

**THE COCONUT MITE, *ACERIA GUERRERONIS*, A
PLAGUE IN AFRICA: PATHWAYS TO ITS MITIGATION
THROUGH BIOLOGICAL CONTROL**

Dissertation

Submitted by

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I affectionately dedicate this thesis to my loving mother, to my wife Denise and my daughters Divine and Fedossia for their love, care and sacrifices.

Glory be to the Lord Almighty

Table of contents

Acknowledgments	4
1. General introduction and thesis outline	6
1.1 Coconut distribution and production.....	7
1.2 Coconut, “The Tree of Life”	7
1.3 The coconut mite <i>Aceria guerreronis</i> and its pest status	9
1.4 References.....	11
2. Objectives and publications of the thesis	17
2.1 Objectives	18
2.2 Core publications	19
3. The coconut mite, <i>Aceria guerreronis</i> , in Benin and Tanzania: occurrence, damage and associated acarine fauna.....	21
4. Season- and fruit age-dependent population dynamics of <i>Aceria guerreronis</i> and its associated predatory mite <i>Neoseiulus paspalivorus</i> on coconut in Benin	45
5. Comparative demography and diet breadth of Brazilian and African populations of the predatory mite <i>Neoseiulus baraki</i> , a candidate for biological control of coconut mite	74
6. Intraguild predation and cannibalism between the predatory mites <i>Neoseiulus neobaraki</i> and <i>N. paspalivorus</i> , natural enemies of the coconut mite <i>Aceria guerreronis</i>	98
7. Morphological variation and reproductive incompatibility of three coconut-mite-associated populations of predatory mites identified as <i>Neoseiulus paspalivorus</i> (Acari: Phytoseiidae).....	117
<i>Presentations at scientific meetings related to the thesis (abstracts published)</i>	141
Summary	143
Abstract German (Zusammenfassung).....	145
Curriculum Vitae	148

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CHAPTER 1

1. General introduction and thesis outline

1.1 Coconut distribution and production

Coconut, *Cocos nucifera* L., is widespread throughout the tropics along the coast and inland of almost all tropical countries, between the tropics of Cancer and Capricorn. Its wide distribution has been favored by its usefulness for humans as well as its ability to adapt to different agro-ecological regions, float on seawater and germinate on beach once washed ashore. The country of origin of the palm is still a controversy; various regions have been mentioned as such by various scientists, from South America to Melanesia, Asia and Madagascar (Ohler, 1999). Ethnological and entomological indications place the centre of diversity in the area of south East Asia and Melanesia (Ohler, 1999). The continental coast and larger islands of Malaysia would be the sites of domestication. Although pantropical, when drifted as far north as Norway, coconut is still capable of germinating. Coconut has been reported from regions with a mean annual precipitation of 700-4200 mm and mean annual temperature of 21-30°C.

Coconut is a perennial plant with a relatively long life cycle and an economic life span of about 60 years depending on growing conditions. According to FAOSTAT (FAO, 2008), the total area harvested in 2006 was approximately 11 million hectares. The Asian and Pacific Coconut Community comprising 17 major coconut producing countries, accounts for more than 90% of the over 54 million tons of world production (APCC: <http://www.apccsec.org/>). The three largest coconut producing countries of the world are Indonesia, Philippines and India (FAO, 2008). Coconut production in Africa accounts for about 3% of the world production, the major producers being located in Western Africa (Ghana, Cote d'Ivoire and Nigeria) and eastern Africa (Tanzania and Mozambique) (FAO, 2008).

1.2 Coconut, “The Tree of Life”

The coconut tree, *Cocos nucifera* L., has a long history of providing man with useful materials for his daily life. It is one of the ten most useful trees in the world. From top to root, every part of the coconut tree is in a way or another essential in farmers' households (Foale, 2003). The growing tip of the palm makes a tasty treat, the “millionaire's salad”; the sheath protecting unopened flowers is often used to fashion shoes, caps and even a kind of pressed helmet for soldiers (Ohler, 1999; Foale, 2003).

