Original article

Population dynamics of the woolly whitefly *Aleurothrixus floccosus* (Maskell) on sweet orange varieties in Nigeria and association of *A. floccosus* with the entomopathogenic fungi *Aschersonia* spp.

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***RESUMEN ESPAÑOL**, p. 392*

Population dynamics of the woolly whitefly *Aleurothrixus floccosus* (Maskell) on sweet orange varieties in Nigeria and association of *A. floccosus* with the entomopathogenic fungi *Aschersonia* spp.

Abstract — **Introduction**. Various whitefly species attack sweet oranges in Nigeria. The economic importance of these whiteflies varies from one area to another depending on prevailing environmental conditions. Trials were established to assess the relative susceptibility of sweet orange varieties to woolly whitefly *Aleurothrixus floccosus* (Maskell) attack, its population dynamics and the impact of its entomopathogenic fungi *Aschersonia* spp. in population management. **Materials and methods**. Four sweet orange varieties were used for the trials: Agege, Bende, Pineapple and Valencia. The population dynamics of *A. floccosus* and its fungi-colonised nymphs were monitored in 2006 and 2007 by sampling 24 leaves per tree at 14-day intervals. Average monthly precipitation, relative humidity and temperature were collated for the period of our study. **Results and discussion**. No significant difference was observed among the populations of *A. floccosus* nymphs or the level of nymphal infection by *Aschersonia* spp. in the sweet orange varieties. Higher numbers of woolly whitefly eggs and adults were observed on the Agege and Bende varieties in 2006 and 2007, but the results were only significantly different in 2007. In both years, the populations of eggs, nymphs and adults of *A. floccosus* and its entomopathogenic fungi *Aschersonia* spp. were relatively high in the dry season months of January to March, dropped during the rainy seasons and rose again from October. Evaluation of *Aschersonia* spp. impact showed active colonisation of whiteflies. Generally, our results showed that weather factors such as rainfall and relative humidity negatively influenced the woolly whitefly population. **Conclusion.** The association between the woolly whitefly population and fungi was beneficial and suggests that *Aschersonia* spp. could be considered as a potential biological control agent for A. *floccosus*.

Nigeria / *Citrus sinensis* / variety trials / insect control / Aleyrodidae / population dynamics / biological control agents / entomogenous fungi / *Aschersonia* / parasitism / nymphs

Dynamique des populations de l'aleurode *Aleurothrixus floccosus* (Maskell) sur des variétés d'orangers du Nigéria et colonisation de cet aleurode par des champignons entomopathogènes *Aschersonia* spp.

Résumé — **Introduction**. Diverses espèces d'aleurodes attaquent les orangers au Nigeria. L'importance économique de ces aleurodes varie d'une région à l'autre selon les conditions environnementales dominantes. Des essais ont été établis afin d'évaluer la sensibilité relative de variétés d'oranges douces à l'attaque de l'aleurode laineuse *Aleurothrixus floccosus* (Maskell), l'évolution de ses populations et l'impact de champignons entomopathogènes du genre *Aschersonia*. **Matériel et méthodes**. Quatre variétés d'oranges douces ont été utilisées pour nos essais : Agege, Bende, Pineapple et Valencia. La dynamique des populations d'*A. floccosus* et de ses nymphes parasitées par *Aschersonia* spp. a été suivie en 2006 et 2007 par échantillonnage de 24 feuilles par arbre à intervalles de 14 jours. Des données sur la moyenne des précipitations mensuelles, de l'humidité relative et de la température ont été collectées pendant la période de notre étude. **Résultats et discussion**. Parmi les variétés d'oranges douces étudiées, aucune différence significative n'a été observée entre les populations de nymphes d'*A. floccosus* ou au niveau des infections nymphales par *Aschersonia* spp. Un plus grand nombre d'œufs et d'adultes d'aleurodes a été observé sur les variétés Agege et Bende en 2006 et 2007, mais les résultats n'ont été significativement différents qu'en 2007. Au cours des deux années d'observation, les populations d'œufs, de nymphes et d'adultes d'*A. floccosus* et de ses champignons entomopathogènes (*Aschersonia* spp.) ont été relativement élevées en saison sèche (janvier à mars) ; elles ont chuté pendant la saison des pluies pour augmenter de nouveau à partir d'octobre. L'évaluation de l'impact des champignons *Aschersonia* spp. a montré qu'ils colonisaient activement les aleurodes. En règle générale, les facteurs météorologiques tels que précipitations et humidité relative ont influencé négativement le développement des populations d'aleurodes. **Conclusion**. La colonisation des populations d'aleurodes par les champignons entomopathogènes a été efficace pour son contrôle ; les champignons *Aschersonia* spp. pourraient donc être considérés comme agents potentiels de lutte biologique contre *A. floccosus*.

