

Morphometric Characterization of the Mango Tree's Mealy Cochineal, *Rastrococcus invadens*, on the Mango Tree in Senegal

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Abstract: Fruit production in Senegal is mainly for national consumption. The major critical point of mangoes and citrus fruits production is fruit ravaging insects such as *Rastrococcus invadens* (Homoptera, Pseudococcidae) (Williams, 1986). Originated in Southeast Asia, this cochineal was first identified in Senegal. Since then it has spread throughout this country and particularly in Natural Casamance region and Thies region. In each region we chose two farms in two different localities. In Casamance, we chose one farm in Diatock locality and another one in the locality of Oussouye. In Thies region, we sampled in Santhie and Khay localities. In each farm, we chose the mango tree which represents the major host plant of *R. invadens*. From each plant we collected 10 specimens. This enabled us to get 20 specimens from Casamance and 20 other specimens from Thies. Specimens were coded with regard to both the area and the type of plant they were collected from. The present work aims to take stock of the morphological and morphometric characteristics of the pest into space and time, as well as its socio-economic consequences since it has been reported in Senegal. The results revealed morphometric groups more or less distinct especially between the Niayes zone and low Casamance.

Keywords: *Rastrococcus invadens*, mango tree, morphometry, agro-ecological zones

1. Introduction

Fruit production in Senegal is mainly for national consumption. This production would reach 100 to 120 000 tonnes per year and concerns mangoes, citrus fruits, and bananas in proportion of 67; 23; and 5% respectively [1]. Nevertheless, the lack of statistics does not allow us to point at these figures as reliable values [1].

Currently, the major critical point of mangoes and citrus fruits production is fruit ravaging insects such as *Rastrococcus invadens* [2]. The mango tree's mealy cochineal has become pandemic and represents an agronomic, economic, social, and biodiversity threat.

Exports of mangoes account for only 5 to 6% of national production [3].

Although the small volume exported with respect to production, Senegal, over the last 10 years, has significantly increased his foreign trade to Europe especially, reaching, on average 5,000 to 6,000 tons per year, despite the 50% fall in 2010 (around 3,000 tons) due to the quality mangoes as well as the attacks of insect pests that compromised the marketing campaign [3].

In Senegal, since 1981 (first exports in the French market), fruit production has become the third agricultural source of

income in southern Senegal, behind cotton and cashew nut [4]. In fact, from 71 tons exported in 1981), Ivory Coast exports yearly more than 10 000 tonnes since 1999 [5] for an estimated annual production of 100 000 tonnes [6]. However, mangoes production is threatened by phytosanitary problems, of which the most important are fruit flies, belonging to the Tephritidae family, and the mealy cochineal, *Rastrococcus invadens* Williams (Homoptera: Pseudococcidae) [7]. The latter was accidentally introduced in Africa in the early 1980s from the Southeast Asia, its area of origin [2].

The mealy cochineal was first observed in Togo and Ghana before spreading in most of West African countries, damaging mango trees and other fruit trees [8].

Its appearance was reported in Ivory Coast, in 1989 [9]. Highly polyphagous, *R. invadens* quickly became one of the major enemies of mango trees and several other fruit trees including citrus trees and various ornamental and shade plants.

R. invadens is a species of bisexual and ovoviviparous cochineal that lives in colonies on the leaves of host plants [10]. On mango trees, mealy cochineal can also be found on leaves' petioles, fruits and peduncles [10].

Various studies have been carried out in order to set up an integrated pest management program. Morphometric data are useful additional information that enables an accurate

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identification of the different stages of development of an insect [11].

The present work aims to take stock of the morphological and morphometric characteristics of the pest into space and time, as well as its socio-economic consequences since it has been reported in Senegal.

2. Material and methods

2.1 Sampling

Two populations of *R. invadens* [2] were compared during this analysis: one population from Natural Casamance region and another from Thies region. In each region we chose two farms in two different localities. In Casamance, we chose one farm in Diatock locality and another one in the locality of Oussouye. In Thies region, we sampled in Santhie and Khay localities.

In each farm, we chose the mango tree which represents the major host plant of *R. invadens*.