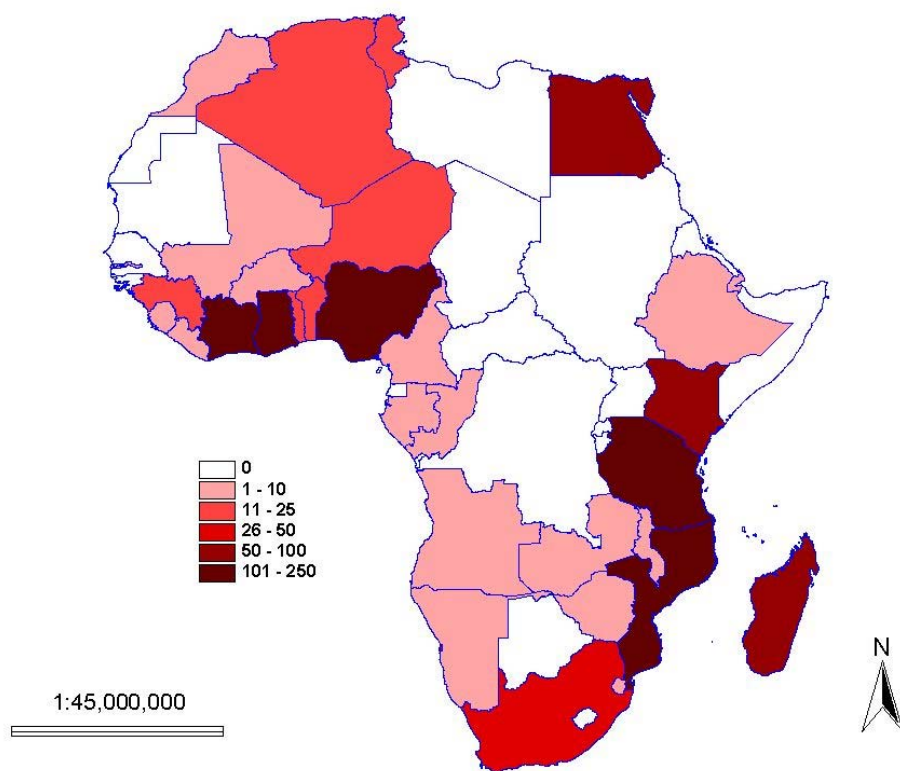


Figure 1: Coconut distribution and production (millions of tons) in Africa

Source: Mapped with Arcview 3.3, 2002, based on data from FAOSTAT (August 2008) (© Koffi Negloh).

Opened flowers provide honey for bees. The coir of the coconut fruits can be woven in strong twine rope and used for padding mattresses, upholstery and life-preservers. The coir is resistant to sea water and is used for cables and rigging on ships, for making mats, rugs, bags, brooms, brushes, and olive oil filters in Italy and Greece. If the fruit is allowed to germinate its cavity fills with a spongy mass called bread that can be eaten raw or toasted in the shell over fire. The fruit shell is hard and fine-grained and may be carved into all kinds of objects (Ohler, 1999).

In many tropical countries, the coconut fruit is an important part of the daily diet; the 5-month old fruit produces a pure and sterile liquid, crystal clear and cool sweet, the coconut water, which is rich in growth substances, minerals and vitamins. The nutmeat of immature coconut fruits is like a custard in

flavor and consistency, and is eaten or scraped and squeezed through cloth to yield a 'cream' or 'milk' used to prepare various dishes (Duke, 1983).

The main product is the oil, extracted from the copra (dried kernel of fruit), which is rich in glycerine and used to make soaps, shampoos, shaving creams, toothpaste, lotions, paints, synthetic rubber, plastics, margarine, ice cream etc. (Duke, 1983). The nutritional and health aspects of coconut oil have been and are thoroughly investigated. Coconut oil has long been erroneously considered harmful to human health but studies in the late 1980s (Enig, 2011; Kabara, 2011) demonstrated that coconut oil, like palm oil, is a healthy fat. Researchers demonstrated that coconut oil may reduce the risk of atherosclerosis, heart disease, cancer, and other degenerative conditions. It helps prevent bacterial, viral, and fungal infections, as a result of containing the antimicrobial component, lauric acid, which is solely found in coconut oil and in breast milk. Coconut oil is rich in medium-chain triglycerides, which provide an immediate source of fuel and energy, and enable the human body to metabolize fat efficiently (Trum Hunter, 2011). The leaves of coconut trees are commonly used for roof thatching. The midribs of the leaves are used for brooms. Coconut wood is more and more being used for building houses and other uses such as furniture or tool handles.

1.3 The coconut mite *Aceria guerreronis* and its pest status

Although a great number of different insects and mites have been observed feeding on the coconut palm, most of them are only sporadic guests. To date the most intractable and most damaging pest of coconut fruit is by far the eriophyid mite, *Aceria guerreronis*, commonly called “the coconut mite”, which was first observed on coconut in the state of Guerrero, Mexico in 1960 (Keifer et al., 1965). Until recently, the pest was known only from Africa and America (Howard et al. 1990; Mariau, 1977; Julia and Mariau, 1979). At the end of the 1990s it was reported for the first time from Sri Lanka and southern India (Ramaraju et al., 2002; Fernando et al., 2002), causing considerable damage to coconut in these countries. *Aceria guerreronis*, recently shown to be likely native to Brazil (Navia et al., 2005), is a tiny (0.2 mm long) worm-like organism, which populations develop beneath the perianths (floral bracts) of coconut fruits, feeding on the epidermal meristematic tissues. The earliest symptom of coconut mite damage is the appearance of white streaks originating from beneath the perianth of fruits

(Figure 4). The streaks eventually enlarge and develop into necrotic and suberized tissues on the fruit surface (Figures 3A and 5) (Cardona and Potes, 1971). The damages may result in deep fissures in the fruit pericarp, distortion of the fruit, reduction in fruit size and weight, and a decline in copra yield (Julia and Mariau 1979, Hall et al., 1980). Yield losses attributable to *A. guerreronis* damages range from 10 to 70% in many countries (Hernandez, 1977; Moore et al., 1989). In studies by Mariau and Julia (1970), cited in Julia and Mariau (1979), copra losses induced by *A. guerreronis* in Benin ranged from 6 to 18%, with a grand average of 10%. However, this loss was very likely underestimated, as damage to coconut is currently widespread and more severe in major growing areas of this country (Negloh, personal observations). Two surveys conducted in Tanzania in 1993 and 1996 revealed damage by *A. guerreronis* on 70 to 100% of sampled fruits, with an associated crop loss of 34% (Seguni, 2002). Coconut farming is one of the most important branches of agricultural production in the coastal region of Tanzania (Seguni, 2002). According to FAO, harvested areas in 2006 reached 312796 ha in Tanzania (FAO, 2010).