Nigéria / *Citrus sinensis* / essai de variété / lutte anti-insecte / Aleyrodidae / dynamique des populations / agent de lutte biologique / champignon entomopathogène / *Aschersonia* / parasitisme / nymphe

1. Introduction

Feeding pressure by certain species of leaf feeders contributes significantly to fruit yield decline in citrus production. Under severe infestation, diebacks and stand losses are often experienced. Furthermore, some leaf feeders transmit diseases and thus compound control initiatives. In Nigeria, the most important leaf feeders of established citrus orchards include whiteflies, scale insects, leaf miners and mites [1]. Among these pests, whiteflies contribute significantly to fruit yield losses during heavy infestations [2–4]. Adults and nymphs of whitefly cause damage by direct feeding on plant sap and, when present in large numbers, can cause leaf fall. Upsurge in population is usually associated with copious production of honeydew. The latter aids the development of sooty mould, which hampers photosynthesis [5–7].

Among whiteflies present in Nigeria, the woolly whitefly *Aleurothrixus floccosus* (Maskell) had never been reported or identified on citrus and other horticultural crops. Its presence in Nigeria passed unnoticed due to the existence of other closely related species. The woolly whitefly has also been reported to attack other crops such as guava [8, 9].

The immature stages (nymphs) are flattened and oval shaped, resembling scales. As they age, they cover themselves with "woolly" white waxy filaments. Four nymphal stages are undergone by the woolly whitefly. The first instar or crawler is about 0.3 mm in length; it is the only nymphal stage that is mobile. The second to fourth instars are sedentary; they will often be completely obscured by copious amounts or waxy filaments, droplets of honeydew, and cast skins. The fourth instar serves as the "pupal stage". It is about 0.6 mm in length. The morphology of this insect as described above has long-lasting significance on its control.

Insecticidal control of woolly whitefly sometimes proves difficult, especially with the fourth nymphal stage (pupa) and the surrounding waxy secretions because of restricted penetration of the insecticides. Control can be enhanced by combining with light oil. It can also be improved by timing application at early instar stages. Visible control results can only be noticed after a period of about four weeks or more after the commencement of insecticide application. Therefore, control with insecticide may be quite expensive for small-scale farmers.

Some natural enemies (including entomopathogenic fungi) are known to check the population of these leaf pests [4, 10]. However, their impact is often hampered by human actions such as the indiscriminate application of broad-spectrum pesticides. Environmental factors are also known to influence the abundance and spread of many pests and their natural enemies. Notably, rainfall and relative humidity have been reported to influence woolly whitefly populations [11, 12]. Temperature also influences the population of many pests and their pathogens. However, in the case of the woolly whitefly, it played little role. Instead, it regulated the population of some parasites of the woolly whiteflies [13].

The aim of our study was to monitor the population dynamics of the woolly whitefly *A. floccosus* and its entomopathogenic fungi *Aschersonia* spp. on sweet orange varieties and to assess the sweet orange varietal effect on the woolly whitefly population as well as the impact of entomopathogenic fungi on the woolly whitefly population. Furthermore, the environmental factors affecting the abundance of woolly whitefly and its fungi were monitored with the aim of developing control strategies that are directed to the more vulnerable stages of the pest.

2. Materials and methods

The trials were carried out in 2006 and 2007 at the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. The site was located at 3° 5' E, 7° 3' N and 168 m above sea level; it has a bimodal rainfall pattern with an annual mean accumulation of about 1,278 mm. The mean annual temperature is 26.2 °C with a radiation of 10.7 $MJ \cdot m^{-2}$.

