(RESEARCH ARTICLE)

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Morphometric characterization of *Rastrococcus invadens* on four varieties of mango trees (Pout, Thies)

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Abstract

Rastrococcus invadens (Homoptera, Pseudococcidae) or mealybug is an insect pest of mango and several other fruit trees including citrus. The cochineal is native to Southeast Asia and was first identified in Senegal in Dakar in 1995. Since then, it has been widely distributed throughout the country and more particularly in the two most fruit-producing areas: Casamance and Thies. To properly conduct our study, we chose the four most infested mango varieties namely Kent, Keitt, Sewe and Boukodiekhal. All these populations are from the locality of Pout (Thies region). On each plant we collected 10 people. This allowed us to have a total of 40 individuals. The individuals harvested are coded according to the zone and the type of plant in which they were harvested.

This study revealed almost two more or less distinct morphometric groups made up on the one hand by populations from improved varieties (Kent and Keitt) and on the other by those from local varieties (Sewe and Boukodiekhal).

Keywords: *Rastrococcus invadens*; Mango tree varieties; Morphometric characterization; Niayes area

1. Introduction

The mango tree (*Mangifera indica*) is a flowering and fruiting plant (spermaphyte). It belongs to the Dicotyledonous class, the Sapindales order and the *Anacardiaceae* family. It is native to northern India at the foot of the Himalayan range [1].

It is a tree with a large, rounded, dense, spreading crown that can reach 30 m in height with a well-individualized monopod trunk [2]. It has a pivoting root system with some ramifications for a good anchoring to the ground well adapted to the search for water table under conditions of water stress [3]. In Senegal, the mango has been identified as one of the horticultural sector value chains with interesting potential on the American, European and sub-regional markets [4]. Indeed there are several varieties among which one can quote: kent, keitt, sewe, boukodiekhal, mango papaya. Studies have shown that the first four varieties are the most attacked by *R. invadens* [5]. The objective of this

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work is to take stock of the morphological and morphometric characters of the pest of fruit plants in a single area. agro ecological on the four varieties of mango trees most attacked.

2. Material and methods

2.1. Presentation of mango varieties

In Senegal, the mango has been identified as one of the value chains of the horticultural sector with interesting potential on the American, European and sub-regional markets [4]. Indeed there are several varieties among which one can quote: kent, keitt, sewe, boukodiekhal, mango papaya. Our study focuses on four varieties most attacked by *R. invadens*, namely: The Kent variety, which represents more than 70% of exported mangoes, produces a large fruit with a purplish-red exterior color with a tender and juicy orange flesh, without fiber. Its taste quality is excellent. It resists well to maritime transport; The Keitt variety is the second variety exported by the countries of the sub-region. Later, of a pinkish-red color, it has a firm yellow flesh, without fibers, but its skin is thin and fragile, sensitive to handling. However, its slow maturation process makes it suitable for maritime transport. The Boukodiekhal variety is a local variety generally not appreciated for export. It has a green color, becoming slightly orange, with orange flesh, and presents fibers with very good taste quality; The Sewe variety is also a local variety. It presents fruits of very small size and yellow in color when ripe with a sweet taste but very rich in fiber and not exported to the international market.

2.2. Presentation of the insect

Rastrococcus invadens is an insect native to Southeast Asia (Figures 1). It belongs to the phylum Arthropods, to the class of Insects, to the order of Orthoptera, to the superfamily Coccoidea and to the family of Pseudococcidae.
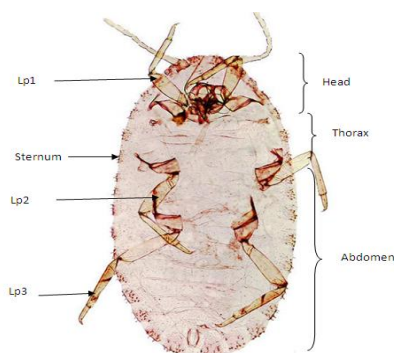

Figure 1 *Rastrococcus invadens* (5)

2.3. Sampling

The work took place between May and June 2020 on a farm located in the town of Pout.

Four populations of *R. invadens* [6] were compared in this study: a population originating from the Kent variety, one from Keitt, one from Sewe and another from Boukodiekhal. All these populations are from the locality of Pout (region of Thies).

Table 1 Summary table of sampling.