To mitigate the significant impact of the pest *A. guerreronis* on coconut fruit production, control measures initially focused on the use of chemical pesticides in continuous applications. Some chemicals such as monocrotophos and chinomethionate sprayed on the fruit bunches significantly reduced mite damage (Hernandez, 1977; Mariau, 1977; Julia and Mariau, 1979; Moore, 2000), but due to constraints such as the necessity of repeated applications, the associated financial costs and threats to the environment, chemical control appeared to be non-feasible in the long run. Because of the adventive nature of the pest in Asia and Africa, exploring the possibilities of controlling it biologically has been considered a priority (Moraes and Zacharias, 2002). Recent investigations in the presumable area of origin of the pest, Brazil, as well as in areas invaded by the pest revealed several predatory mite species associated with *A. guerreronis* (Fernando et al., 2003, Lawson-Balagbo et al., 2008), of which a few deserve further attention. These are *Neoseiulus baraki* Athias-Henriot, *N. paspalivorus* DeLeon (Acari: Phytoseiidae), and *Proctolaelaps bickleyi* Bram (Fernando et al., 2003; de Moraes et al., 2004; Aratchigüe, 2007; Lawson-Balagbo et al., 2007ab, 2008).

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Figure 2: A young hybrid coconut plantation in southern Benin (© Koffi Negloh)

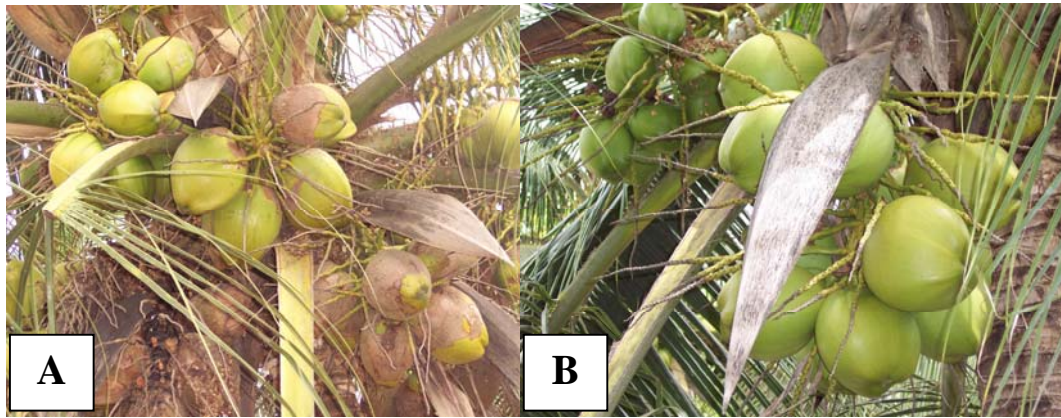


Figure 3: Bunches of *Aceria guerreronis*-damaged (A) and -undamaged (B) coconut fruits (© Koffi Negloh)



Figure 4: Freshly damaged young coconut fruit (© Koffi Negloh)



Figure 5: Different levels of *Aceria guerreronis* damage on coconut fruits (© Koffi Negloh)



Figure 6: Colony of *Aceria guerreronis* beneath coconut bracts (© Koffi Negloh)

CHAPTER 2

2. Objectives and publications of the thesis

2.1 Objectives

The overall objective of the present thesis was to investigate the occurrence and ecology of natural enemies associated with the coconut mite, *Aceria guerreronis*, in Africa and identify possible pathways to the mitigation of the damage caused by this pest. The core of the thesis comprises five publications.

The first step was to explore Southern Benin in West Africa and Eastern Tanzania in East Africa, which are the main coconut growing areas on the African continent, to assess the distribution of the pest *Aceria guerreronis* and the extent of its damages in order to update the knowledge about the status of this pest gathered in Africa in the 1980s. We furthermore examined the associated acarine fauna with special focus on natural enemies of the family Phytoseiidae, which were the most common type of natural enemies found in association with the pest under the bracts, though the occurrence and distribution of phytoseiid species varied within and between the two countries.

Based on the results of the first publication, the survey in Benin and Tanzania, in the second publication, we investigated the population dynamics of the pest *A. guerreronis* and its most common natural enemy, the predatory mite *Neoseiulus paspalivorus*, in Benin. In this study, we focused on the fluctuations of the pest and predator populations as a function of seasons and fruit ages. The numerical and proportional fruit infestations by both pest and predator were investigated, and the ages of coconut fruit at which the pest and the predator first occurred on the fruits were determined.

In the third publication, we explored the life history of individuals of two populations of *N. baraki*, one from Benin and the second originating from Brazil, where a similar survey to the one described in the first publication of the current thesis, had been carried out in the course of a related doctorate study. The study focused on determining the demographic parameters of both populations on five different food sources, including *A. guerreronis*, the target pest of this thesis. *Aceria guerreronis* turned out to be the preferred prey of both populations and resulted in maximum predator population growth rates.

The fourth publication investigated trophic interactions, namely cannibalism and intraguild predation (IGP), of the predatory mites *Neoseiulus paspalivorus* and *N. neobaraki*, the latter being the third phytoseiid predator associated with *A. guerreronis* and encountered in Benin and Tanzania. *Neoseiulus neobaraki* was the most common in Tanzania, which triggered our interest to include this species in the

investigations of this publication. Cannibalism and IGP were tested on predator larvae in presence and absence of the shared herbivorous prey *A. guerreronis*. Both predator species appeared to refrain from cannibalism more than from IGP when *A. guerreronis* was provided. IGP was mutual but asymmetric with *N. neobaraki* being a much greater IG predator of *N. paspalivorus* than vice versa.

In the fifth publication, we assessed the morphology and reproductive compatibilities of three geographically separated populations of *N. paspalivorus*, originating from Benin, Ghana and Brazil, to determine whether they belong to the same or different species. Morphometric and biological traits were compared and inter-population crosses, with subsequent progeny examinations, carried out. The outcome indicated that the three populations differed and may thus represent three different species.

