Effect of mango mealybug and sooty mould attack on mango and the impact of the released *Gyranusoidea tebygi* Noyes on yield

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Effect of mango mealybug and sooty mould attack on mango and the impact of the released *Gyranusoidea tebygi* Noyes on yield.

Abstract — **Introduction**. The mango mealybug *Rastrococcus invadens* is a pest of horticultural crops, especially mango. Though this fact has been demonstrated and its parasitoid, *Gyranusoidea tebygi*, released for its control in many countries, quantitative information on the damage inflicted by the mealybug and post-release mango fruit production are still scanty. This study was therefore undertaken to investigate the damage caused by mango mealybug and its associated sooty mould on mango plants and to assess mango fruit production after the release of *G. tebygi* in 1989. **Materials and methods**. Laboratory experiments were set up to determine the effect of different populations of mango mealybug (0, 5, 10, 20, and 40 adults/cage) on the chemical constituents of mango leaves. The mould associated with the mango mealybug was identified and its effect on leaf temperature was studied. A mango orchard was studied for fruit production from the time of fruit-lessness in 1990 to 1998 when fruit yield peaked. The resultant effect of the parasitism of mango mealybug by *G. tebygi* was monitored on the chemical composition of mango leaves during this period. **Results**. Protein, fat, carbohydrate, ash, crude fibre and moisture contents were depleted with increase in mealybug population. The isolated mould fungus *Capnodium mangiferae* was found to abe negatively but significantly correlated with mango mealybug population and positively correlated with mango fruit yield. Parasitism was highly correlated with mealybug population and positively correlated with mango fruit yield. Parasitism was highly correlated with mealybug population and positively, and was considered a major factor in the control of the pest and the subsequent increase in mango fruit yield. Rainfall did not have a significant impact on yield, mealybug population or sooty mould score. **Discussion**. The injury inflicted by *R. invadens* and its associated mould, and the enhancement of mango fruit production by the activities of *G. tebygi* on the mea

Nigeria / Mangifera indica / pest control / Rastrococcus invadens / Gyranusoidea tebygi / biological control organisms / yields

Effet des attaques de la cochenille farineuse et de la fumagine sur manguiers et impact de la libération de *Gyranusoidea tebygi* Noyes sur leur rendement.

Résumé — **Introduction**. *Rastrococcus invadens*, la cochenille farineuse du manguier, est un ravageur des productions horticoles, et du manguier en particulier. Bien que ce fait ait été démontré et que son parasitoïde, *Gyranusoidea tebygi*, ait été libéré dans de nombreux pays afin de contrôler le ravageur, des informations quantitatives sur les dommages qu'il inflige à la production en mangues sont encore minces. L'étude engagée a permis d'étudier les dommages provoqués sur manguiers par ce parasite et par la fumagine qui lui est associée et d'évaluer l'évolution des rendements après un lâcher de *G. tebygi* en 1989. **Matériel et méthodes**. Des expériences en laboratoire ont été mises en place pour déterminer l'effet de différentes populations de cochenilles farineuses (0, 5, 10, 20, et 40 adultes par cage) sur la composition chimique de feuilles de manguiers. La fumagine associée à la présence de l'insecte a été identifiée et son effet sur la température de la feuille a été étudié. Un verger de manguier a été étudié pour sa production en fruits à partir de 1990, date à laquelle il était improductif du fait des infestations, jusqu'à l'année 1998 marquée par un pic de rendement. L'effet résultant du parasitisme de la cochenille farineuse par *G. tebygi* a été suivi sur la composition chimique des feuilles de manguier pendant cette période. **Résultats**. Les protéines, lipides, hydrates de carbone, cendres, fibres brutes et teneurs en eau se sont épuisés en même temps qu'augmentait la population de cochenille farineuse. Le champignon isolé, *Capnodium mangiferae*, a entraîné une élévation de la température des feuilles des jeunes manguiers infectés. La densité de population de *G. tebygi* a été corrélée négativement mais sensiblement avec la population de la cochenille farineuse du manguier et positivement corrélée avec le rendement en mangues. Le parasitisme a été fortement corrélé avec la population de cochenilles et avec le rendement ; il a été considéré comme un facteur important de contrôle du

