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Research Article

Morphometric identification of cowpea weevil populations, *Bruchidius atrolineatus* (Coleoptera-Bruchinae) from three varieties of cowpea, using a discriminant analysis (FDA).

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Abstract: *Bruchidius atrolineatus* Pic. (Coleoptera-Bruchinae) is a major pest of cowpea in the Sahel. The attacks began in the fields and continue in the stocks. In this work, a morphometric study to characterize populations of this species from different cowpea varieties is performed. Individual's male from seeds and pods of three varieties of cowpea (TN5/78 HTR and a local variety) were compared using a discriminant analysis (FDA) on 17 morphological variables. The average size of each variety was measured. The data analysis shows that these groups differ from one another primarily by size. Their decreasing rank order size is: local variety, TN5 / 78 and HTR. The individuals from HTR are clearly distinguishable from all others by the length of the first abdominal sternum (Los) longer. The maximum length of the antenna (Lan), the largest dimension of the femur 3 (Lf3), the largest dimension of the tibia 3 (Lt3), the largest radial dimension of the cell of posterior flange (Loc), and length of the pygidium (LPY) lead to distinguish individuals of the local variety. Other variables allow differentiating individuals from TN5 / 78. This is the maximum length of the head (Lmt), the length and width of pronotum (Lop and Lap), the maximum width of the elytron (Lae), the maximum radial width of the cell (Lac), the largest dimension of the coxa 3 (CML), the maximum width of the first abdominal sternum (Las) and the width of pygidium **(**Lpy**).** The analysis of raw data by a reference variable (maximum width of pygidium, LPY) data, AFD reveals differences in form between individuals of different populations. Four variables explain the differentiation observed after data analysis. These are Lmt, Lap, Lf3 and LPY. Thus, this study shows that morphometric characters of *B. atrolineatus* vary depending on the variety which individuals belong. However, a genetic study seems necessary to clarify the characteristics of these different populations.
Keywords: Cowpea; *Bruchidius atrolineatus*; Morphometric analysis; Sahel; AFD (Discriminant Factorial Analysis).

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp. is one of the most widely cultivated food legumes in the world. The studies about this crop in Niger the have shown that the production fluctuates from one year to another. Indeed, it was estimated at 1,329,514 tons in 2012 against 1,517,142 tons in 2011 and 1,773,423 tons in 2010 [1]. It is often consumed by people at both cities as villages, cowpea is rich in protein, and helps offset food shortages.

After harvesting, conservation cowpea is difficult due damage by Coleoptera Bruchinae which recognized species as the most devastating *Bruchidius atrolineatus* Pic. and *Callosobruchus maculatus* Fab. occur in the cultures at the end of the rainy season and breed during the fruiting period cowpea.

Between these two species, *B. atrolineatus* is the one responsible for the first damage in cowpea storage stores. In cultures, females have *Bruchidius atrolineatus* opportunistic behavior by depositing their eggs on the most abundant growth stage in crops. But in a situation of choice, 60% of eggs are deposited on the green pods[2]. These eggs are deposited along the sutures of the pods (Alzouma 1987). During storage of cowpea crops, the initial infestation by weevil rate is generally less than 5%[3,4].

This rate increases to 30% after one month of storage, then 80 to 100% in the period of 5 to 6 months of storage if no control measures are taken. Studies on the distribution of egg-laying of this species of beetle conducted in the region of Niamey in Niger showed that

80-90% of harvested cowpea pods carried *B. atrolineatus* eggs[5].

To control these insects including *B. atrolineatus* which is responsible for the first damage in stores, several control methods have been developed including varietal resistance which is the ability of a plant variety to get good productivity despite the presence pests.

According to Seck [6], the use of improved resistant or tolerant varieties to insect development is the most popular method with low income farmers.

Therefore, there is today in Africa extension of several cowpea varieties from producers to reduce pest pressure in general and particularly the damage caused by weevils in both fields and storage structure.

It is therefore raises the question of the effects of these varieties in the offspring of the pest: In fact, the parameters of the offspring of a pest may be strongly influenced by the characteristics of the variety on which the pest development occurred.