From each plant we collected 10 specimens. This enabled us to get 20 specimens from Casamance and 20 other specimens from Thies. Specimens were coded with regard to both the area and the type of plant they were collected from. Data are clustered in Table 1 below. In general, morphometric analysis of *R. invadens* larvae is usually destructive. Actually, it requires prior death of the specimens and their fixation in alcohol [12].

2.2 Morphometric study

We chose 10 measurable variables with a reasonable degree of accuracy. These mainly include body length, body width, length and width of head, length of rear, median, and front legs. To these are added lengths of abdomen, thorax, and average diameter of sternum (Figure 1).

Table 1: Choice of values

Body	Head	Thorax	Abdomen
Lc :Body length	LT : Head length	Th : Thorax length	La :length of abdomen
lc : body width	lt : Head width	Lp1 : length of the first pair of legs	Ls1 : length of first sternum
		Lp2 : length of the second pair of legs	
		Lp3 : length of the third pair of legs	

The relevant parts were mounted on a binocular stereoscope equipped with a camera connected to a computer. Observations were made on L3 (3rd larval stage) specimens which correspond to pre-pupa for males. Specimens are then cleaned in alcohol 70 before proceeding to measurements, each relevant part being carefully separated from the next one.

Each specimen of a given sample is associated to a code, using the capital letter of the gender name followed by the first letter of the specific epithet of the corresponding plant, the first letter of the locality of origin and finally the first letter of the region of origin (Table 2).

Table 2: Summary table of the sampling

Codes	Plant species	Localities	Number of specimens	Regions
CDM	Mango tree	Diatock	10	Casamance
COM	Mango tree	Oussouye	10	Casamance
TSM	Mango tree	Santhie	10	Thies
TKM	Mango tree	Khay	10	Thies

CDM: Casamance-Diatock-Mango tree **COM:** Casamance-Oussouye-Mango tree **TSM:** Thies-Santhie-Mango tree **TKM:** Thies-Khay-Mango tree.

2.3 Statistical analyses

2.3.1 Raw measurements

A discriminant factor analysis (DFA) of populations with raw measurements of variables in regard to the sampled agro-ecological zones was carried out with the help of the software R version 3.2.3 [13]. This analysis enables to set off the contribution of each variable with respect to their bald measurement, in order to see the most discriminating ones, and to bring out morphometric groups regarding the agro-ecological zones, too.

2.3.2 Converted measurements

Size effect

According to [14] size effect appears by a circle of correlation that groups all the variables in a single plane for a given axis. This concerns a very undesirable effect which metrical studies try to overcome. The principle of elimination is then to bring all specimens to the same size so as to observe on the PCA only differences in shape.

Data conversion

Eliminating the size effect that affects almost all biometric studies was done using the following approach by Santos (2015):

- Log-transformation of data: initial data table consists of the variables X1, X2, ..., Xp, subsequently a new data table consisting of the variables log (X1), log (X2), ..., log (Xp) has been created.
- For each specimen, the average over all Log-transformed variables was calculated. This average score is a good idea of the "size" for this specimen.
- Finally, for each specimen, the average size obtained with Log-transforms was deducted from each of these raw measurements.

Size effect is thus eliminated and only the difference in shape will be observed on the PCA. Decreasing the weight of this factor (Size) results in a decrease of the global discrimination between populations and the reduction of the distance between centres of gravity of populations. This transformation was performed in Excel version 2011.

2.3.2.1 Discriminant Factor Analysis (DFA)

A discriminant factor analysis (DAF) of the populations with the transformed data of the variables according to the localities and host plants sampled was carried out with the software R version 3.2.3 [13]. This analysis aims to bring out the contribution of each variable after elimination of the size effect in order to see the differences in form between

population groups revealed by the discriminating power of each of the variables.

2.3.2.2 Correspondence Factor Analysis (CFA)

Factorial Correspondence Analysis (CFA) is performed to visualize relationships between specimens of different populations from converted data and test for possible metric similarities between these populations. It is a multi-varied analysis method that considers converted measurements of all populations as variables [15]. For this purpose, a graphical representation is produced from the transformed data that are adapted using the Genetix version 4.05.2 program [16] to estimate the distribution of morphological diversity at all levels (individuals, populations and total population).

