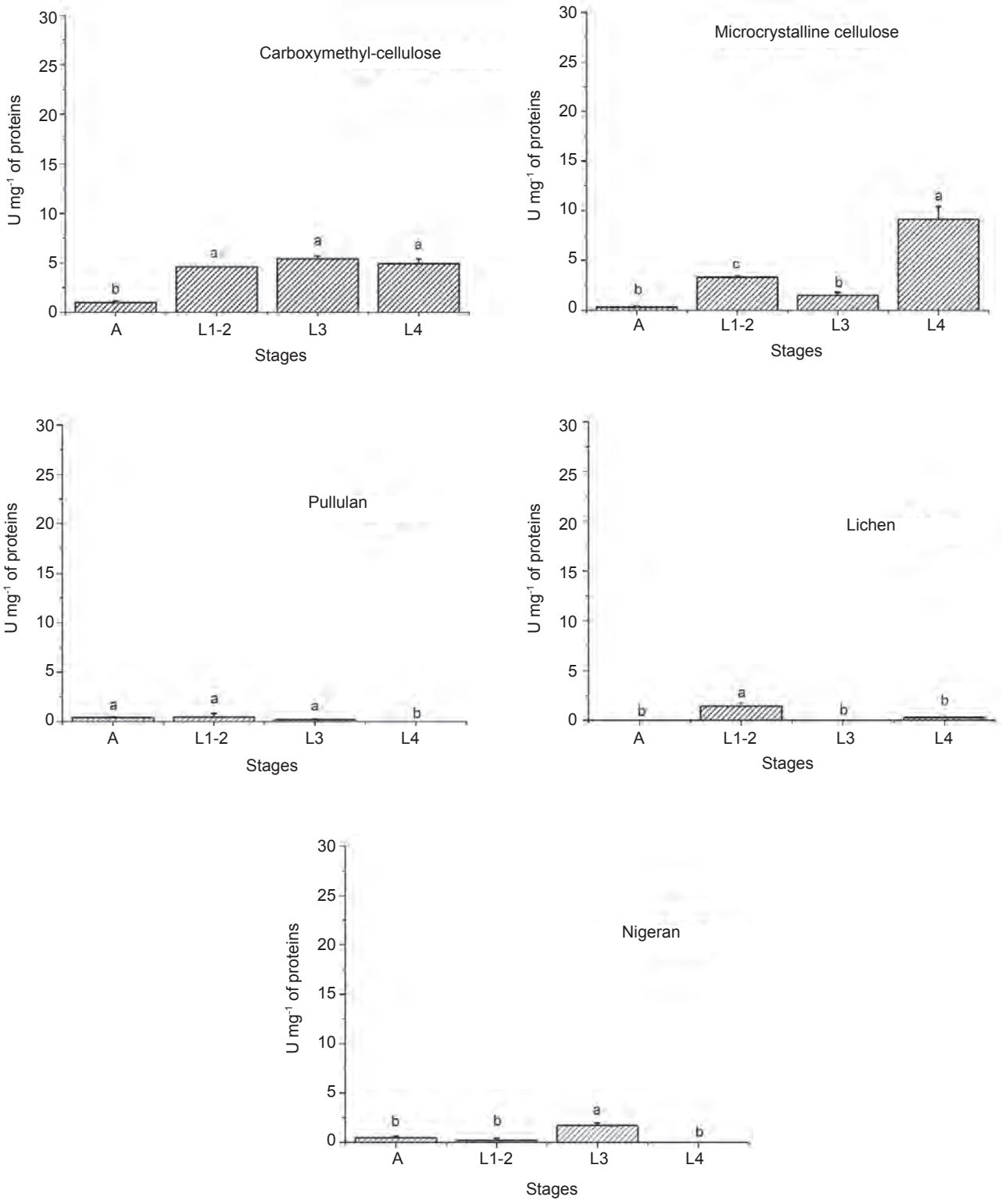

Figure 1b. Polysaccharidase activities of C. lameensis *at different stages (carboxymethyl-cellulose, microcrystalline cellulose, pullulan, lichen, nigeran) (A: adults; L1-2: larvae instar-2; L3: larvae instar 3; L4: larvae instar 4).*