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1. Introduction

The mango mealybug, Rastrococcus invadens Williams (Homoptera: Pseudococcidae), was accidentally introduced into West Africa from Southeast Asia [1] and it quickly spread through Togo, Benin and Ghana [2]. The mealybug was first noticed in Nigeria in 1987 and has since spread through the southern parts of the country [3, 4]. The insect sucks sap from leaves and, where it occurs in large numbers, honeydew deposits encourage the growth of sooty mould on the foliage leading to a marked reduction in fruit production [5]. Infestation poses a serious threat to subsistence farmers in areas where mangoes and citrus are often the most readily available food providing energy and vitamins in the diet. Yield losses are largely unquantified but our surveys have revealed that, in some parts of Nigeria, losses were as high as 100% [6]. Some of the control options adopted in Nigeria included cultural (pruning and, in some instances, felling of the affected trees), chemical and biological control. The biological control involved the importation of an endo-parasite of mango mealybug, an encyrtid wasp, Gyranusoidea tebygi Noyes [7], from Togo in 1989 [4] and, subsequently, from the International Institute for Tropical Agriculture, Biological Control Programme (IITA/BCP), Cotonou, Republic of Benin. This parasitoid, which attacks the first and the second instars of the mango mealybug [8], has been released all over the infested parts of the country and its performance has been satisfactory [4].

Reports on the impact of the parasitoid on mango fruit yield, and various yield losses and changes in physiochemical properties caused by mealybug infestation are still scanty. This was partly because progressive assessments of yield losses caused by the mango mealybug were not possible due to the suddenness of the infestation and the fast rate of its multiplication in the absence of natural enemies. Another reason was because of the inability of the growers to provide accurate estimation of the fruit yields prior to the mealybug invasion. The quantitative assessment of yield reduction as a result of mealybug infestation and that of increase in yield as a result of control efforts is no doubt of commercial interest. This would provide information, at least in part, on the extent of damage and the approximate population of mealybugs at which various quantitative degrees of damage are inflicted. Therefore, a long-term study was undertaken to assess the impact of *G. tebygi* on mango fruit yield and that of mango mealybug and its associated mould on some physiochemical characteristics of mango leaves after mealybug invasion and the release of its natural enemy.

2. Materials and methods

2.1. Analyses of the infested leaf chemical contents

A local variety of mango (Ogbomoso) propagated by grafting was used for the experiment. Twenty Ogbomoso grafted mango seedlings were planted in 5 L-polythene bags filled with topsoil, one per pot, and kept outdoors. At six months, when the seedlings had fully taken, they were caged in a screen house, one per cage, and throughout the duration of the study, five leaves were maintained on each plant. Groups of first instar mealybugs born within an interval of 24 h were transferred from the laboratory culture to the leaves of caged plants. The cages $(1.0 \text{ m} \times 1.0 \text{ m} \times 1.0 \text{ m})$ were wooden, had wire mesh on all the sides and were provided with a door each to facilitate easy access into them.

After settling for 1 or 2 days on the leaves, the number of mealybugs was reduced to 0 (control), 5, 10, 20 and 40 representing the treatments. Different treatment cages were arranged in a completely randomised block design in the screen house with four replicates. The number of insects in each cage was kept constant by replacing, from the laboratory culture, dead individuals with fresh ones of approximately the same age. At the maturity of these mealybugs, all the young ones produced by them were removed immediately at least twice daily. The experiment was maintained for 2 months in the screen house; then, analyses for the chemical contents and chlorophyll concentrations in the leaves were carried out.

For the analysis of the chemical contents of the leaves, two leaves from each of the caged plants were weighed, ground and dried in the oven at 150 °C for 6 h; then, the samples were stored in screw-capped bottles. Two grams of the dried samples were digested by the wet digestion method. These digested materials were made up into four solutions in different 100 mL-graduated flasks and the nutrient contents (crude protein, crude fibre, fat, carbohydrate, ash and moisture contents) were determined [9].