Table I.
Varietal effect on the abundance of the whitefly *Aleurothrixus floccosus* and its entomopathogenic fungi *Aschersonia* spp. on sweet orange varieties in 2006 and 2007 (Nigeria).

Citrus variety	Mean number of *A. floccosus*						Mean number of pupae infected by *Aschersonia* per leaf		% of nymphs infected by *Aschersonia* per leaf	
	Eggs		Nymphs		Adults					
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Agege	2.6 a	4.4 a	1.2 a	1.1 a	3.0 a	3.3 a	3.0 a	2.7 a	71.4 a	71.1 a
Bende	3,4 a	4.5 a	1.9 a	2.3 a	2.4 a	3.4 a	3.0 a	2.3 a	61.2 a	50.0 a
Pineapple	2.2 a	1.0 b	0.9 a	3.6 a	2.4 a	1.6 b	2.5 a	2.2 a	73.5 a	37.9 a
Valencia	2.0 a	1.5 b	1.5 a	1.6 a	2.0 a	1.6 b	2.9 a	2.1 a	65.9 a	56.7 a

Means in the same column followed by the same letters are not significantly different ($P > 0.05$) using Student-Newman-Keuls tests.

For our studies, the sweet orange varieties used were in full production (about 30 years old) and chosen based on earlier infestation studies. Agege, Bende, Pineapple and Valencia Late sweet orange varieties arranged in a randomised complete block design and replicated four times in a portion of a citrus orchard were studied. The varietal effect on woolly whitefly infestation and the impact of its natural enemies *Aschersonia* spp. (entomopathogenic fungi) were assessed for two years.

Three candidate trees aligned next to each other were selected per variety, tagged and used throughout the study. The trees had not been treated with pesticides for 3 years. The phenology of the woolly whitefly and its colonisation by the fungi *Aschersonia* spp. was monitored from January to December during the two years of investigation by sampling 24 leaves per tree at 14-day intervals; there were four replicates per variety, thus totalling 96 leaves sampled per variety. This aspect involved counting eggs, nymphs (including pupae that are the 4th nymphal instars) and adults of the woolly whitefly in detached leaves. For each tree, leaves were sampled at four points along the circumference of the canopy from the outer and inner portions of a branch (*i.e.*, 6 leaves per twig). For each tree, sampled leaves were detached, put in separately labelled paper bags and taken to the laboratory for whitefly and infected nymph population counts using a binocular stereomicroscope. Whitefly stages were distinguished following the description of Gill [14]. Average monthly precipitation, relative humidity and temperature were collated for the period of study by the meteorology station at NIHORT.

Data on woolly whitefly population counts and number of *Aschersonia*-infected nymphs were transformed using square root transformation $(x + 0.5)^{0.5}$. The percentage of colonised *A. floccosus* was estimated as:

$$\frac{\sum \textit{infected nymphs}}{\sum \textit{all developmental stages of woolly whitefly} + \textit{infected nymphs}} \times 100$$

Each set of data was subjected to ANOVA. Means of significantly different tests were separated by applying Student-Newman-Keuls tests using SAS software [15]. Correlation analysis was computed between the population of woolly whitefly nymphs and level of infection by *Aschersonia* spp. Correlation and multiple regression studies were also done for the relationship between the populations of various whitefly developmental stages, its entomopathogenic fungi and weather factors. These included rainfall, relative humidity and temperature. All statistical tests were judged significant at $P \leq 0.05$.