2.2 Core publications

- (1) Negloh K, Hanna R, Schausberger P (2008) Comparative demography and diet breadth of Brazilian and African populations of the predatory mite *Neoseiulus baraki*, a candidate for biological control of coconut mite. Biol Control 46: 523-531. DOI 10.1016/j.biocontrol.2008.04.022.
- (2) Negloh K, Hanna R, Schausberger P (2010) Season- and fruit age-dependent population dynamics of *Aceria guerreronis* and its associated predatory mite *Neoseiulus paspalivorus* on coconut in Benin. Biol Control 54: 349-358. DOI 10.1016/j.biocontrol.2010.06.007.
- (3) Negloh K, Hanna R, Schausberger P (2011) The coconut mite, *Aceria guerreronis*, in Benin and Tanzania: occurrence, damage and associated acarine fauna. Exp Appl Acarol 55: 361-374, DOI 10.1007/s10493-011-9474-0.
- (4) Negloh K, Hanna R, Schausberger P (2012) Intraguild predation and cannibalism between the predatory mites *Neoseiulus neobaraki* and *Neoseiulus paspalivorus*, natural enemies of the

coconut mite *Aceria guerreronis*. Exp Appl Acarol. submitted.

- (5) Nazer Famah NS, Hanna R, Zannou I, de Moraes GJ, Negloh K, Sabelis MW (2011) Morphological variation and reproductive incompatibility of three coconut-mite-associated populations of predatory mites identified as *Neoseiulus paspalivorus* (Acari: Phytoseiidae) Exp Appl Acarol 53: 323-338. DOI 10.1007/s 10493-9413-5.

CHAPTER 3

3. The coconut mite, *Aceria guerreronis*, in Benin and Tanzania: occurrence, damage and associated acarine fauna

The coconut mite, *Aceria guerreronis*, in Benin and Tanzania: occurrence, damage and associated acarine fauna

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Abstract

The coconut mite *Aceria guerreronis* (Eriophyidae) is considered the most important pest of coconut fruits in Africa; however, quantitative knowledge about its distribution and abundance is lacking. We conducted four diagnostic surveys – three in Southern Benin and one along the coast of Tanzania - to determine the distribution of *A. guerreronis* and the severity of its damage to coconut fruits, as well as the diversity and abundance of other associated mites and potential natural enemies. *Aceria guerreronis* was found in all visited plantations with the percentage of damaged fruits varying considerably among plantations – 67-85% in Benin and 43-81% in Tanzania. Overall, 30-40% of the fruit surfaces were damaged by *A. guerreronis*. Damage severity increased with fruit age and negatively affected fruit weight of 7 to 12 months-old fruits. *Aceria guerreronis* was by far the most abundant mite on coconut fruits but its abundance depended on fruit age. The highest densities of *A. guerreronis* were observed on 3 to 4 months-old fruits. *Neocypholaelaps* sp. (Ameroseiidae) was the most abundant mite on inflorescences. Three species of predatory mites (Phytoseiidae) - *Neoseiulus baraki*, *N. neobaraki* and *N. paspalivorus* - were the most commonly found predatory mites beneath the coconut bracts in association with *A. guerreronis*. *Neoseiulus neobaraki* was the prevailing predator in Tanzania while *N. paspalivorus* was the most frequent predator in Benin. Other mites found beneath the bracts were the herbivore *Steneotarsonemus furcatus* (Tarsonemidae) and the detritivore and fungivore *Tyrophagus putrescentiae* (Acaridae).

Keywords: *Cocos nucifera*, Eriophyidae, phytoseiidae, predatory mites, mite diversity.

Introduction

Coconut, *Cocos nucifera* L. (Arecaceae), is widespread throughout the tropics. Its geographical distribution has been favored by its adaptability to wide range of climatic and vegetational regions, floatability in seawater followed by germination on beaches once washed ashore, and its usefulness to humans (Foale 2003). With approximately 11 million ha harvested worldwide in 2006 (FAO 2010), coconut palm is considered one of the ten most important trees for humankind in the world, providing food and income for hundreds of millions of people (e.g. APCC 2010).

Aceria guerreronis Keifer (Eriophyidae), commonly called the coconut mite, presently is the most important pest of coconut fruits (Howard et al. 2001). This mite pest had been known for decades from the Americas and Africa (e.g. Mariau and Julia 1970; Howard et al. 1990), but it has been detected only recently in India and Sri Lanka, two major coconut producing countries (Fernando et al. 2002; Ramaraju et al. 2002). *Aceria guerreronis*, likely originating from South America (Navia et al. 2005), is a tiny worm-like organism that typically inhabits the area beneath the perianth (i.e. the floral bracts) of coconut fruits feeding on the tender meristematic tissue (Howard and Abreu-Rodriguez 1991; Aratchige 2007; Lawson-Balagbo et al. 2007a). Physical injuries resulting from feeding develop into necrotic and suberized tissues on the fruit surface. Infested fruits later become distorted and stunted due to uneven growth, leading to reductions in copra yield and premature fruit drop (e.g. Julia and Mariau 1979; Hall 1981). Yield losses attributable to damage by *A. guerreronis* range from 10 to 70% in many countries (e.g. Hernandez 1977; Moore et al. 1989). In the 1960s in Benin, copra losses due to *A. guerreronis* ranged from 6 to 18%, with an overall average of 10% (Mariau and Julia 1970). However, this loss was likely underestimated, as damage to coconut is presently widespread and more severe in major growing areas of Benin (K. Negloh, personal observations, years 2004 to 2010). Results of two surveys conducted in Tanzania in 1993 and 1996 revealed that 70 to 100% of sampled fruits were damaged by *A. guerreronis*, with an associated crop loss of 34% (Seguni 2002). These are substantial losses in a country (Tanzania) where coconut farming is one of the most important branches of agricultural production in the coastal region (Seguni 2002). In 2006, harvested coconut areas in Tanzania were estimated at 312.796 ha (FAO, 2010).