To carry out this, the study is to compare: the morphometric parameters of adult *Bruchidius atrolineatus* from three (3) cowpea varieties, including a local variety and two improved varieties.

According Delobel[7], morphometric analysis is one that is easier to access, but it is also the largest.

MATERIALS AND METHODS

Studied populations of B atrolineatus.

During this work, the parent strain populations *Bruchidius atrolineatus* used comes from the seeds of a local variety of cowpea purchased in the 5th District of the City at Niamey market. Adults of *B. atrolineatus* emerging from these seeds were subsequently used to infect healthy seeds of each of three varieties namely a local variety and the two improved varieties TN5/78 and HTR). 48 hours after infection, the insects are removed and seeds bearing eggs are allowed to incubate in the laboratory until the emergence of adults and a sample of adult males is used for morphometric analysis.

The three samples consist of:

- A population of the local variety noted L
- A population of TN5-78 variety and noted T
- A population of HTR variety noted H.

For each population, the analysis focused on 30 individuals, for a total of 90 individuals examined during the study. The characteristics of the varieties used are shown in Table-1.

Table-1: Average size and color of seeds used

Varieties	Average size (cm)	Grain colour
Locale variety	0,76±0,89a	White
TN5/78	0,71±0,84b	Red
HTR	0,70±0,73b	White

Choice of the variables to measured and led operation.

We chose 17 measurable variables with a reasonable degree of accuracy. I1 primarily acts lengths of parts of the body of the insect. The observations were carried out on male individuals killed with alcohol 90°. The specimens are then washed in distilled water and dissected at once to proceed to measurements, each part being carefully separated from the close parts. It is about direct measurement on the insect thanks to a binocular magnifying glass and a graph paper. The 17 selected variables are distributed as follows:

The head is represented by four (4) variables which are:

- The Length of the antenna (Lan);
- The minimal distance enters the eyes ventralement (dvy);
- The minimal distance enters the eyes dorsalement (Lmf) and
- The maximum length of the head (Lmt).

The thorax is represented by nine (9) variables which are:

- The length of the pronotum in the center (Lop);
- The width of the pronotum (Lap);
- The greatest dimension of the femur3 (Lf3);
- The greatest dimension of tibia 3 (Lt3);
- The maximum length of the elytron (Loe);
- The maximum width of the elytron (Lae);
- The greatest dimension of the radial cell of the posterior wing (Loc);
- The maximum width of the radial cell (Lac) and
- The greatest dimension of the coxa 3 (Lmc).

The abdomen out as the head is also represented by four (4) variable which is:

- The maximum length of the abdominal first sternite (Los);
- The maximum width of the abdominal first sternite (Las);
- The maximum length of the pygidium (Lpy) and

- The maximum width of the pygidium (lpy).

Statistical analysis of data

The analyses were carried out thanks to software MINITAB 16 version 2013. The method used is the discriminating factorial analysis (AFD) which combines the variance analysis and analyzes in principal components (ACP)[8]. It makes it possible as well as possible to separate several groups using several variables by carrying out discrimination from the populations defined a priori but also to assign an individual whose origin is unknown with a given population. The analysis in principal components (ACP) is a mathematical method of data analysis which consists in searching the directions of space which represent best the correlations between random variables. Initially, the raw data were subjected to an analysis of the completely encased variances. This test makes it possible to note that the minimal distance between the eyes ventralement (dvy) and dorsalement (Lmf) do not present a better discrimination. The value of P applied to these two variables is higher than 0.05 and are consequently rejected. Discriminating factorial analysis at summer carried out on the 15 preserved variables. However, the use of these raw data to analyses like the AFD, even if it makes it possible to define a factor cuts then the form does not allow a good interpretation of the results. Indeed the variables not having a whole a good quality of representation in the plan having the strongest power of discrimination, the distribution of the individuals will be due only to phenotypical differences directly related to the respective mean sizes of the three populations. The data were thus transformed by using the logarithm of the relationship between each measurement and the maximum width of pygidium considered as variable of reference. Because significantly correlated compared to more the share of the other variables (multiple Coefficient of correlation for lpy is 0.99). This transformation was proposed by many authors [8] order to reduce the effect of the size.