For chlorophyll contents, two leaves from each caged plant were separately weighed, ground and the chlorophyll contents extracted in 10 mL 80% acetone in water. The extracts were centrifuged (1400 g) for 3 min and decanted. A corning 253 colorimeter was used to measure the optical density of the decanted supernatant at (643 and 663) nm against the 80% acetone blank. The colorimeter is an analog instrument covering the wavelength range (400-700) nm and the wavelength selection is by a calibrated interference wedge filter. During the absorbance measurements, care was taken to see that there were no air bubbles in the samples to avoid inaccuracies in the absorbance values. Care was also taken to ensure that the glass tubes containing the blank and chlorophyll extracts always had the same orientation with respect to the incident beam of light. This eliminated any variation in absorbance values arising from variations in the thickness of the walls of the glass tubes [10]. The concentration of chlorophylls a and b were then computed by using the following formulae:

chlorophyll $a = [(212.7D_{663} - 2.69_{645}) \cdot V]/1000 W$ and chlorophyll $b = [(212.9) D_{645} - 4.68_{663}) \cdot V]/1000 W,$

where D is the optical density at the wavelength state, V is the total volume of the chlorophyll extracted and W, the weight of fresh leaf tissue extracted [11]. Separate samples were analysed for total chlorophyll and chlorophylls *a* and *b*.

2.2. Identification and isolation of the mould fungus

Typical mango leaves showing sooty mould symptoms were collected from the field and the sooty mould was then scraped into a sterile petri dish by the use of a spatula. The mould was plated on sterile potato dextrose agar (PDA) incubated at 28 °C for 6 days; then, the organism was grown in pure culture. A pure single colony of the organism was grown on PDA slants and was kept at 4 °C for further studies. Identification was done by colony and morphological characteristics [12].

2.3. The effect of the mould on leaf temperature

Twenty potted grafted seedlings of a local variety of mango (Ogbomoso) were used for the study. Forty adult females of mango mealybug were placed on 16 of the potted mango plants. After 7 days of honeydew production on the leaves, the mango mealybugs were removed by using a camel hairbrush. Each of the leaves carrying the honeydew was inoculated with 0.5 mL of conidia suspension (10^7 conidia per mL) of the culture of the organism. There were five treatments of varying proportions (percentage) of leaf surface area covered by sooty mould, namely, 0 (no sooty mould for the control), 25%, 50%, 75% and 100% of the leaf surface area; each of these treatments was replicated four times. After the inoculation, the plants were caged singly in wooden cages (44 cm \times 45 cm \times 45 cm) with sleeves. The caged plants were kept in the insectary at (28 ± 2) °C and (60 to 80)%relative humidity under a 12:2 light/dark regime. Ten drops of diluted honey were applied weekly to each of the infected leaves by the use of a glass rod to maintain the culture of the mould on the leaves. When the mould had fully developed (at 1 week), weekly temperature data were thereafter collected for 12 weeks at about 18:00 h. Temperature readings inside the cages were taken using a mercury-in-glass

thermometer and on the leaves (at the base of the mould) using a clinical thermometer.

2.4. Assessment of mango fruit production

The field used for this study was located in Ibadan in the southern part of Nigeria. Plantations of mango in this area are not common but single mango trees are widely distributed as shade trees in front of houses and along roads (mostly wild trees). The sampling site was one of the places where the ceremonial release of 400 adults of G. tebygi took place in August 1989 before the natural enemy was released all over the country. The field referred to above was a small mango plantation with ten 20-yearold mango trees located in the Onireke area which is about 3 km from the National Horticultural Research Institute (NIHORT). Ibadan. The field contained the Ogbomoso mango variety and, in December 1989, it was heavily infested resulting in fruitlessness. The mango field was sampled and yield was progressively assessed from the time of fruitlessness occasioned by the high mealybug population in January 1990 to December 1998 when mango mealybug and the sooty mould had disappeared.

It was not possible to have a control at the experimental site where there was no mealybug but, for purposes of comparison, clean mango trees of the same variety and age were monitored for yield in a NIHORT's mango orchard from 1990 to 1998.

2.5. Mealybug sampling

Two sampling procedures involving stratification by leaf age [13] were used to assess mango mealybug populations at the experimental site.

The first sampling was destructive and randomised; the samples were taken from the terminal segment of the shoot (new leaves). Twenty leaves per tree from four randomly selected shoots (five leaves/ shoot) were taken each month from three trees in the orchard and counts of mealybugs (adults and nymphs) were made under a stereomicroscope. The number of mummified mealybugs (dead parasitised mealybugs containing a live pupa of *G. tebygi* or a secondary parasitoid) was also counted.

In the second procedure, sampling was conservative; the samples were taken from four randomly selected shoots from three other mango trees and were from the previous segment (old leaves). Five leaves from each shoot making a total of twenty leaves per tree were randomly collected every month and mealybugs were counted as in the first sampling.

The data from the two sampling techniques were pooled before analysis of variance tests were carried out.