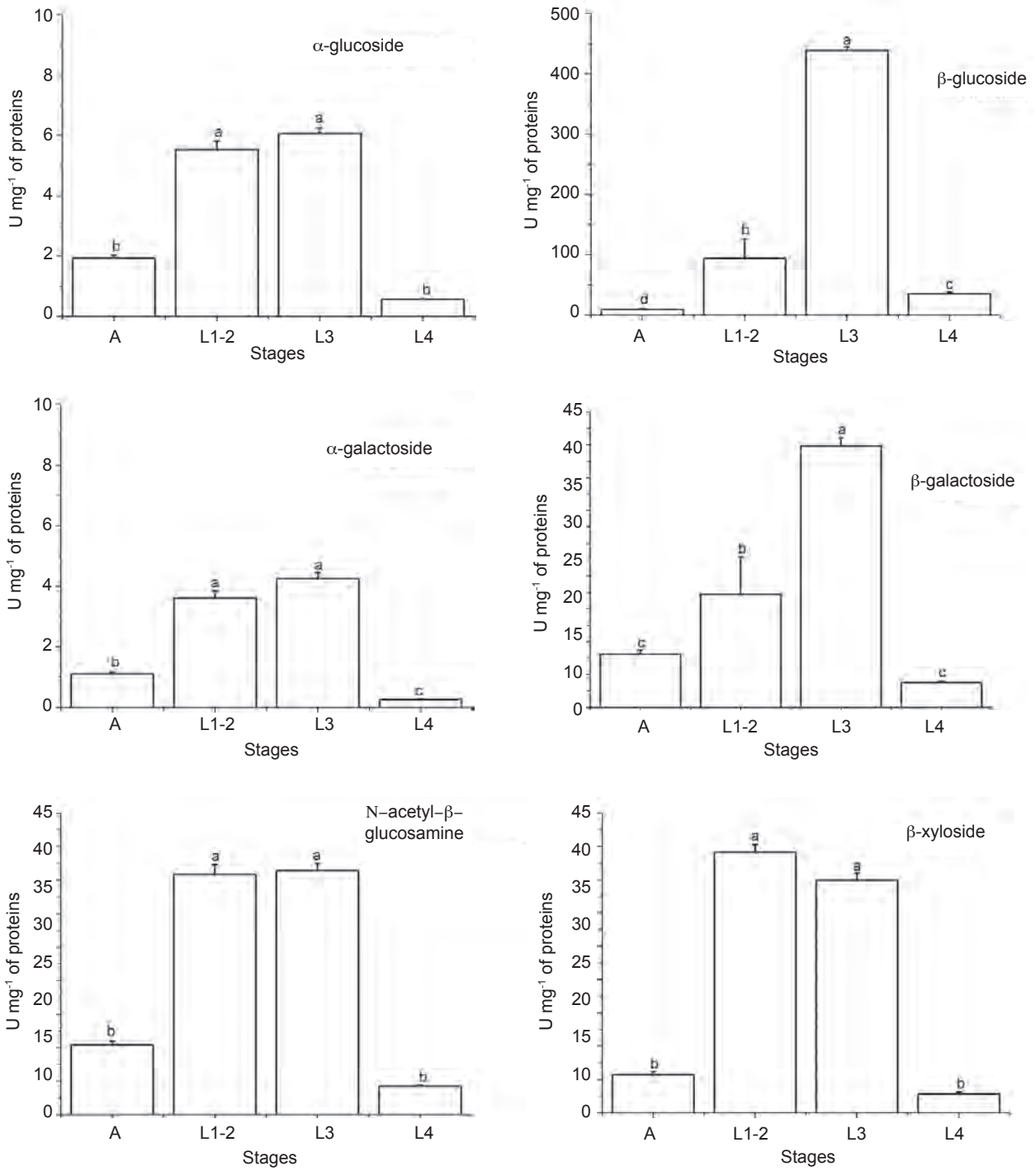

Figure 2. Heterosidase activities of C. lameensis *at different stages (A: adults; L1-2: larvae instars1-2; L3: larvae instar 3; L4: larvae instar 4). Specific activity values are expressed as micrograms glucose equivalent per minute per milligram protein.*

axis 2). The discriminative analysis carried out on 10 000 permutations shows that the PCA is highly significant (P=0.00001). The correlations circle shows, on axis 1, an opposition between L1-2, L3 and L4, adult which had low enzyme activities. On axis 2, L1-2 with high polysaccharidase actictivites are in opposition to L3 characterised by high oligosacharidase activities.