Common control measures against *A. guerreronis* were traditionally based on repeated applications of pesticides. Although some chemicals such as monocrotophos and chinomethionate

sprayed on fruit bunches significantly reduce mite damage (Hernandez 1977; Mariau 1977; Julia and Mariau 1979), the need of repeated applications renders chemical control economically and environmentally non-feasible in the long run. Several researchers therefore recommended biological control as an environment-friendly alternative to pesticides (e.g. Moore 2000; Moraes and Zacarias 2002; Perring 2002; Lawson-Balagbo et al. 2007ab). Various predatory mites, particularly from the family Phytoseiidae, have been found associated with *A. guerreronis* (Mariau 1979; Moraes and Zacarias 2002; Moraes et al. 2004; Aratchige 2007; Lawson-Balagbo et al. 2007ab, 2008) but their effect on *A. guerreronis* populations has not been extensively addressed (Reis et al. 2008; Negloh et al. 2010). Entomopathogenic fungi such as *Hirsutella thompsonii* Fisher have been found infecting *A. guerreronis* (Julia and Mariau 1979; Hall et al. 1980; Cabrera 2002; Moore 2000; Gopal and Gupta 2001; Gopal et al. 2002; Fernando et al. 2007). Recent efforts to develop *H. thompsonii* as a biopesticide against *A. guerreronis* show promising results but the duration of spore viability remains a major obstacle (Edgington et al. 2008).

In the present study, we report on diagnostic surveys conducted in coconut growing areas in southern Benin and along the coast of Tanzania to assess the current status of *A. guerreronis*. These surveys are essential components of a multi-institutional research project on the development of biological control strategies against the coconut mite in Africa and elsewhere in the world. Our objectives were to determine the occurrence and abundance of *A. guerreronis*, the incidence and severity of damage caused by this pest, and the diversity of mites associated with it on coconut fruits with emphasis on potential natural enemies.

Materials and Methods

Three diagnostic surveys were conducted in 66 plantations of the three major growing areas of southern Benin, - Mono, Atlantique and Oueme provinces, (located between 06°15'20'' N, 01°42'54'' W and 05°50'50'' N, 02°36'39'' W) - from July to August 2004, February to March 2005, and June to July 2005 (Fig. 1A). These periods corresponded respectively with the onset of the short dry season, the end of the long dry season and the long rainy season in all provinces. The Beninese plantations were selected randomly at distances of 2 to 10 km., depending on plantation frequency. In August 2005 one diagnostic survey was conducted in Tanzania, along the Indian Ocean coast from Manza (04°49'17'' S, 039°08'59'' E) (district of Tanga in the north) to Ziwani (10°20'90'' S, 040°14'99'' E) (district of Mtwara in the south) (Fig. 1B). This period coincided with the end of the rainy season in the north (from Manza to Saadani) and the dry season from Bagamoyo, at approximately mid-coastal zone, to Ziwani (Mtwara) in the south. The eighteen Tanzanian plantations were selected randomly at 10 to 45 km distance from each other. The longer distances between plantations in Tanzania were necessary due to the much bigger length of the coastal areas in Tanzania compared with Benin. In most surveyed plantations, minimal management was applied, mainly including weeding. In few plantations, farmers tethered cows to the palms in order to fertilize the soil with their excrements or they dug holes around the palm trees and dumped dead organic matter such as shed palm leaves and cut weeds in the hole. In all plantations, mature coconut fruits were harvested at intervals of 3 to 4 months.

Incidence and severity of damage caused by A. guerreronis

Incidence and severity of fruit damage caused by *A. guerreronis* were assessed *in situ* on 10 randomly selected palms per plantation by classifying all coconut fruits on each tree on the basis of the extent of characteristic *A. guerreronis* damage visible on fruit surfaces. Binoculars were used where trees were not reachable by a ladder. Coconut fruits were grouped into four grades – based on the percentage of fruit surface damaged by *A. guerreronis* (Moore et al. 1989): grade 1 (0%), grade 2 (1 to 10%), grade 3 (11 to 25%), grade 4 (26 to 50%), grade 5 (>50%).