2.3.2.3 Confusion matrix for cross-validation results

The confusion matrix summarizes the reclassifications of specimens to infer both rates of good and bad ranking. This makes it possible to determine the "correct%" which is the ratio of the number of well-ranked specimens to the total number of specimens. Cross-validation is done using transformed data according to agro-ecological zones and plant species sampled with the XLSTAT software version 2016.03.30882 [17].

2.3.2.4 Hierarchical Ascending Classification (CAH)

It represents a method of trees construction that is often delicate and difficult to generalize if the learning data are poorly representative of reality. Automatic classification methods that do not require learning are of great interest when data are completely unknown. They thus make it possible to release subsequent classes that are not obvious a priori. Therefore, the CAH consists of grouping specimens regarding their resemblance or difference. The ascending hierarchical classification is carried out in Excel version 2011.

3. Results

3.1 Raw Data

3.1.1 Contribution of the variables with raw measurements in terms of the localities of origin of specimens collected from mango tree

The discriminant factor analysis obtained from specimens collected from mango tree shows that the first two factorial axes (dimension) best explain the morphometric variability with 87.74% of inertia power. Following the factorial axis 1 (dimension 1), we find that variables such as Lc (F1 = 13.1), lc (F1 = 12.3), LT (F1 = 11.8), lt (F1 = 11.3), (F1 = 11.3), Lp2 (F1 = 9.69), Lp3 (F1 = 9.67), Lp1 (F1 = 8.86), and Ls1 (F1 = 8.24) have largely participated in the construction of the first factorial axis with 69.17% of the power of inertia. Only the variable Th (F1 = 3.66) contributes slightly to the construction of the first axis. The factorial axis 2 (dimension 2), with a low power of inertia (18,57) is constructed largely by the variables Th (F2 = 30.4), Lp1 (F2 = 15.3), Lp2 (F2 = 15.3), Lp3 (F2 = 14.1), Ls1 (F2 = 10.9) and It (F2 = 8.01). Other variables such as Lc (F2 = 4.37), La (F2 = 0.72), lc (F2 = 0.57) and LT (F2 = 0.17) contribute little to the construction of this axis. On the first factorial axis, all the

variables are positively correlated. Obviously, the size effect affects our ACP. (figure1) A globally positive correlation for the variables, along the factorial axis of dimension 1, seems to suggest an influence of the data by the "size effect".

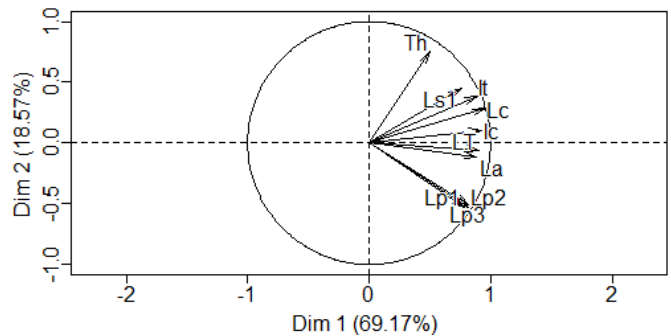


Figure 1: Contribution of mango tree populations' variables

3.1.2 Discrimination of populations according to raw measurements according to the localities of individuals from the mango tree

Following the two factorial axes with a power of inertia of 87.74%, the AFD (Discriminant Factor Analysis) reveals two groups. A group consisting of the populations of Santhie and Khay and another group composed by that of Diatock and Oussouye with a zone of significant introgression between the two populations. Khay specimens have some resemblance to those from Diatock.