Codes	Plant species	Localities	Number of individuals	Regions
TPVk	Kent	Pout	10	Thies
TPVki	Keit	Pout	10	Thies
TPVs	Séwé	Pout	10	Thies
TPVkb	Boukodiekhal	Pout	10	Thies

TPVk:Thiés-PoutVarietékent ;TPVki :Thiés-Pout Varietékeit ; TPVs:Thiés-PoutVarietéSéwé ;TPVb:Thiés-PoutVarietéboukodiékhal.

On each plant we collected 10 individuals. This allowed us to have a total of 40 individuals. The individuals harvested are coded according to the zone and the type of plant in which they were harvested. The data are grouped together in

Table 1 below. In general, morphometric analysis of *R. invadens* larvae is destructive. Indeed, it requires the prior death of individuals and their fixation in alcohol [7].

2.4. Morphometric study

We have chosen 10 measurable variables with a reasonable degree of precision. These are essentially the length of the insect's body, body width, head length and head width, length of the hind legs, middle legs and front legs. Add to this the length of the abdomen, length of the thorax and the average diameter of the sternum (Table 2). The parts concerned were mounted on a binocular magnifying glass incorporated with a camera connected to a computer. The observations were carried out on individuals of the L3 (3rd larval stage) which corresponds in the male to the pre-pupa. The harvested specimens are then cleaned in 70 alcohol before taking the measurements and each piece being carefully separated from neighboring pieces. Each individual in a sample is paired with a code, using the first letter of the region in uppercase followed by the first letter of the locality of origin and finally the first letter of the plant species considered, (Table 2).

Table 2 Choice of values

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

2.5. Statistical analyzes

2.5.1. Gross measurements

After the choice of the number of axes to be retained, according to the elbow criterion (which makes it possible to obtain the best factorial plan), a principal component analysis (PCA) of the populations with the raw measurements of the variables with the R software version 3.2 .3 from [8]. The purpose of the PCA is to output the contribution of the variables on each factorial axis, to visualize the correlations between the variables.

2.5.2. Transformed measurements

Size effect and data transformation

The size effect results in a correlation circle that groups all the variables into a single plane for a given axis. This is an unwelcome effect, one that metric studies seek to overcome [9].The principle is therefore to eliminate this effect and reduce all individuals to the same size, in order to observe only differences in shape on the PCA. The elimination of the size effect in this study was performed according to the approach proposed by [10]; [9]; [11]; [12]; [13]

- Data log-transformation: our initial data table is made up of the variables X1, X2,..., Xp, we subsequently created a new data table made up of the variables log (X1), log (X2),..., log (Xp);
- For each individual, we calculated the average over all the log-transformed variables. We consider this average score to be a good idea of the "size" for this individual;
- Finally, for each individual, we subtracted from each of these raw measurements the average height obtained with the log-transforms.

The size effect is thus eliminated and only the difference in shape will be observed on the PCA. The reduction in the weight of the factor (Size) leads to a reduction in the overall discrimination. This transformation was carried out in Excel version 2013.

2.5.3. Discriminant factor analyzes (DFA)

A discriminant factor analysis (DFA) of the populations with the transformed data of the variables is carried out with R software version 3.2.3. It is performed with variables whose contribution is greater than or equal to $(1 / P) \times 100$. It aims to see the best factor for grouping individuals according to their morphology.

2.5.4. Determination of variables discriminating populations

To determine the variables that significantly differentiate the morphology of populations, a Shapiro-Wilk test of normality of the data is first performed on each transformed log variable. When the p-value of the test is less than 0.05, the corresponding log-transformed data does not follow the normal distribution and the Kruskal-Wallis test (nonparametric) is performed for a multiple comparison of the mean ranks of the populations of the corresponding variable. The Wilcoxon test (nonparametric) with the Bonferroni adjustment is performed to test the significance of the deviations in the mean ranks of the log-variables transformed by pair of populations. When the p-value is greater than 0.05, the log-transformed data follows a normal distribution and the Anova test is performed for a comparison of the mean of the transformed log variable.