A similarity dendrogram (*Figure 4*) realised on the PCA values were used to estimate oppositions between samples. The first section segregated L1-2 samples from all other samples. The second section regrouped all L3 samples in a cluster distinct from that of L4 and A (adults). Finally, L4 and A (adults)

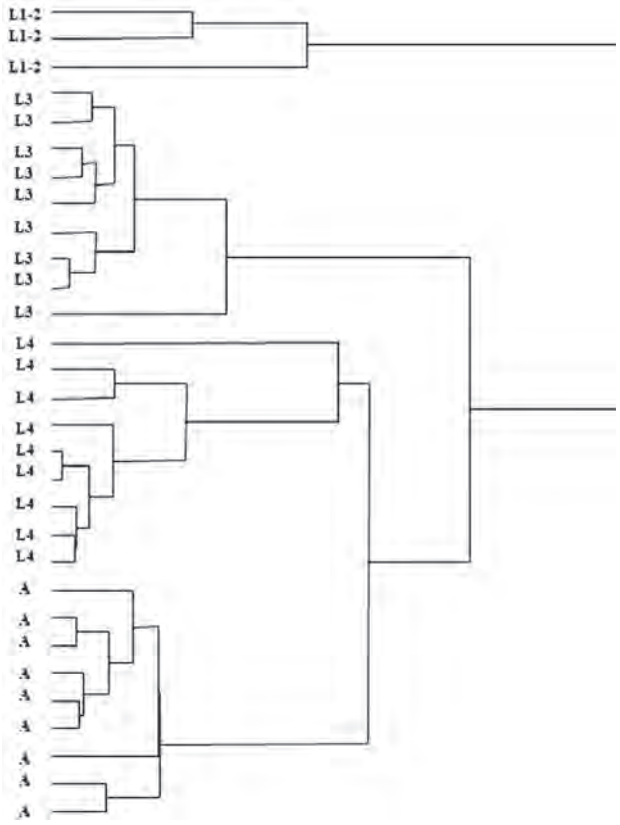

Figure 3. Dendrogram of similarity calculated on the basis of principal component analysis (PCA) values of the enzymatic activities of the stages of C. lameensis *(A: adult; L1-2:instars1-2; L3: instar 3; L4: instar 4).*

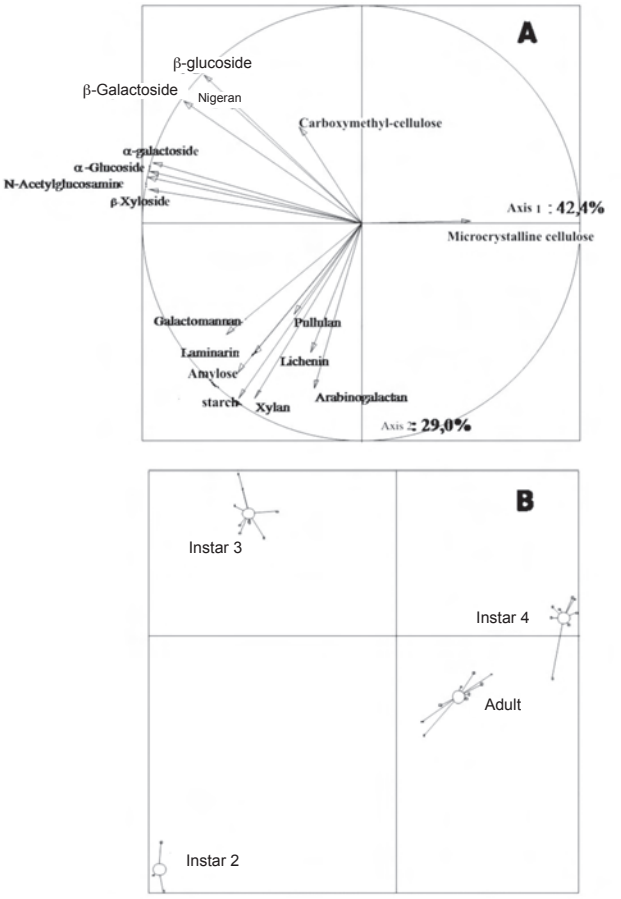

Figure 4. Results of the principal components analysis (PCA). A: Correlation circle. B: Ordination of the samples in the plane defined by axes 1 and 2 of the PCA.

were more or less close to each other, L3 constituted an intermediate group and L1-2 were very different from the other groups.

DISCUSSION

The digestive processes in insects which are adapted to a wide range of diets occur in several stages (Applebaum, 1985). Polymeric food molecules are the target of specific hydrolases, which break them down to oligomers and then dimers and monomers (Terra, 1990). The relationship between insect gut structure and foraging strategy has been studied for several hundred years; however, we know little about how, during the different larval instars of an insect, carbohydrases are linked to gut morphology and larval behaviour.