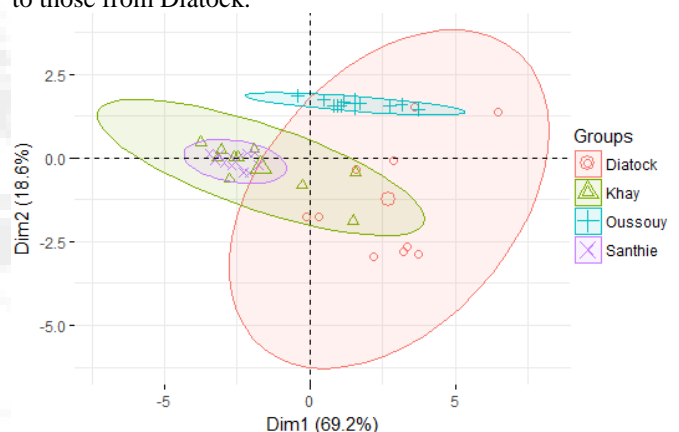


Figure 2: Representation in the main AFD plan of the populations of *Rastrococcus invadens* on the mango tree

3.1.3 Variables allowing the discrimination of mango tree populations

In regard to the Tukey test, among the 10 variables studied, except the length of thorax (Th), all the other variables make it possible to differentiate Diatock and Khay populations. Between Diatock and Santhie, except for the body width (lt) and the length of the thorax (Th), all the variables make it possible to discriminate between these two populations. Between Oussouye and Khay, the differentiation noted is due to variables such as body length (Lc), body width (lc), head length (Lt), head width (lt) and length of the first sternum (Ls1). Between Oussouye and Santhie, variables such as body length (Lc), body width (lc), head length (Lt), length of the abdomen (La), thoracic length (Th) and length of the first sternum (Ls1) discriminate their populations.

Table 3: Morphometric study of specimens (*Rastrococcus invadens*) collected from mango tree

localities variables	Diatock	Khay	Oussouye	Santhie
Lc	3.95±0.6 ^b	2.86±0.43 ^a	4.12±0.33 ^b	2.67±0.15 ^a
lc	2.47±0.57 ^b	1.75±0.31 ^a	2.28±0.35 ^b	1.54±0.14 ^a
Lt	1.09±0.18 ^b	0.64±0.20 ^a	1.05±0.86 ^b	0.56±0.07 ^a
lt	1.72±0.41 ^b	1.09±0.21 ^a	2.01±0.13 ^b	1.02±0.08 ^b
La	2.26±0.22 ^c	1.91±0.40 ^{ab}	2.06±0.25 ^{bc}	1.64±0.13 ^a
Lth	0.61±0.46 ^a	0.38±0.23 ^a	1.01±0.04 ^b	0.46±0.14 ^a
Lp1	1.57±0.29 ^b	0.89±0.14 ^a	1.08±0.07 ^a	0.91±0.06 ^a
Lp2	1.68±0.24 ^b	1.06±0.26 ^a	1.14±0.12 ^a	0.98±0.09 ^a
Lp3	1.74±0.24 ^b	1.17±0.33 ^a	1.20±0.16 ^a	1.04±0.09 ^a
Ls1	0.37±0.12 ^{bc}	0.30±0.04 ^{ab}	0.41±0.04 ^c	0.27±0.03 ^a

3.2 Converted data

3.2.1 Contribution of the variables with converted measurements in terms of the localities of origin of specimens collected from mango tree

Unlike raw data, the factor analysis with the converted data shows a reduction of the inertia percentage of 14.87% for the first dimension (factorial axis 1) following a decrease in the discriminating power of most of contributory variables namely : LT (F1 = 13.4), F1 (F1 = 13.2), Lc (F1 = 12.1), Lp2 (F1 = 12.1), Lp3 (F1 = 11.7), Lp1 (F1 = 10.8), La (F1 = 10.7), F1 (F1 = 9.15) and Ls1 (F1 = 6.79).

The second factorial axis with a very noticeable decrease (6.33%), shows a situation almost identical, compared to the results with the raw data, with an increase in the discriminating power of almost all the variables and a significant contribution of some variables such as Th (F2 = 29.6), lt (F2 = 18.5), Lc (F2 = 12.4), Lp1 (F2 = 11.5), Lp2 (F2 = 11.2) and Lp3 (F2 = 10.0).

The best quality of representation is always obtained with the plane formed by axis 1 and 2 with a total inertia percentage of 79.2%.