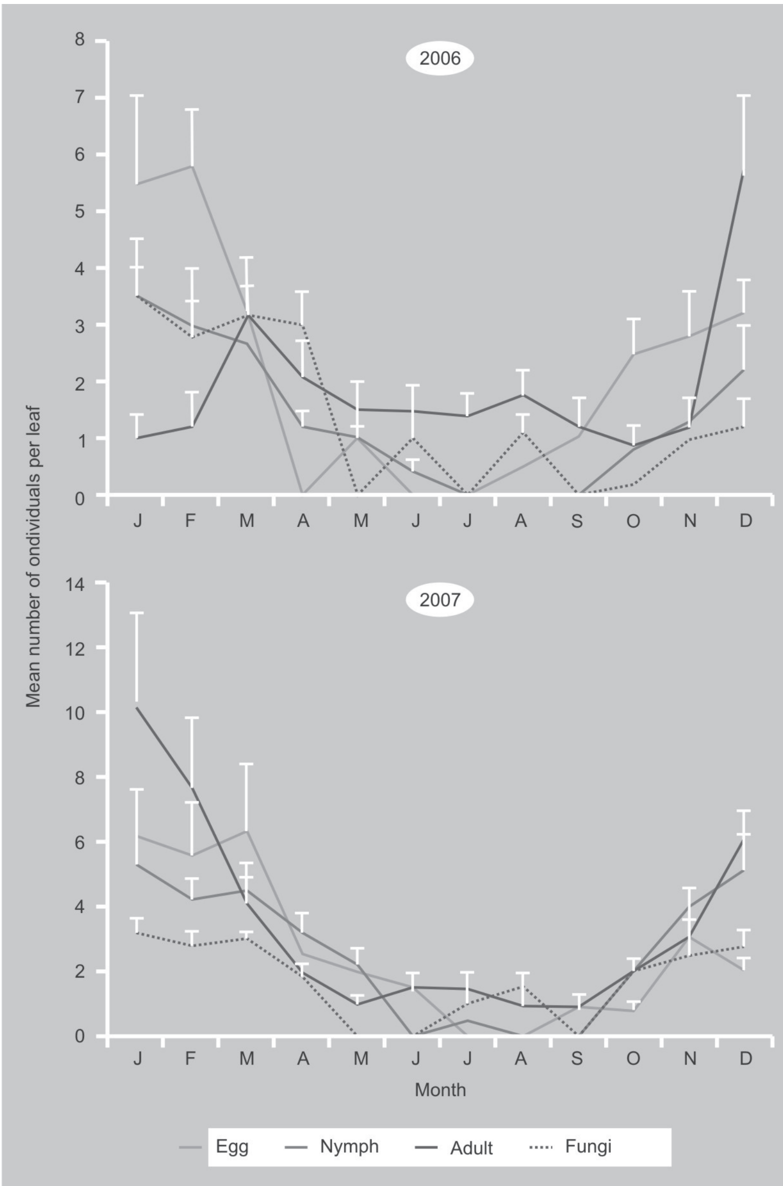

Figure 1.
Population dynamics of the whitefly *Aleurothrixus floccosus* and its entomopathogenic fungi *Aschersonia* spp. in sweet orange varieties in 2006 and 2007 (Nigeria).

3. Results and discussion

Generally, higher numbers of the woolly whitefly eggs, nymphs and adults were observed per leaf on the Agege and Bende sweet orange varieties. Observations also showed that white *Aschersonia* (*Aschersonia goldiana* Saccardo and Ellis) dominated about 95% of infected whitefly nymphs compared with red *Aschersonia* (*Aschersonia aleyrodis* Webber). However, there were no significant differences among the sweet orange varieties in the numbers of the various woolly whitefly stages or the fungi *Aschersonia* spp. in 2006 (*table I*).

In 2007, significantly ($P < 0.05$) higher numbers of eggs and adults of the woolly whitefly were observed on the Bende and Agege varieties than on the Pineapple and Valencia varieties (*table I*). Although the number of nymphs observed on Pineapple was higher than that of the other varieties in 2007, it was not significantly different. The numbers and percentages of nymphs infected by *Aschersonia* spp. per leaf were not significantly different among the varieties in 2006 and 2007. Woolly whitefly eggs and adults were not infected by *Aschersonia* spp. in the two years of observations. Similar to our present findings, Fransen has reported that *Aschersonia* spp. were less effective in infecting the eggs of *Trialeurodes vaporariorum* (Westwood) than the various stages of the nymphs [16]. This was attributed to physical barriers caused by the corium of the egg against the proteases produced by the entomopathogenic fungi [17].

It has been reported that many fungi infect the whitefly complex [10], especially fungi belonging to Deuteromycetes or Coelomycetes. These fungi can infect and kill different whitefly stages and reproduce mainly by asexual spores (conidia). They can be cultured in artificial media, and thus have great potential as biological control agents in Integrated Pest Management [16, 18, 19].