RESULTS

Analysis of the raw data

The results shown in Figures 1 and 2 show a principal component analysis of the conserved variables. A group of variables allow distinguishing the population with the studied local variety. This is the maximum length of the antenna (Lan = 1.57 ± 0.19mm), the largest dimension of the femur 3 (= Lf3 1,01mm) and tibia 3 (= 0.81 ± Lt3 0.12 mm) , the largest radial dimension of the cell of the posterior wing (= 3.27 ± 0,46mm Loc), the maximum length of the elytron (Loe = 1.60 ± 0.17 mm) and the maximum length of pygidium (1.56 ± 0.27 mm LPY =). The population from HTR is characterized by a variable, length of the first abdominal sternum (Los) longer. The other eight variables allow to differentiate individuals from TN5 / 78. This is the maximum length of the head (Lmt), length (lop) and width (Lap) pronotum, the maximum width of the elytron (Lae), the maximum radial width of the cell (Lac), the largest dimension of the coxa 3 (Lmc), the width of the first abdominal sternum (Las) and the maximum width of pygidium (LPY).

The variables with the greatest overall discriminating power are by decreasing order of F: Lap (F = 74.494), Loc (F = 64.644) Lf3 (F = 49.113), Lmt (F = 45.691) Lt3 (F = 25.794), Lake (F = 16.273), Lan (F = 13.691) LPY (F = 13.102), Lop (F = 11.903), Lae (F = 7.940), Loe (F = 6.234), Lmc (F = 5.800) Las (F = 5.800), LPY (F = 5.800) and Los (F = 4.374). The probability of erroneous classification of these variables is less than 0.001%. Examination of multiple correlations between variables shows that the variable Lpy (maximum width of pygidium) is the best correlated with all other variables (multiple correlation coefficient is 0.99).

Axis 1 (Horizontal) does not allow a better separation of different populations or separating the TN5/78 population to that of HTR. Axis 2 (vertical) allows to separates better the population of local variety to that of HTR. Overall individuals from the local variety are larger and followed by those of TN5/78 and HTR. Mahalannobis distance (distance between the center of gravity) between strains is as follows:

Locale Variety _TN5/78 (0,952)
Locale Variety _HTR (0.948)
TN /78_HTR (1.708).

Analysis of transformed data

The results in Figure 3 and Figure 4 show the principal component analysis (PCA) transformed data. Examination of F shows that the discriminating power of some new variables has increased significantly. This is the case of the maximum length of the head (Lmt) (F = 56.588), the width of pronotum (Lap) (F = 78.487), the largest dimension of the femur 3 (Lf3) (F = 57.216) and the length of pygidium (Lpy) (F = 13.66).

Though the discriminating power of most new variables is lower than it was previously. This is the length of the antenna (Lan) (F = 13.298) of the maximum length of pronotum (Lop) (F = 11.410), the largest dimension of tibia 3 (Lt3) (F = 21.254), the maximum length of the elytron (Loe) (F = 4.651), the width of the elytron (Lae) (F = 6.988), the maximum radial length of the rear cell of the wing (Loc) (F = 48.692), the maximum radial width of the cell from the rear wing (Lac) (F = 14.343) , the largest dimension of the coxa 3 (Lmc) (F = 5.644), and the length of first abdominal sternum (Los) (F = 3.110).

The correlation examination between the variable and the discriminant axis shows one horizontal axis (Figure 3) does not allow a better differentiation between the various the different populations. On the other hand, the vertical axis 2 separates the best from

the local variety population to that of HTR variety. The analysis of these new variables (Figure 4) shows that the maximum length of the wing wchich was in the group of variables differiciating the population of the local variety is ranged in those with characteristics of the population with HTR. The population of HTR is thus distinguished by two variables that are: The maximum length of the scissor and the length of the first abdominal sternum.

The population of the local variety is characterized with these transformed data by five (5) variables instead of the previous six. These five variables are: The maximum length of the antenna (Lan), the largest dimension of the femur 3 (Lf3) 3 and tibia (Lt3), the largest radial dimension of the cell of the posterior flange (Loc) and the maximum length of pygidium (LPY).