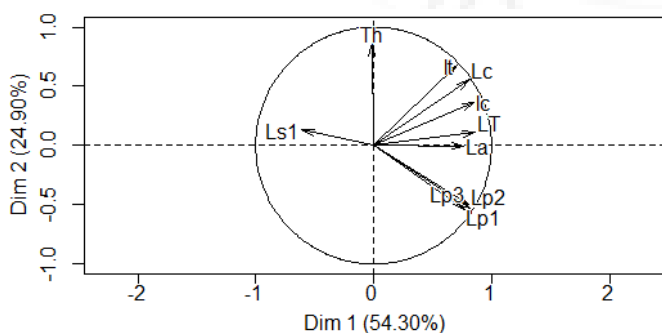


Figure 3: Contribution of mango tree population variables

3.2.2 Discrimination of the populations, according to the converted measurements, in terms of the localities of origin of specimens collected from mango tree

With the converted measurements, according to the two factorial axes, the localities show a strong discrimination. Thus with an increase in the percentage of inertia, the first dimension allows a discrimination of the populations of Khay, Diatock and Oussouye. On the other hand, the second dimension reveals discrimination between the populations of

Oussouye, those of Khay and Santhie, and between the population of Santhie and that of Diatock

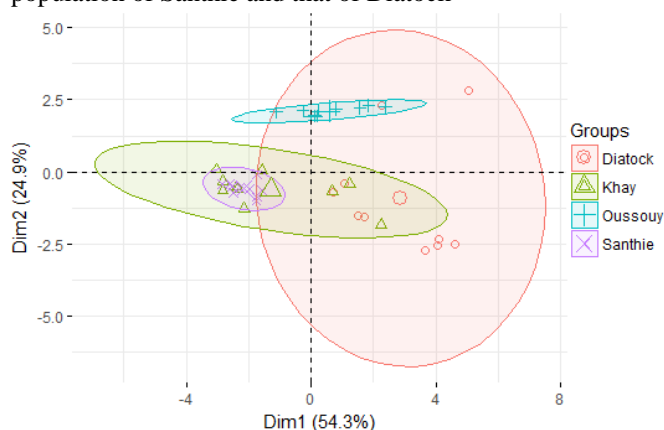


Figure 4: Representation in the main AFD plan of *Rastrococcus invadens* populations on the mango tree

3.2.3 Variables allowing the discrimination of mango tree populations

According to the Tukey test, among the 10 variables studied, except the length of the thorax (Th), all the other variables make it possible to differentiate the Diatock and Khay populations. Between Diatock and Santhie, except for the width of the body (lt) and the length of the thorax (Th), all the variables make it possible to discriminate between these two populations. Between Oussouye and khay, the differentiation noted is due to variables such as body length (Lc), body width (lc), head length (Lt), head width (lt) and length of the first sternum (Ls1). Between Oussouye and Santhie, variables such as body length (Lc), body width (lc), head length (Lt), length of the abdomen (La), thoracic length (Th) and length of the first sternum (Ls1) discriminate their population.

Table 4: Variables allowing the discrimination of mango populations (converted data)

	Casamance	Thies	Total	% correct
Casamance	20	0	20	100.00%
Thies	0	20	20	100.00%
Total	20	20	40	100.00%

3.2.4 Confusion matrix for the results of cross-validation of populations

The confusion matrix summarizes reclassifications of specimens to infer the rates of good and bad ranking. This makes it possible to determine the "correct%" which is the ratio of the number of well-ranked specimens to the total number of specimens. Thus, specimens from different populations are well ranked in their original populations.