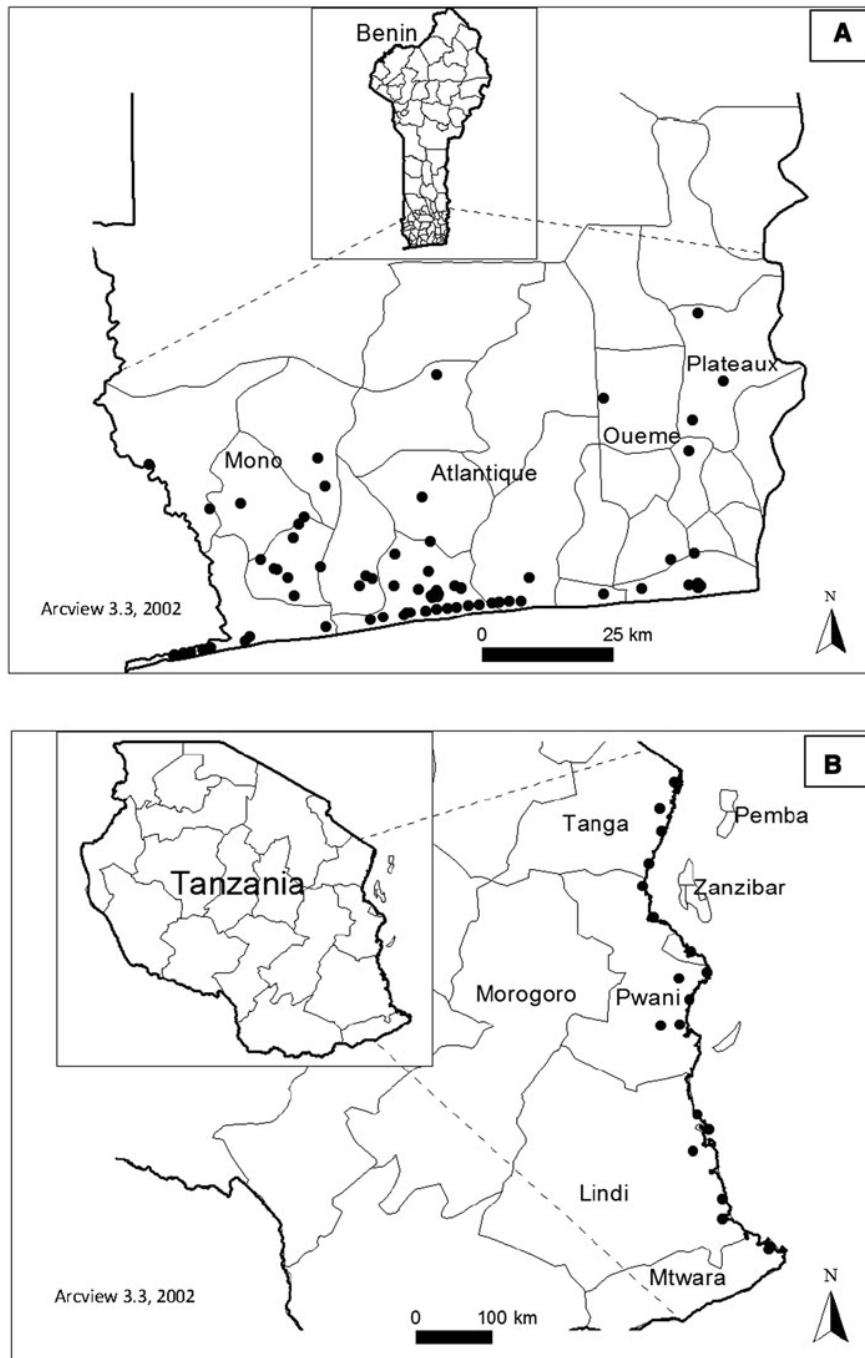


Fig. 1 Sites sampled from July 2004 to July 2005 in Benin (a) and in August 2005 in Tanzania (b) (Mapped with Arcview 3.3, 2002 by K. Negloh, IITA)

Distribution and abundance of A. guerreronis and identification of associated mites

In each plantation, coconut fruits were sampled from 10 and 12 palms in Benin and Tanzania, respectively, to assess the distribution and abundance of *A. guerreronis* and identify other mites inhabiting coconut fruits and inflorescences. Fruit sampling was based on fruit bunch age classes (FBAs) defined as follow: FBA₁ (fruit bunches 1 to 3); FBA₂ (fruit bunches 4 to 6); FBA₃ (fruit bunches 7 to 9) and FBA₄ (fruit bunches 10 to 12) (Negloh et al. 2010). The number of a particular bunch corresponds approximately to its age in months. Fruit classification was based on the knowledge that a new inflorescence (the prospective bunch) is produced approximately every month (Moore and Alexander 1987; Foale 2003; K. Negloh personal observations). Each succeeding bunch, from the top of the palm, is therefore a month older than the previous one. Bunch 1 is that of just fertilized fruits (approximately 1 month). Inflorescences were not included in FBAs but sampled separately. Fruit samples were collected from FBA₁₋₃ in Tanzania and from FBA₁₋₄ in Benin. We considered only the first 3 FBAs in Tanzania because of the long travel distances, the limited time allotted to the survey and the fact that previous observations in Benin indicated that older fruits harbor very few mites and do not show significant damage variations (Negloh et al. 2010). One fruit was sampled from each FBA plus one branch of inflorescence per palm when available. Samples were taken only from palms bearing at least one bunch of each FBA.

Sampled fruits were examined on-site with a 10X magnification head lens immediately after removal from the palm. Mites found on the fruit surface were collected with a brush and preserved in 75% ethanol. Each fruit was then labeled, placed in a paper bag and brought to the laboratory for further processing. In the laboratory, the bracts of each fruit were sequentially and carefully removed to uncover the meristematic zone. Mites other than *A. guerreronis* present beneath the bracts were counted and stored in 75% ethanol for further slide-mounting and species identification. Abundance of *A. guerreronis* was assessed using a methodology similar to that developed by Siriwardena et al. (2005). Bracts as well as the meristematic zone of the fruit and some distance away on the exocarp (2-5 cm depending on fruit size) were rinsed with 30 ml detergent solution into a small container. The solution was vigorously shaken to obtain a homogenous distribution immediately before 1.0 ml aliquot was drawn from the solution and placed in counting cells (Costar® Brand Cell Culture Clusters, 24 cells of 3.4 ml volume each). All individuals of *A. guerreronis* present in the 1.0 ml aliquot were

counted using a stereomicroscope. Other mites in the solution were counted and added to those previously collected in alcohol. Abundance of *A. guerreronis* per fruit was then estimated by multiplying the obtained values by a factor of 30 (the total volume of the rinse solution). Inflorescences were dissected under a stereomicroscope and mites found were collected and preserved in 75% ethanol. For each sampled fruit, characteristics such as its age, FBA, damage grade, and weight were recorded.