The population from TN5 / 78 is distinguished as above by eight variables, which some of them have improved their discriminating power. The rate of ranks well is less than previously because of it was observed TN5 / 78 individual which is placed in the group of the population with the local variety. The Mahalannobis distances between strains obtained with these new data have increased dramatically. These distances are as follows:

Variety locale_TN5 / 78 (0.961)
Variety locale_HTR (1.85)
TN5 / 78_HTR (2,492).

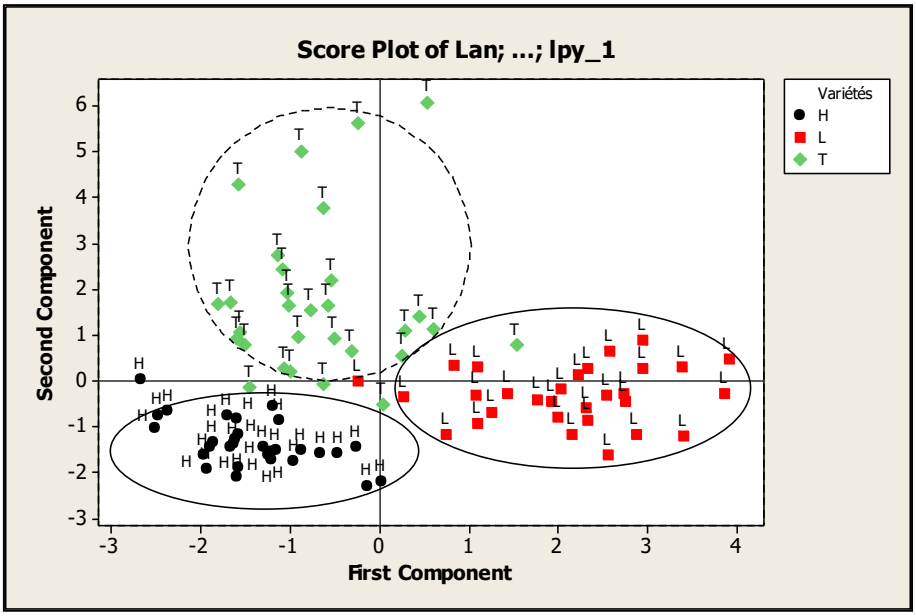

Fig-1: Chart of the individuals (Given rough): L: Populations male of *B. atrolineatus* resulting from the local variety; T: Population resulting from TN5/78 and H: Populations resulting from HTR

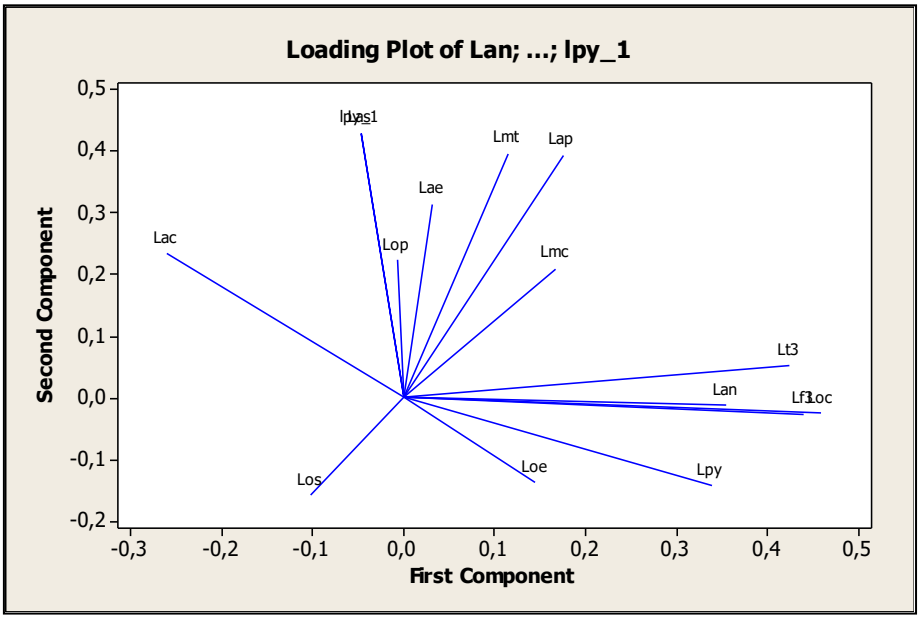

Fig- 2: Chart of the measured variables (variable rough)