Woolly whitefly egg and nymph populations were high in January and February 2006 (5.5 and 3.5 individuals per leaf, respectively, on average). These populations started declining and became very low from April-August (rainy season). Thereafter, the population of eggs and nymphs began to rise notably again in September and increased steadily until December (dry season) (*figure 1*). In 2006, the adult woolly whitefly population followed the same trend as observed with the other stages; its population rose from January with 1.3 adults per leaf to March with 3.3 adults per leaf (dry season) and declined steadily until May. The population remained low (but not as low as those of the eggs and nymphs did) up to November when it

Table II.
Correlation matrix (coefficient of correlation *r*) for the relationship between weather factors and developmental stages of woolly whitefly (*Aleurothrixus floccosus*) and its fungi pathogen (*Aschersonia* spp.) on sweet oranges in 2006 and 2007 (Nigeria).

Developmental stages	Rainfall		Relative humidity	
	2006	2007	2006	2007
Egg	– 0.60296 *	– 0.76948 **	– 0.4178	– 0.69629 **
Nymph	– 0.77372 **	– 0.84886 ***	– 0.60726 *	– 0.62908 *
Adult	– 0.38334	– 0.79898 **	– 0.31623	– 0.83429 ***
Whitefly (Σ of all stages)	– 0.72617 **	– 0.8819 ***	– 0.602 *	– 0.79289 **
Fungi	– 0.57717 *	– 0.85432 ***	– 0.42609	– 0.55295 *

*, **, ***: significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

increased drastically during the dry season. The dynamics of the *Aschersonia* spp. population (manifested as number of infected nymphs) followed the same trend as nymphal population. *Aschersonia* level was high from January to April (dry season), followed by a period of general decline from May to October, and then a gradual increase until December 2006 (*figure 1*).

In 2007, relatively higher *A. floccosus* egg, nymph and adult populations as well as *Aschersonia* level of infection were observed in January compared with populations of 2006. This was in continuation with the increases observed from November 2006. Due to the presence of high populations of whitefly adults in January, the number of eggs and nymphs per leaf increased slightly in March (*figure 1*). Many generations may be observed in a year depending on environmental conditions. In 2007, the populations of eggs, nymphs and adults of *A. floccosus* started declining after March; egg and nymphal populations became generally very low on leaves between June and September with a corresponding low level of fungal infection (*figure 1*). Thereafter, a gradual increase in the various stages of woolly whitefly and fungi was observed until December. These results corroborate those obtained in 2006 and earlier surveys carried out on the spiralling whitefly *Aleurodicus dispersus* Russel (in central Nigeria) [4]. Similarly, the seasonal distribution of *A. dispersus* reported by Rashid *et al.* [11] showed a peak infestation period of December to February and a decline period of April to August.

Generally, our results showed that weather factors such as rainfall and relative humidity influenced the woolly whitefly population. This was similar to results reported by Rashid *et al.* [11]. Correlation studies between the populations of various developmental stages of the woolly whitefly and its fungi and monthly precipitation or relative humidity showed significant negative relationships (*table II*). These relationships were more pronounced, with significantly higher levels in 2007 (r = –0.76948 to r = –0.8819) than in 2006 (r = –0.38334 to r = –0.77372) (*table II*), probably due to increase in the incipient populations during the 2007 dry season. Changes in rainfall level influence *A. floccosus* and *A. dispersus* populations [11, 12]. The rains tend to affect whitefly developmental stages and thus reduce population, while the favourable breeding conditions of the dry season result in population increases.

The high relative humidity recorded during the 2006 and 2007 periods followed the same trend of negative influence on the populations of woolly whitefly and fungi as rainfall did, with significant coefficients of