2.6. Sooty mould score

From the two samples, the average percentage of the leaf surface covered by sooty mould was estimated as a mean score/leaf on the basis of a five-point scale: 1, no sooty mould; 2, 1 to 25% of the leaf surface; 3, 26 to 50%; 4, 51 to 75%; and 5, 76 to100% [14].

2.7. Yield assessment

In the study area, mango has two flowering periods, one in each season, resulting in two harvests. Yield was calculated for each year based on the weight of the total number of fruits produced annually from 1990 to 1998.

2.8. Analysis of chemical contents of infested orchard mango leaves

Ten randomly selected leaves of mango were collected from the sample trees in the orchard, every December from 1990 to 1998, for analysis of their nutrient and chlorophyll contents using the same procedure described above.

2.9. Statistical tests

An analysis of variance (ANOVA) was conducted for the means of variables and means of significantly different variables were separated using Duncan's New

Table I.

The proximate analysis of mango leaves infested with *Rastrococcus invadens*, the mango mealybug, at different population levels in the screen house.

Number of mealybugs	Moisture content (%)	Protein (g)	Fat C (g)	Carbohydrate (g)	Ash C (g)	rude fibre (g)	e Chlorophyll (mg·g ⁻¹ dry weight)		
							Total	Chlorophyll a	Chlorophyll b
0	80.4 a	3.6 a	1.9 a	8.3 a	2.6 a	3.2 a	2.4 a	1.6 a	0.7 a
5	78.6 b	3.0 b	1.6 b	7.5 b	2.5 a	3.0 a	2.4 a	1.5 a	0.7 a
10	78.1 b	2.0 c	1.5 b	6.4 c	2.0 b	2.6 b	2.3 a	1.4 a	0.8 a
20	76.6 c	1.1 c	0.8 c	5.9 d	1.2 c	2.2 b	2.3 a	1.4 a	0.8 a
40	74.2 d	0.8 d	0.6 c	5.0 d	0.8 d	2.0 c	2.1 b	1.2 b	0.8 a
Standard error of the mea	an 1.0	0.5	0.3	0.6	0.4	0.2	0.1	0.1	0.2
Coefficient of variation	3.0	57.1	43.3	19.7	43.7	19.6	4.3	10.5	49.2

Means followed by the same letters along the same column are not significantly different from one another, according to the Duncan's New Multiplication Range Tests (P < 0.05).

Data in percentages were transformed using arcsine \sqrt{x} before analysis.

Multiple Range Test (P < 0.05). Data in percentages were transformed using arcsine \sqrt{x} before analysis.

3. Results

The results of the screen house study revealed significant differences in the nutrient contents of the leaves between the control and the treatments at different levels of mealybug infestations. Appreciable reduction occurred in the nutrients as mealybug population increases from 0 to 40 adults/ cage. Except for chlorophyll a at 40 mealybugs/plant, chlorophyll contents were generally not influenced by mealybug population (table I). The sooty mould fungus isolated and identified was Capnodium mangiferae. The results of its artificial inoculation on mango leaves in the screen house showed that leaf temperatures were generally raised from 29.1 °C for leaves with no sooty mould to 30.9 °C for leaves with 100% mould coverage (table II).

In 1990, yield reduction was found to be 100% in the study area and the total number of mealybugs was about 256.2 per leaf (*figure 1*). There was a progressive increase

in yield from 1990 to 1998 and a reduction in the population of the mango mealybug within the same period as the percent parasitism increased from 1.7 in 1990 to 100 in 1998. From 1995 when mealybug extinction occurred, the yield pattern was similar to that of the uninfested trees in NIHORT's orchard (*figure 1*). The annual mean score for sooty mould cover also progressively decreased from 100% in 1990 to 0% in 1995 to 1998 and was significantly correlated

Table II.

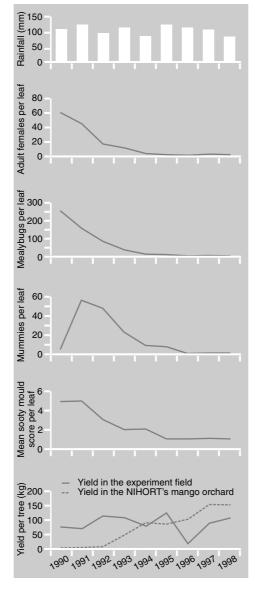
Assessment, in the laboratory, of the effect of the mango leaf surface area covered by sooty mould on leaf temperature [standard error of the mean = 0.3 °C; average cage temperature was (29.1 + 1.0) °C].