Table 5: Confusing mastery of cross-validation of specimens from mango tree

localities variables	Diatock	Khay	Oussouye	Santhie
Lc	3.82±0.53 ^b	2.89±0.39 ^a	3.98±0.30 ^b	2.71±0.12 ^a
lc	2.32±0.52 ^c	1.77±0.26 ^{ab}	2.14±0.31 ^{bc}	1.57±0.13 ^a
Lt	0.95±0.19 ^b	0.67±0.16 ^a	0.91±0.05 ^b	0.60±0.07 ^a
lt	1.57±0.35 ^b	1.12±0.17 ^a	1.87±0.09 ^c	1.06±0.07 ^a
La	2.11±0.17 ^b	1.94±0.36 ^{ab}	1.91±0.22 ^{ab}	1.67±0.12 ^a
Lth	0.47±0.41 ^a	0.41±0.21 ^a	0.86±0.05 ^b	0.50±0.13 ^a
Lp1	1.43±0.28 ^b	0.91±0.10 ^a	0.94±0.04 ^a	0.95±0.04 ^a
Lp2	1.54±0.22 ^b	1.09±0.22 ^a	1.01±0.09 ^a	1.02±0.06 ^a
Lp3	1.60±0.21 ^b	1.20±0.28 ^a	1.06±0.12 ^a	1.08±0.07 ^a
Ls1	0.23±0.08 ^a	0.33±0.04 ^b	0.28±0.01 ^{ab}	0.31±0.03 ^b

3.3 Hierarchical Ascending Classification (HAC)

The hierarchical ascending classification brings out several morphometric groups based on similarities, from variables. On the mango tree 3 groups were noted: a group where we find the populations of Diatock and Oussouye, a group made up only of Diatock populations and a third group including populations of Diatock, Khay and Santhie. The population of Oussouye is only found in one group; which shows then that it is a homogeneous population. This same result is also observed in the Khay and Santhie populations. On the other hand, the population of Diatock is very heterogeneous because it is found in all groups.

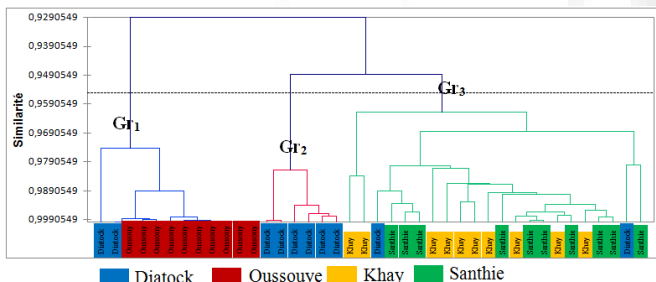


Figure 5: Ascending classification of specimens from the mango tree
 Gr1: group 1 Gr2: group 2 Gr3: group 3

3.4 Correspondence factor analysis (CFA)

The discriminant factorial analysis reveals that the first five factorial axes explain all the morphometric variability of this cochineal. However, the plan formed by the first three axes best explains the discriminative situation of agro-ecological zones with an inertia of 99.6%. The first factorial axis with an inertia of 36.39 %, discriminates the group formed in majority by the individuals of Diatock (Casamance). The second factorial axis with inertia of the order of 34.39% discriminates the groups containing all the specimens from Khay. The third factorial axis with an inertia of 28.82%, allows the discrimination of the group which contains all the specimens from Santhie and Oussouye.

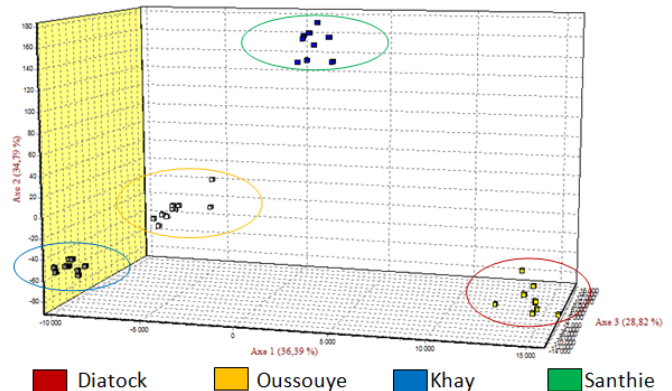


Figure 6: Simulation of the morphometric variability of specimens of *R. invadens* on mango tree following the first three axes of the AFC

4. Discussion

Morphometric measurements carried out on *R. invadens* specimens show various values. For most variables, measurements vary more or less according to a certain average. However, variables such as thoracic length (Th) and length of first sternum (Ls1) show consistent values in all specimens. These two variables do not allow to discriminate specimens from different populations. Thus, out of the 10 variables chosen, only 8 variables (Lc, lc, Lt, lt, La, Lp1, Lp2 and Lp3) are used to obtain relevant information on the morphometric variability of the species.