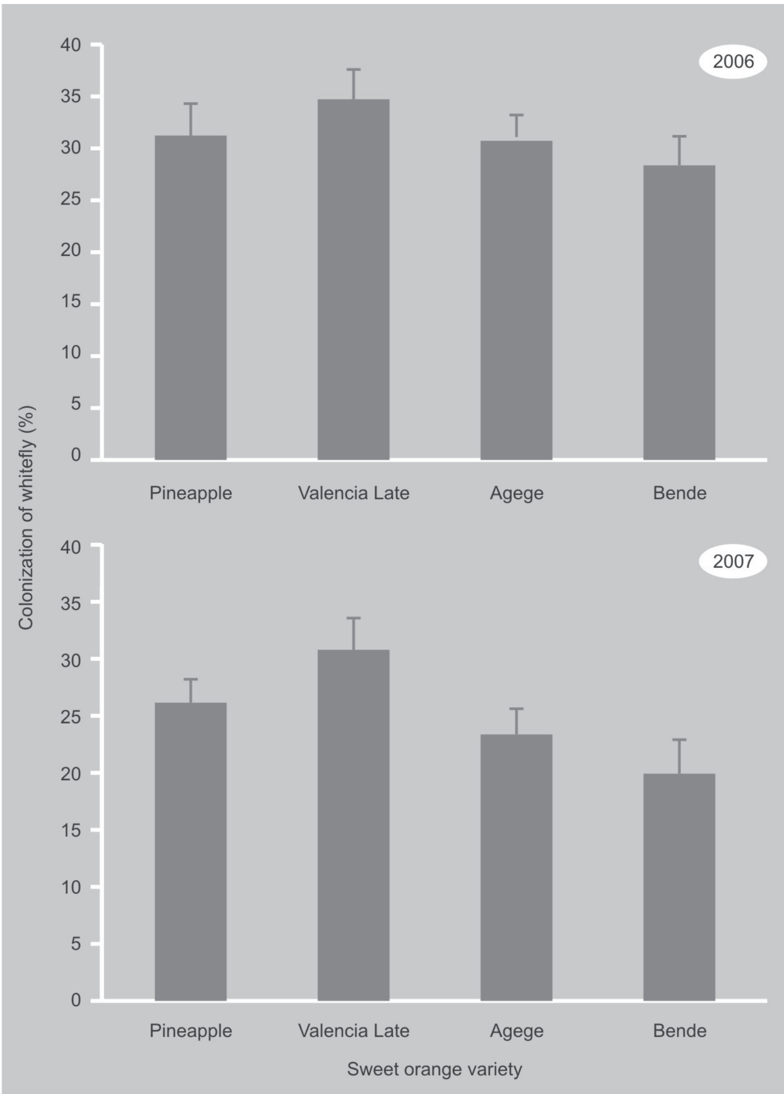

Figure 2.
Percentage colonisation of the whitefly *Aleurothrixus floccosus* by the entomopathogenic fungi *Aschersonia* spp. on sweet orange varieties in 2006 and 2007 (Nigeria) ($n = 4$, $P > 0.05$).

correlation for most of the stages: $r = -0.31623$ to $r = -0.60726$, $n = 12$, in 2006; and $r = -0.55295$ to $r = -0.83429$, $n = 12$ in 2007 *(table II)*. These results were similar to those of Gerling *et al.* [20] in which significant fluctuations due to extreme relative humidity, both high and low, were unfavourable for the survival of immature stages of whiteflies.

Temperature (which ranged from 24.8 °C to 29.1 °C) did not significantly ($P > 0.05$) influence the population of various woolly whitefly stages. The coefficients of correlation r ranged from 0.070365 to 0.469008 in 2006 and 2007 ($n = 12$, $P > 0.05$). This result was similar to that of Miklasiewicz and Walker whereby temperature played a major part in regulating the population of woolly whitefly parasites rather than the woolly whiteflies themselves [13]. When the various weather factors were entered into multiple regression analyses with the various developmental stages of the woolly whitefly and its fungi, significant correlations were obtained in 2006 and 2007 except with adult whiteflies of 2006 for which the correlation was not significant ($R^2 = 0.2$, $n = 12$; $P > 0.05$). High significant coefficients of determination R^2 were obtained in the regression analyses in 2006 ($R^2 = 0.603$ to $R^2 = 0.811$, $n = 12$, $P < 0.05$) and 2007 ($R^2 = 0.736$ to $R^2 = 0.918$, $n = 12$, $P < 0.01$). This shows the combined effect of rainfall, relative humidity and temperature on population dynamics.