3. Results

3.1. Gross measurements

3.1.1. Choice of the number of axes to be used

To obtain the number of axes to retain, we used the elbow criterion. The purpose of this criterion is to obtain the maximum conserved inertia with the minimum of factors. In our study two axes are retained, because the cut (the elbow) takes place at K = 2.
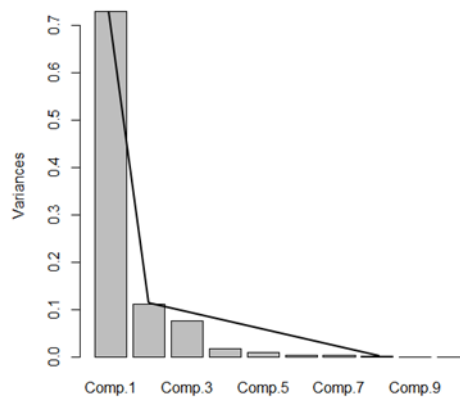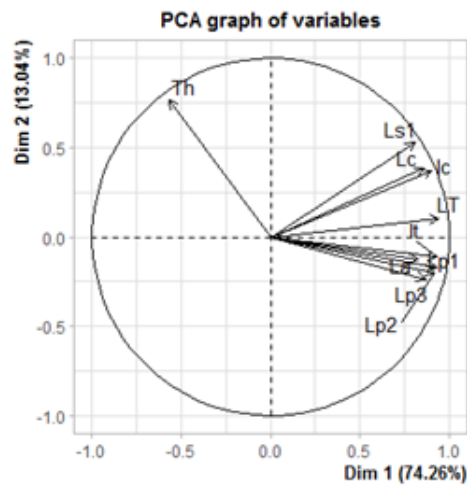

Figure 2 Choice of the number of factors

3.2. Principal component analysis

The principal component analysis of individuals from different varieties of mango trees reveals that the first two factor axes (dimension) best explain the morphometric variability with 87.3% of the inertial power. Along the factorial axis 1 (dimension 1) variables such as LT (F1 = 11.9), lt (F1 = 11.7), Lp1 (F1 = 11.6), Lp2 (F1 = 11.3), lc (F1 = 10.9), Lp3 (F1 = 10.1), largely participate in the construction of the first factorial axis with 74.26% of the power of inertia. On the other hand, variables such as Lc (F1 = 9.9) La (F1 = 9.2), Ls1 (F1 = 8.7), and Th (F1 = 4.38) contribute little to the construction of the first axis. The factorial axis 2 (dimension 2), with a low power of inertia (13.04%) is largely constructed by the variables Th (F2 = 44.6), Ls1 (F2 = 21.17), LC (F2 = 11.21), lt (F2 = 10.51) and Lc (F2 = 11.9). The other variables such as Lp3 (F2 = 4.27), Lp2 (F2 = 3.009), Lp1 (F2 = 2.16), La (F2 = 1.18) lt (F2 = 0.94), LT (F2 = 0.82) contribute little to the construction of this axis. On the first factorial axis, all the variables are positively correlated except for the length of the thorax (Th). Obviously, the size effect affects our PCA (principal component analysis) (Figure 3). An overall 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 3 Correlation of the variables according to their contribution to the construction of the two dimensions (Dim. 1 and Dim. 2) with the raw data of the populations

3.3. Transformed measurements

*3.3.1. Choice of the number of axes to be retained after data transformation*

After transformation of the data, with the elbow criterion, two factorial axes are retained because K = 2.
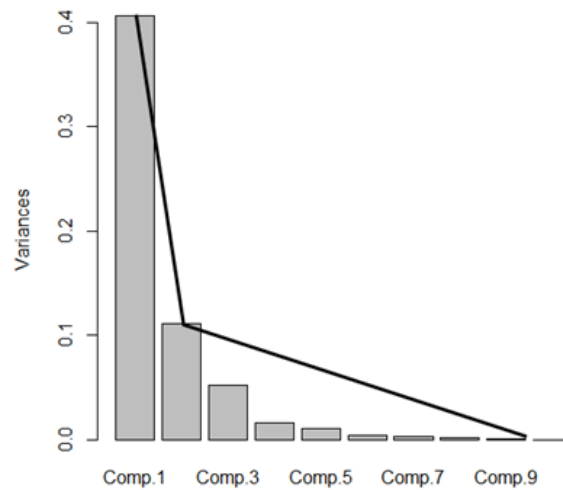
Figure 4 Choice of the number of factors after transformation