Statistical analyses

All statistical analyses were performed using SAS 9.1 (SAS Institute, 2005). Logistic regressions were used to assess variations in the incidence of damage among plantations, survey periods and growing areas. Based on grouping of fruits in damage grade classes, a Severity Index (SI) was calculated for each palm as $SI = \frac{\sum (X_i * i)}{\sum X_i}$ where X_i is the number of damaged fruits of grade i (i varies from 2 to 5; undamaged fruits were not included). SI values were used in general linear models (GLM) to compare damage severity among survey periods and coconut growing areas for each country separately. The same analysis was performed on arcsine-transformed proportions of damaged fruits per palm. Furthermore, based on season similarities (occurrence of the dry season) the results of the Tanzania survey, conducted in August, were compared to those of the second survey in Benin, which was conducted from February to March.

For each country, log-transformed densities of *A. guerreronis* (only mobile stages) were compared among fruit ages by analysis of variance (ANOVA). Mixed model analyses were used to assess the effect of survey period (random effects) and growing area (fixed effects) on *A. guerreronis* abundance and damage severity. The effect of damage severity on fruit weight within each FBA was assessed with general linear model (GLM) and subsequent Bonferroni multiple comparison tests on pooled data for both countries.

Results

Incidence and severity of damage caused by A. guerreronis

In Benin coconut fruit damage by *A. guerreronis* was observed on 90 to 100% of palms across plantations and throughout the three surveys. Overall mean percentage of damaged fruits per palm was $73 \pm 0.01\%$ (Fig 2a), but fruit damage incidence differed among provinces (mean \pm SE: 67 ± 0.02 , 70 ± 0.01 and $85 \pm 0.01\%$ damaged fruits per palm in Atlantique, Mono and Oueme, respectively) (logistic regression: $\chi^2 = 3923.72$, $P < 0.0001$). Of the coconut fruit showing visible mite damage, $39 \pm 0.01\%$ were severely damaged ($\geq 25\%$ of the fruit surface damaged) (Fig. 2a). Damage severity indices differed among survey periods (GLM: $F_{2,815} = 6.99$, $P < 0.0001$) and growing areas (GLM: $F_{2,815} = 4.36$, $P = 0.0131$). The interaction between survey period and growing area was also highly significant ($F_{4,815} = 18.90$, $P < 0.0001$), indicating that the difference in the severity indices among survey periods varied with the growing area. Mean separation using Bonferroni showed no difference between Mono and Oueme and between the first and second survey ($P > 0.05$). Overall mean severity indices were 3.70 ± 0.05 , 3.83 ± 0.04 and 3.82 ± 0.05 , respectively, in Atlantique, Mono and Oueme.

Incidence of *A. guerreronis* damage along the Tanzanian coast was 100%. All plantations surveyed and all palms in those plantations showed visible symptoms of *A. guerreronis* damage. All other plantations inspected informally midway between two surveyed sites were damaged also (K. Negloh, personal observations). Damage incidence assessed on-site varied among plantations from 43 ± 0.02 to $81 \pm 0.01\%$ of fruits damaged per palm. Overall, $63 \pm 0.01\%$ of coconut fruits per palm were damaged and $43 \pm 0.01\%$ of them were severely damaged, i.e. $\geq 25\%$ of the fruit surface was damaged (Fig. 2b). Logistic regression revealed highly significant differences among plantations (Maximum Likelihood $\chi^2 = 184.36$; $P < 0.0001$), with almost 40% difference between the maximum and minimum damage incidence. Damage severity indices ranged from 3.20 to 4.10 among plantations with an overall mean of 3.77 ± 0.05 (GLM: $F_{10,88} = 3.33$, $P = 0.001$). Comparisons between the Tanzanian survey and the second Beninese survey showed similarities in damage incidence (73 vs. 63%) (GLM: $F_{1,347} = 0.14$, $P = 0.701$) and severity indices (3.77 ± 0.05 vs. 3.88 ± 0.03) (GLM: $F_{1,347} = 3.22$, $P = 0.074$).