Evaluation of the actions of *Aschersonia* spp. showed active colonisation of whiteflies in the years 2006 and 2007. The percentage of colonisation of whitefly (including all developmental stages) by *Aschersonia* spp. ranged between 27% and 35% in 2006, and 21% and 32% in 2007 (*figure 2*). There was no significant difference among the percentages of colonised whitefly in the four varieties assessed. However, there was a linear positive relationship between the population of whiteflies and the level of its colonisation by *Aschersonia* spp. in 2006 ($r = 0.8516$, $P < 0.001$, $n = 12$) and 2007 ($r = 0.7989$, $P < 0.01$, $n = 12$). It indicates that, when there is a high *A. floccosus* population, *Aschersonia* spp. colonisation of the population remains high. This relationship is beneficial if *Aschersonia* is to be considered as a biological control agent. Further population reduction may need the complement of other natural enemies. These include predators [21] and parasitoids (parasitic wasps) such as *Encarsia* and *Eretmocerus* spp. [10], which have shown promise in the control of many species of whiteflies including the woolly whitefly [22].

4. Conclusion

Sweet orange varieties had no significant effect on the population of woolly whitefly. Weather factors, which included rainfall and relative humidity, negatively affected woolly whitefly populations. The latter decreased during the rainy season and increased during the dry season. Naturally occurring *Aschersonia* spp. contributed to the reduction of the woolly whitefly populations on citrus. The maximum 34.5% colonisation of woolly whitefly by *Aschersonia* spp. found in our study suggests that there is room for the introduction of other natural enemies to further enhance the reduction of the woolly whitefly population.

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Dinámica de las poblaciones de la mosca blanca *Aleurothrixus floccosus* (Maskell) en variedades de naranjos de Nigeria y colonización de dicha mosca blanca por hongos entomopatógenos *Aschersonia* spp.

Resumen — **Introducción**. Diversas especies de la mosca blanca atacan los naranjos en Nigeria. La importancia económica de dichas moscas blancas varía de una región a otra, según las condiciones medioambientales dominantes. Se establecieron ensayos con el fin de evaluar la sensibilidad relativa de ciertas variedades de naranjas dulces frente al ataque de la mosca blanca lanosa *Aleurothrixus floccosus* (Maskell), la evolución de sus poblaciones y el impacto de hongos entomopatógenos del género *Aschersonia*. **Material y métodos**. Se emplearon para nuestros ensayos cuatro variedades de naranjas dulces: Agege, Bende, Pineapple y Valencia. Se hizo un seguimiento de la dinámica de las poblaciones de *A. floccosus* y de sus ninfas parasitadas por *Aschersonia* spp. en 2006 y 2007, mediante muestreo de 24 hojas por árbol, en intervalos de 14 días. Durante el periodo de nuestro estudio, se recopilaron datos sobre la media de las precipitaciones mensuales, la humedad relativa y la temperatura. **Resultados y discusión**. Entre las variedades de naranjas dulces estudiadas no se observó ninguna diferencia significativa, ni entre las poblaciones de ninfas de *A. floccosus* ni a nivel de las infecciones ninfales por parte de *Aschersonia* spp. Se observó un mayor número de huevos y de adultos de la mosca blanca en las variedades Agege y Bende en 2006 y 2007, pero los resultados fueron significativamente diferentes sólo en 2007. En el trascurso de los dos años de observación, las poblaciones de huevos, de ninfas y de adultos de *A. floccosus* y de sus hongos entomopatógenos (*Aschersonia* spp.) fueron relativamente elevadas en la estación seca (enero a marzo); bajaron durante la estación de las lluvias y aumentaron de nuevo a partir de octubre. La evaluación del impacto de los hongos *Aschersonia* spp. mostró que colonizaban activamente las moscas blancas. Por regla general, los factores meteorológicos, tales como las precipitaciones y la humedad relativa, influenciaron negativamente el desarrollo de las poblaciones de la mosca blanca. **Conclusión**. La colonización de la mosca blanca por los hongos entomopatógenos fue eficaz para su regulación; por lo tanto, los hongos *Aschersonia* spp. podrían considerarse potenciales agentes de lucha biológica contra *A. floccosus*.

Nigeria / *Citrus sinensis* / ensayos de variedades / control de insectos / Aleyrodidae / dinámica de poblaciones / agentes de control biológico / hongos entomopatogenos / *Aschersonia* / parasitismo / ninfas