Leaf surface area (%) covered by sooty mould	Average leaf temperature (°C)
No sooty mould	29.1 a
1 to 25	29.3 ab
26 to 50	29.6 b
51 to 75	30.0 c
76 to 100	30.9 d

Means followed by the same letters along the same column are not significantly different from one another, according to the Duncan's New Multiplication Range Tests (P < 0.05).

Figure 1.

Seasonal flunctuation (January 1990 to December 1998) in *Rastrococcus invadens* abundance in an experimental field (Ibadan, Nigeria) expressed as various parameters and assessed according to the total annual rainfall and the annual yield per tree in this experimental field and a NIHORT's mango orchard not infested.



with mealybug population (r = 0.94) and yield (r = 0.85). The relationship between mealybug population and yield was negative and significant, r = -0.78 (P < 0.05). Rainfall did not have any significant impact on mango mealybug population, parasitism, sooty mould score and yield during the period of the study. Increases were noticed in the nutrient contents of the leaves between 1990 and 1998. With a reduction in the mealybug population by *G. tebygi*, there were increases in the protein, fat, carbohydrate, ash, crude fibre and moisture contents of leaves (*figure 2*). Chlorophyll contents, however, increased very slightly from 1990 to 1998 (*figure 2*).

4. Discussion

The results showed a gradual but significant increase in fruit yield over a period of 9 years as parasitism increased. The highly significant correlation between mealybug population and yield on the one hand, and mealybug population and parasitism on the other, showed that mealybug infestation was largely responsible for the drastic reduction in yield observed before 1995; it also showed that *G. tebygi* was responsible for the reduction of the *R. invadens* population and the increase in fruit production observed during this period.

From a similar study, the duration of the presence of *G. tebygi* showed a positive correlation with plant growth as measured by the production of new leaves [14]. This improvement in the growth of trees also occurred when the percentage of physiologically active trees was calculated. Though yield was not measured in that study, it can be inferred that production of new leaves and the reduction of the population of mango mealybug would bring about an increase in fruit production, as observed in this study.

The results of the proximate analysis of the chemical contents of the leaves both in the laboratory and in the field showed there was a reduction in protein, fat, carbohydrate, ash, crude fibre and moisture contents of the leaves exposed to the mealybug. The infestation of leaves by the mango mealybug had a negative influence on the chlorophyll contents of infested leaves only at very high infestation level. This depletion of chlorophyll content further reduced the photosynthetic sites and therefore the amount of sugar manufactured.

The mould fungus isolated, *Capnodium mangiferae*, has been reported to be very common together with *Meliola* spp. on citrus and mango [12]. The fungus has been reported to grow on honeydew produced

by sap-sucking insects (e.g., aphids and mealybugs) on the leaves and is therefore responsible for the blackness of the leaves associated with mango mealybug on mango trees. Therefore, its control would be achieved by controlling the insect pests that produce the honeydew on which it grows [15]. The positive correlation between the sooty mould score and mango mealybug population suggested that the reduction in sooty mould observed between 1990 and 1998 was as a result of the reduction in the population of the mango mealybug by G. tebygi; it is a case of successful biological control. Rain was found to have little impact on the sooty mould score due to the non-significant relationship between the two factors and, therefore, might not be a key factor in sooty mould reduction. This is because, in an uncontrolled situation, a constant supply of honeydew by the mealybug will make the sooty mould too thick to be washed off by rain. The non-significant relationship between parasitism and sooty mould score means that any reduction in sooty mould scores due to the control of R. invadens by G. tebygi may become evident only after the affected leaves have been shed.

The sooty mould and the resultant black cover reduce photosynthesis [13], obviously by reducing the amount of light available for the process. In addition to this, the increase in temperature observed on infested leaves may be due to the metabolic activities and the blackness of the fungus that is capable of radiating heat [8]. Under field conditions where temperatures are higher than the ordinary room temperature (up to about 35 °C) the magnitude of temperature increase in the infested leaves could be more and could last longer than what has been reported.

The major impact of mealybugs in the agricultural cropping system is reduction in harvestable product. The contributing mechanisms can be grouped into two categories. First they contribute to reduced productivity by directly consuming transportable carbohydrates and other nutrients carried in the phloem. Secondly, they produce honeydew which can directly contaminate harvestable mango fruits.