Feeding Activity

Feeding activity concerned mainly the stages L1-2 and L3 and at the end of this activity, the gallery length was about 4.9 cm. According to Morin and Maraiu (1971) the three first larval instars last for 30 days; then, we concluded that during this period, feeding activity or larval mining could reach 17 mm per day. In contrast, during L4, no significant activity was observed. As this insect 'eats its own way', thus, food acquisition only happened from the L1 through the L3 instar.

Enzymatic profiles

Among insects, differences in feeding behaviour between life stages are well-known (*e.g.* Michaud, 2005), but how dietary breadth changes within a life stage of an insect remains poorly understood (Scriber and Slansky, 1981; Barton Browne, 1995). In this study, an enzymatic approach was used to investigate food dietary. Insects generally have a wide spectrum of digestive enzymes that are spatially and temporally expressed in the midgut (Terra and Ferreira, 1994; Terra *et al.*, 1996). The profile of digestive enzymes according to the different larval developments of *C. lameensis* until the adult stage showed a progressive decrease. The presence of digestive carbohydrases was detected in the early instars (L1-2) of the larval development of *C. lameensis*. The enzyme profile was characterised by high polysaccharidase and

oligosaccahridase activities. According to the PCA analysis, the most descriminant enzyme activities were laminarinase, xylanase, galactomannanase and amylase. Laminarin is a plant polysaccharide composed of glucose residues linked together through β-1, 3-linkages; and xylan is a polymer of xylose residues linked trough β-1-4 linkages resulting in the hardening or thickening of plant cell walls. Thus, the presence of xylanase and laminarinase demonstrated the ability of *C. lameensis* larvae to degrade the main hemicelluloses of the plant cell wall. Moreover, galactomannanase activities are consistent with the fact that mannan polymers are one of the major constituents of the cell wall of *Elaeis guineensis*. According to Dusterhoht *et al.* (1992) major polysaccharides in palm kernel meal were linear mannans with very low galactose substitution (78% of total non-starch polysaccharides), followed by cellulose (12%) and small amounts of (4-O-methyl)-glucuronoxylans and arabinoxylans (3% each). Starch, which is the most common food reserve glycan, was also consumed by L1-2. In contrast, activities measured on arabinogalactan, pullulan, lichenan and nigeran were too low to have a real contribution in the digestive metabolism. Regarding L3, instar PCA analysis showed that these larvae were more characterised by oligosaccharidase than polysaccharidases. Such results suggested that L3 larvae feed on plant materials which are more or less degraded. The β-glucosidase was the main oligosaccharidase in comparison with others tested. This enzyme is involved in cellulose hydrolysis. The conversion of cellulose into glucose is known to consist of two steps. In the first step, β-1, 4 glucanase (endo and exo cellulase) breaks the glucosidic linkage to cellobiose, which is a glucose dimer with a β-1, 4 bond. Subsequently, this β-1, 4 glucosidic linkage is broken by β-glucosidase. Logically, a high β-glucosidase activity is associated with a high cellulase activity as observed for many insects (*e.g.* Martin, 1983; Watanabe and Tokuda, 2001; Willis *et al.*, 2010) but our results were not consistent with this general rule. Indeed, cellulase activity of L3 was not the main activity in comparison with the other polysaccharidases tested. Conversely, to a high cellulase activity, corresponded a low β-glucosidase activity for L4. At this stage of experiment, such results remain unclear and need further investigations. Adults were characterised by low activities of enzymes. This decline may result from a greater degradation or a lower synthesis of digestive enzymes produced by a quantitative decrease of the feed intake when larvae are not far from the next moult. This hypothesis was confirmed by the mining activity which declined in the old instars. Ours results showed that there is a close relationship between feeding habits and carbohydrases activities.

Knowledge of the enzymatic profile of *C. lameensis* at different stages of insect development is important. Indeed, it specifies the most destructive stages. It also helps to consider the use of enzyme inhibitor in the management of this pest. Insect digestive enzymes as a target for pest control are innovative (Macedo *et al.,* 2011). Inactivation of digestive enzymes by inhibitors results in blocking of gut amylases including proteases, cellulases, *etc*., leading to poor nutrient utilisation, growth retardation and death (Zibaee *et al.,* 2010; Mehrabadi *et al.*, 2011; Sami, 2014).

CONCLUSION

The enzymatic profile of the different developmental stages of *C. lameensis* is fundamental in understanding the digestive physiology of the pest. The development of new pest management strategies based on inhibition of digestive enzymes, may now be considered. Future studies are expected to initiate this control approach in the field of oil palm.

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