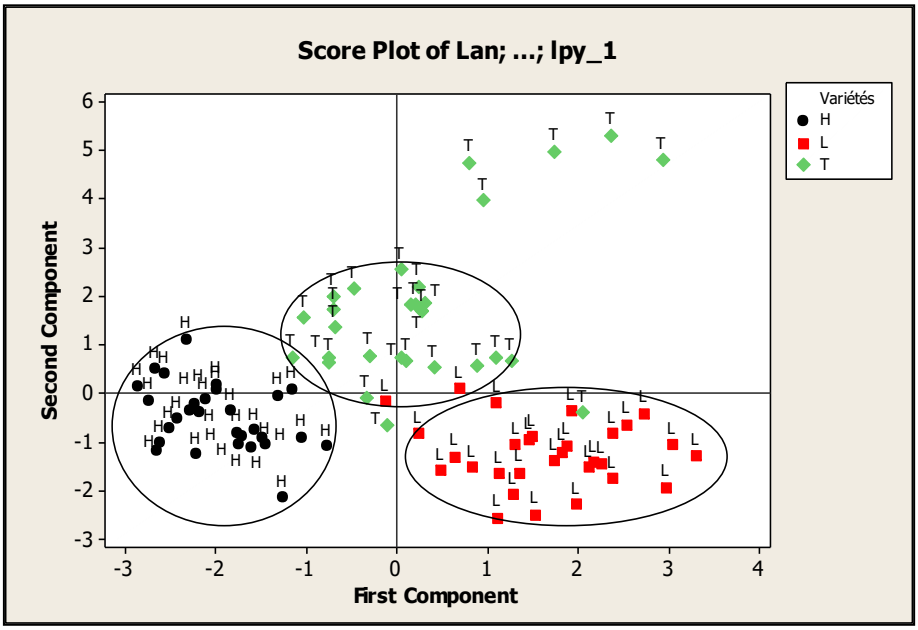

Fig-3: Chart of the individuals (Given transformed): L: Populations male of *B. atrolineatus* resulting from the local variety; T: Population resulting from TN5/78 and H: Populations resulting from HTR

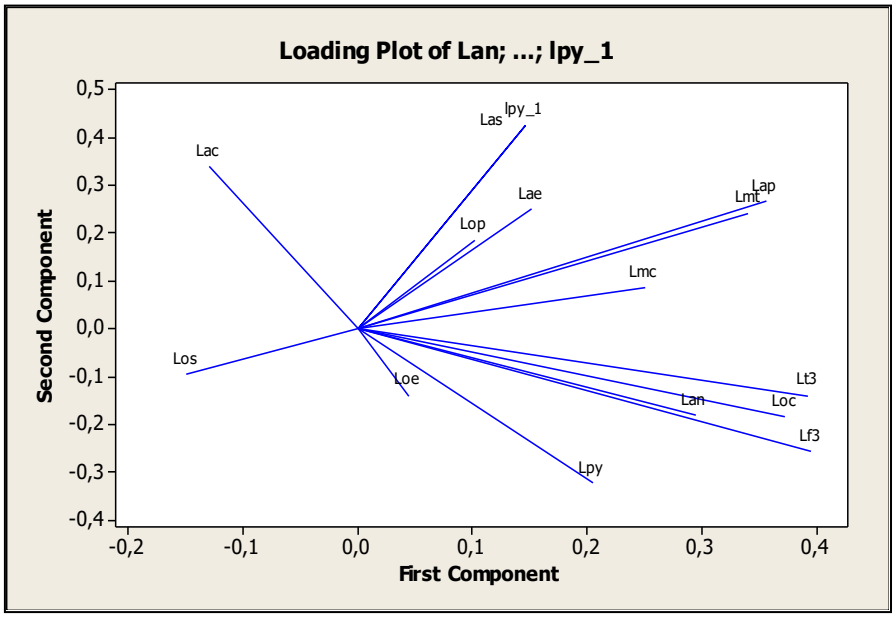

Fig- 4: Chart of the measured variables (Variable transforms)