### 3.4. Principal component analysis of transformed variables

In comparison with the raw data, the principal component analysis of the transformed data shows a reduction in the percentage of inertia of 17.43% for the first dimension (factorial axis 1) following a decrease in the discriminating power of most of the variables at namely Lp3 (F1 = 9.03), lc (F1 = 8.35) and Lc (F1 = 7.76). The second factorial axis with a very noticeable decrease (6.22%), shows an almost identical situation, 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 certain variables such as LC (F2 = 24.18), lc (F2 = 18.61), Lp3 (F2 = 15.01), Ls1 (F2 = 12.41), Lp2 (F2 = 10.64). The other variables such as Lp1 (F2 = 6.04), La (F2 = 5.15), Lt (F2 = 4.36), Th (F2 = 2.37) and lt (F2 = 1.14). Better representation quality is always obtained with the plane formed by axes 1 and 2 with a total inertia percentage of 76.09%.
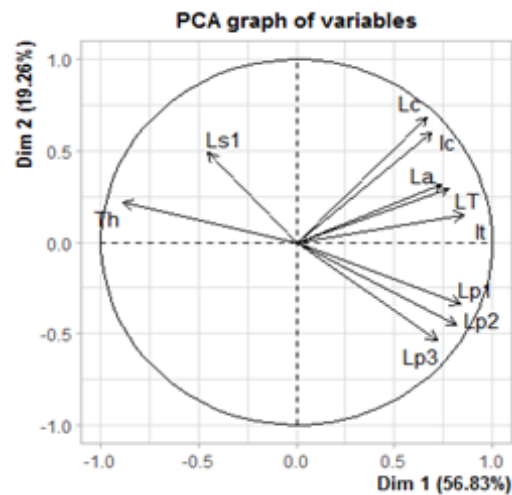
Figure 5 Correlation of the variables according to their contribution to the construction of the two dimensions (Dim. 1 and Dim. 2) with the transformed data of the populations

3.5. Discrimination after transformation

The best quality of representation of discriminant factor analysis (DFA) is obtained by the plane formed by the first two factor axes (Dim1 and Dim2) with 78.1% of the percentage of inertia. The AFD of populations is carried out with variables whose contribution is greater than or equal to $(1 / P) \times 100$ (the principal components) .These variables are LC, lc, LT, lt, Th, Lp1, Lp2, Lp3 and Ls1. Following the first factorial axis (Dim1), with an inertia percentage of 57.6%, the population of Sewe is distinguished from that of Kent and Boulkodiekhal. Following the second factorial axis (Dim 2), with an inertia percentage of 20.5%, the population of Keitt is distinguished from those of Boukodiekhal and Kent.
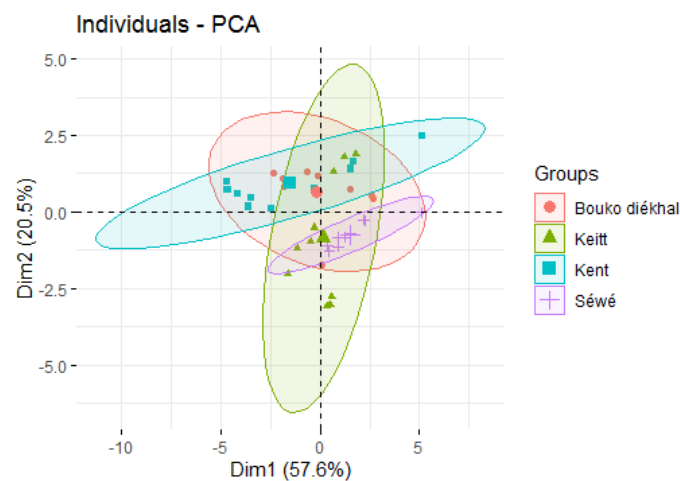
Figure 6 Representation in the main AFD plan of the populations of *R. invadens* after transformation of the variables

3.6. Variables allowing discrimination after transformation

According to the significance test, among the 10 variables studied, except the length of the body (LC), the length of the head (LT), the length of the abdomen (La) and the width of the head (lt) all the other variables make it possible to differentiate the populations of Kent and Keitt on the one hand and those of Sewe and Boukodiekhal on the other. Between these four populations, the variables like body width (lc), length of the abdomen (La), length of the thorax (Th), length of the first pair of legs (Lp1), length of the intermediate legs (Lp2) , length of the third pair of legs (Lp3), and length of the first sternum (Ls1) allow them to be distinguished.

Table 3 Significance test of the means of the transformed log-variables.