The first two factorial axes explain 87.74% of the morphometric variability. Following these same factorial axes, a slight discrimination is observed between 2 groups: specimens of Diatock and Oussouye on the one hand and Khay and Santhie on the other hand. In fact, populations from these different agro-ecological zones do not seem to have a great deal of difference at first glance, because of the proximity of their cloud of points, which could suggest a large gene flow between the different populations. But a more in-depth study of the centroids representative of each population has shown a slight discrimination. However, an apparent correlation of most variables is largely due to a common factor that can be assimilated as a first approximation to a size factor.

Decreasing the weight of this factor results in a slight decrease in overall discrimination between agro-ecological zones, and a slight increase in the distance between centroids. Thus converted data lead to a reduction of the inertia percentage of 14.87%, for the first dimension (factorial axis 1) and 6.33% for the second factorial axis. This offers a better redistribution of variables in relation to their contribution to the axis.

With the converted data, along the factorial axis 1 the first dimension allows a discrimination of the populations of Khay, Diatock and Oussouye. On the other hand, the second dimension reveals a discrimination between the populations of Oussouye, those of Khay and Santhie, and between the population of Santhie and that of Diatock. Correspondence factor analysis shows that the first five factorial axes explain

all the morphometric variability of this cochineal and give a better insight into the discrimination between ecotypes.

The plan formed by the first three axes best explains the discriminative situation of agro-ecological zones with an inertia of 99.6%. The first factorial axis with an inertia of 36.39%, discriminates the group formed in majority by specimens of Diatock (Casamance). The second factorial axis with an inertia of the order of 34.39%, discriminates the groups containing all specimens of Khay. The third factorial axis, with an inertia of 28.82%, allows the discrimination of the group which contains all specimens of Santhie and Oussouye.

From these results we can say that some specimens belonging to a previously defined area have more similarities with other specimens from neighboring agro-ecological zones: this is the case of Santhie and Khay specimens who are all in the same agro-ecological zone but also with specimens from lesser agro-ecological zones like Diatock and those from Thies (Khay and Santhie).

The distance seems to correspond to a discriminative criterion, and thus intervenes in the variation of the morphology of the species and climatic conditions. Apart from this appearance of homogeneity, Khay is revealed as the most homogeneous population while Diatock is the most heterogeneous. This could lead to consider Niayes area as the focus of the infestation [18]. The agro-ecological zone is thus at the origin of this difference in size according to the [19] and development performance of insects is strongly influenced by the nutritional quality [20].

With respect to the agro-ecological zones, we can consider that specimens are more homogeneous in Thies than in Casamance. The morphological homogeneity of the intra-group specimens of Thies is explained by the fact that the plants in Niayes zone constitute the primary speculation of *R. invadens* [18].

Results with the ascending hierarchical classification show 3 groups: a group where we find the populations of Diatock and Oussouye, a group made up only of the populations of Diatock and a third group comprising at the same time populations of Diatock, Khay and Santhie. The population of Oussouye is only found in one group; which shows then that it is a homogeneous population. This result is observed in the Khay and Santhie populations too. On the other hand, the population from Diatock is very heterogeneous because it is found in all groups. This shows that the infestation is more accentuated in Casamance than in Thies [21], knowing that the development cycle of *R. invadens* can be influenced by the availability of food; adult males being long-flight specimens migrate from infested areas in search of new food resources [22], when mango tree is inaccessible or stops production.

5. Conclusion

The study of the morphometric characterization of *Rastrococcus invadens* populations aims to verify whether the distribution of the insect has an impact on its

morphology. It revealed morphometric groups more or less distinct especially between the Niayes zone and low Casamance. However, additional studies are needed to understand what is behind the existence of these more sub-distinct groups. A genetic study is also needed to see whether the morphometric differences detected between groups are observable at the molecular level by using mitochondrial genes that are little variable genes.

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