DISCUSSION

The discriminant analysis performed on the raw data and the processed data show that the three populations are clearly differentiated. However, as revealed by examining the distances between Mahalannobis center of gravity, the two populations of improved varieties differ from each other is less good than the population of the local variety. These are first of all, as revealed by the comparison of analyzed of the raw data, factors associated with size that distinguish the three populations. In general, the outcome of the local variety population is the largest, followed by the population of TN5 / 78.

The population with HTR was the smallest. Specifically, the population of the local variety is characterized by its longer antennae (1.57 mm against 1.37 and 1.49 respectively TN5/78 and HTR), the largest dimension of the femur 3 (Lf3 = 1 01mm against 0.91 and 0.98 respectively TN5 / 78 and HTR) and tibia 3 (± 0.12 mm Lt3 = 0.81 against 0.78 on TN5 / 78 and 0.81 on HTR) longer, the longest dimension of the radial wing of the rear cell (Loc = 3.27 ± 3.13 on TN5 0,46mm against / on HTR 78 and 3.04) longer, the maximum length of the elytron (Loe = 0.17 mm ± 1.62 against 1.60 and 1.39 respectively on TN5 / HTR and 78) and the longer maximum length of pygidium (LPY = 1.56 ± 1.09 against 0.27 mm on TN5 / 78 and 1.44

HTR) longer. The population of HTR, the smallest, is distinguished by the length of the first abdominal sternite (0.65 mm against 0.44 and 0.36 mm respectively on local variety and TN5/78).

The size factor is involved in the differentiation between populations, but it is far from alone. Indeed, analysis of the transformed data shows that once the size factor eliminated, four main characters allow differentiating three populations studied: Lmt, Lap, Lf3 and LPY. The transformation modified the data. This is consistent with the work of Yasui cited by Ouedraogo [3] by a comparative study of the form and not sailer aviary *Callosobruchus maculatus*. According to these authors, the criterion size cannot be only taken into account to differentiate the two forms. It is too variable and depends on the embryonic conditions development and post-embryonic, including those related to intraspecific competition and the water content of seeds. Eliminating size factor results in lower overall discrimination between the three populations, even if the distance between the gravity centers of the three populations increased slightly.

Factors involved to differentiate the size and shape were studied on *Caryedon serratus* in Senegal by Sembène et al. [10]. According to these authors, discrimination observed between populations of *Caryedon serratus* after several host plants in Senegal was linked no doubt to the nutritional quality of these hosts. Indeed, there is no direct relationship between the size of the weevils and seed of TN5/78 and HTR because statistically identical. However, the effect of the amount of nutritive material of the seed on the size of the cowpea weevil should not be neglected since it is observed that adults *Bruchidius atrolineatus* with larger sizes are obtained from the seeds of the local variety which has a significantly larger average size compared to other varieties. Other factors may be involved in this discrimination as the moisture, texture or smell, seed hardness etc. The idea also of larval density on seed resulting in the miniaturization of adult emergence in *Bruchidius atrolineatus* [11] should not be overlooked here, because adults used in this study were randomly selected during rearing and therefore several individuals can emerged from a single seed.

CONCLUSION

The results obtained in this study clearly show that the *Bruchidius atrolineatus* populations from these three varieties are clearly differentiated by their size, even if it is not the only discriminating factor. After data transformation by a reference variable (LPY), the form of the insect is not to be overlooked because four variables have improved their discriminating power and allow the insect to differentiate the three populations based on the form. The 17 variables used are far from the maximum number of variables that have an insect. Therefore a larger number of variables can provide more information on morphometric characteristics of the insect. Finally, the continuation of a genetic study of these results can provide more information about the characteristics of these three populations

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