Varieties / Variables	Boukodiekhal	Keitt	Kent	Sewe	p-value de Shapiro
LC	3.16±0.14[a]	2.73±0.47[a]	2.86±0.49[a]	2.86±0.36[a]	NS
lc	1.72±0.10[a]	1.63±0.34[a]	1.66±0.37[a]	1.66±0.18[a]	**
LT	0.69±0.14[a]	0.69±0.15[a]	0.71±0.18[a]	0.80±0.07[a]	NS
lt	1.06±0.13[ab]	0.91±0.09[a]	0.90±0.23[a]	1.14±0.08[b]	NS
La	1.88±0.33[a]	1.65±0.15[a]	1.69±0.25[a]	1.69±0.18[a]	NS
Th	0.56±0.24[ab]	0.64±0.14[ab]	0.67±0.17[a]	0.82±0.06[b]	**
Lp1	0.69±0.12[a]	0.78±0.13[a]	0.67±0.17[a]	0.82±0.06	**
Lp2	0.72±0.14[a]	0.88±0.07[a]	0.74±0.17[a]	0.91±0.09[a]	**
Lp3	0.76±0.16[a]	0.96±0.08[b]	0.80±0.17[ab]	0.96±0.08[a]	**
Ls1	0.35±0.02[ac]	0.36±0.04[a]	0.45±0.06[b]	0.31±0.03[c]	**

4. Discussion

Morphometric measurements of *R. invadens* show various values. In fact, for most variables, the measurements vary more or less according to a certain average. However, variables such as thorax length (Th) and first sternum length (Ls1) show constant values in all individuals sampled. These two variables therefore do not make it possible to discriminate between individuals from different populations. Thus, of the 10 variables chosen, only 8 variables (Lc, lc, Lt, lt, La, Lp1, Lp2 and Lp3) are used in order to obtain relevant information on the morphometric variability of the species. The first two factor axes explain 57.6% of the morphometric variability. According to the first factorial axis, the population of Sewe is distinguished from that of Kent and Boukodiekhal. Following the second factorial axis (Dim 2), with an inertia percentage of 20.5%, the Keitt population is distinguished from those of Boukodiekhal and Kent. Indeed, the populations of these different varieties do not seem to present first seen great dissimilarities, due to the proximity of their point clouds; which could suggest an important gene flow between the different populations. However, an apparent correlation of most of the variables is due in large part to a common factor which can be assimilated at first approximation to a size factor. The decrease in the weight of this factor results in a slight decrease in the overall discrimination between the four varieties of mango trees. Thus, the transformed data leads to a reduction in the percentage of inertia of 17.43%, for the first dimension (factorial axis 1) and 6.22% for the second factorial axis. This offers a better redistribution of the variables in relation to their contribution to the axis. With the transformed data, along the factorial axis 1, the first dimension allows discrimination of certain populations of Kent and Keitt and most of the populations of Sewe and Boukodiekhal. On the other hand, the second dimension reveals discrimination on the one hand between the Kent and Keitt populations and those of Sewe and Boukodiekhal. Insects from improved varieties (Kent and Keitt) are therefore distinguished from those from local varieties (Sewe and Boukodiekhal).

The organoleptic taste seems to correspond to a discriminatory criterion, therefore it is involved in the variation of the morphology of the species as well as the climatic conditions [14]. This could lead us to consider the mango tree as being the plant most affected. According to [15] the mango mealybug was first identified in Senegal in 1995 on the mango tree. The difference in the size of the insects would be linked to the varieties which each present a specific taste. This confirms the work of [16] which states that the development performance of insects is strongly influenced by nutritional quality.

5. Conclusion

The mealybug is a major constraint on the production of edible fruits in Senegal. This pest, the spread of which is favored by human activity (transport of plant material), very quickly reached a large part of Senegalese territory.

However, the study of the morphometric characterization of the populations of *Rastrococcus invadens* between the different varieties of mango tree made it possible to verify whether the distribution of the insect according to the plant

species has an impact on its morphology. It revealed almost two more or less distinct morphometric groups made up on the one hand by populations from improved varieties (Kent and Keitt) and on the other by those from local varieties (Sewe and Boukodiekhal). But only more studies are needed in order to understand what is behind the existence of these less distinct groups.

Compliance with ethical standards

Disclosure of conflict of interest

No declaration of conflict of interest was noted.

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