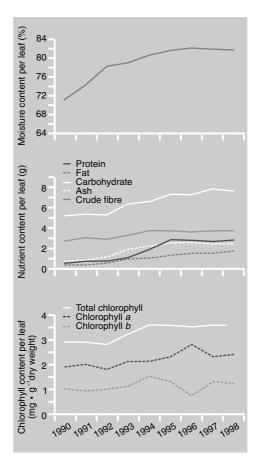


Figure 2.

Evaluation from 1990 to 1998 of the moisture, nutrient and chlorophyll contents in leaves of mango trees from an orchard infested with *Rastrococcus invadens*, then progressively controlled thanks to releases of *Gyranusoidea tebygi*.

Honeydew can also cover leaf surfaces, allowing the subsequent growth of sooty moulds (e.g., Capnodium mangiferae) which severely reduce the productivity of the plant (possibly because less light reaches the cytochrome bearing tissues) [16]. In addition, the sooty mould increases thermal absorption and raises leaf temperature; this in turn reduces leaf efficiency and may even cause premature death of tissues. The combination of these effects culminates in stunted growth resulting in reduction in mango fruit yield or outright fruitlessness of infested trees, as observed in many countries [6, 14, 17-20]. Mango mealybug is thus a very serious pest of high economic and social value that deserves the attention given to it by various governments in Africa.

This long-term study has assessed the impact of *G. tebygi* on *R. invadens* and most importantly on mango fruit production. The

data collected showed that *G. tebygi* has been effective in controlling the mango mealybug; they demonstrated improvement in plant health and growth and also brought about higher fruit yield. The impact of the parasitoid on the target growers' population (by deduction) is an increase in income and enjoyment derived from these trees and fruits. The biological control of the mango mealybug is thus a success in Nigeria; this effort should be sustained and made transferable to new problems [21].

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Efecto de los ataques de la cochinilla harinosa y de fumagina en mangos e impacto de la liberación de *Gyranusoidea tebygi* Noyes en su rendimiento.

Resumen — **Introducción**. *Rastrococcus invadens*, la cochinilla harinosa del mango, es una plaga de las producciones hortícolas y, en particular, del mango. A pesar de que esto ya ha sido demostrado y de que su parasitoide, Gyranusoidea tebygi, haya sido liberado en muchos países para controlar la plaga, aún es escasa la información cuantitativa disponible sobre los daños que ocasiona a la producción de mangos. Este estudio permitió estudiar los daños que este parásito y la fumagina que se asocia con él provocan en los mangos y evaluar la evolución de los rendimientos después de una liberación de G. tebygi en 1989. Material y métodos. Se establecieron experimentos en laboratorio para determinar el efecto de diferentes poblaciones de cochinillas harinosas (0, 5, 10, 20, y 40 adultos por jaula) en la composición química de hojas de mangos. La fumagina asociada a la presencia del insecto fue identificada y se estudió su efecto en la temperatura de la hoja. Se estudió la producción de frutos de un huerto de mangos a partir de 1990, fecha en la que era improductivo debido a la infestación, hasta 1998 marcado por un pico de rendimiento. El efecto resultante del parasitismo de la cochinilla harinosa por G. tebygi fue seguido en la composición química de las hojas de mango durante este período. Resultados. Las proteínas, lípidos, hidratos de carbono, cenizas, fibras brutas y contenidos de agua se agotaron al mismo tiempo que aumentaba la población de cochinilla harinosa. El hongo aislado, Capnodium mangiferae, provocó un aumento de la temperatura de las hojas de los jóvenes mangos infectados. La densidad de población de G. tebygi estuvo correlacionada negativamente pero sensiblemente con la población de la cochinilla harinosa del mango y correlacionada positivamente con el rendimiento en mangos. El parasitismo estuvo fuertemente correlacionado con la población de cochinillas y con el rendimiento; se consideró como un factor importante de control de la plaga y del aumento del rendimiento de mangos que tuvo lugar. Las precipitaciones no tuvieron impacto significativo en el rendimiento, la población de cochinillas y la distribución de fumagina. Discusión. Se analizan los daños causados por R. invadens y la fumagina que se le asocia y la producción mejorada en mangos debido a las actividades de G. tebygi.

Nigeria / Mangifera indica / control de plagas / Rastrococcus invadens / Gyranusoidea tebygi / organismos para control biológico / rendimiento