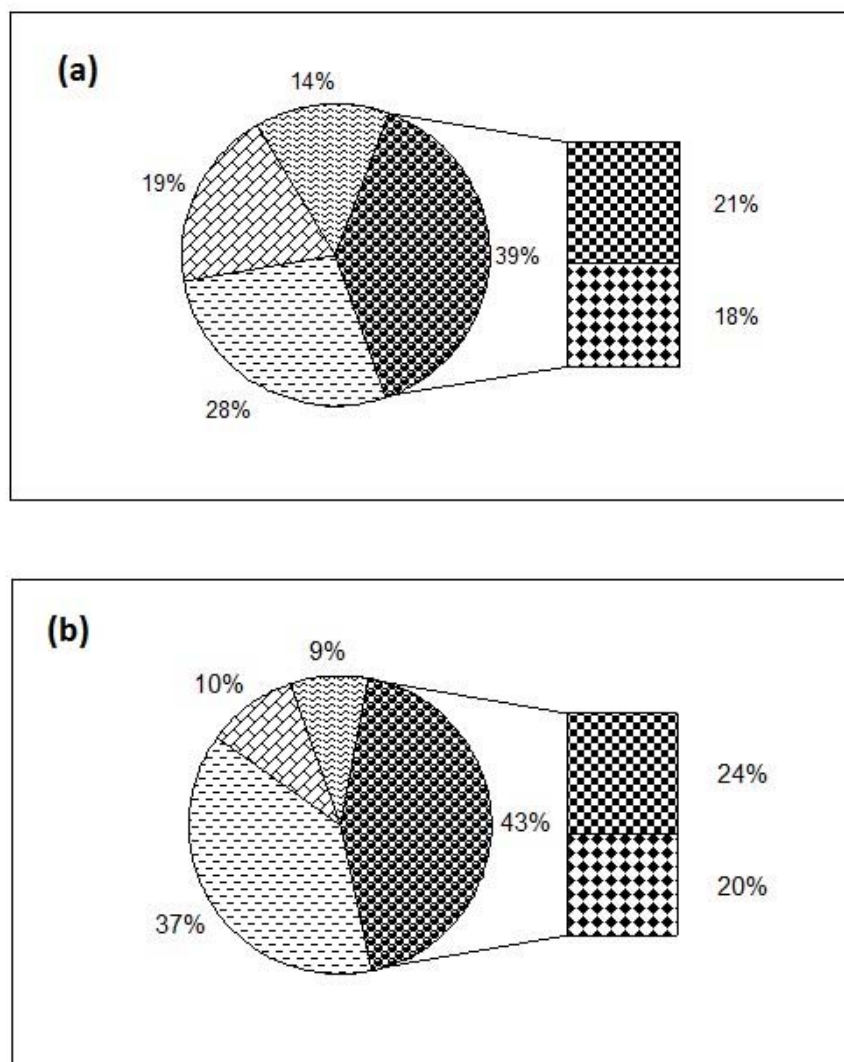








Figure 2: Overall mean percentage of sampled fruits per damage severity grade (1 , 2 , 3 , 4 , 5 ; 4+5 ) in Benin (a) and Tanzania (b). Grades correspond to % fruit surface damaged (Moore et al. 1989) as follows: 1 (0%), 2 (1 to 10%), 3 (11 to 25%), 4 (26 to 50%), 5 (>50%).

In both countries damage severity increased with fruit age (Fig. 3). Damage severity increased almost linearly from 1 to 5 months old, while it remained constant on older fruits (6 to 12 months old). Undamaged fruits were mostly younger fruits (1 to 3 months) with a rare occurrence of severe damage. The highest damage grades were observed on 4 to 12 months old fruits (Fig. 3).

Damage grade affected fruit weight in FBA₁, FBA₃ and FBA₄ (GLM: $F_{4, 710} = 77.01$ $P < 0.0001$, $F_{4,84} = 4.95$, $P = 0.0012$ and $F_{4,75} = 5.45$, $P = 0.0007$ for FBA₁, FBA₃ and FBA₄, respectively) but not in FBA₂ (4 to 6 months old fruits) (GLM: $F_{4,87} = 0.35$, $P = 0.85$) (Fig. 4). Fruit weight differed between damage grades 2 and 5 in FBA₃₋₄, between grades 4 and 5 in FBA₃, and between grades 2 and 4 in FBA₄ (Bonferroni, $P < 0.05$ for each pairwise comparison).

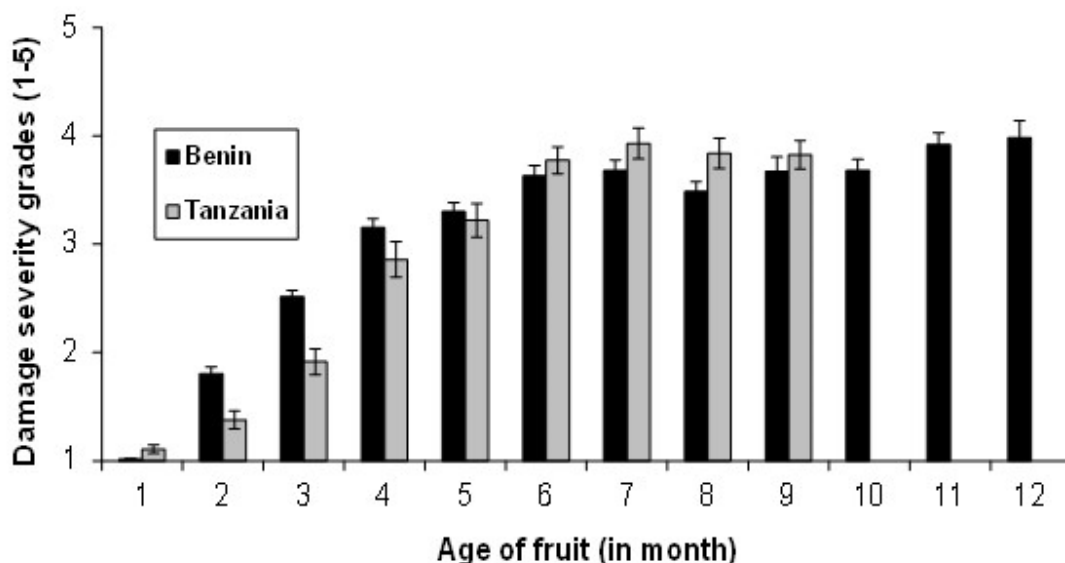


Figure 3: Damage severity grade (1 to 5; mean \pm SE) in relation to coconut fruit age. Grades correspond to % fruit surface damaged (Moore et al. 1989) as follows: 1 (0%), 2 (1 to 10%), 3 (11 to 25%), 4 (26 to 50%), 5 (>50%).

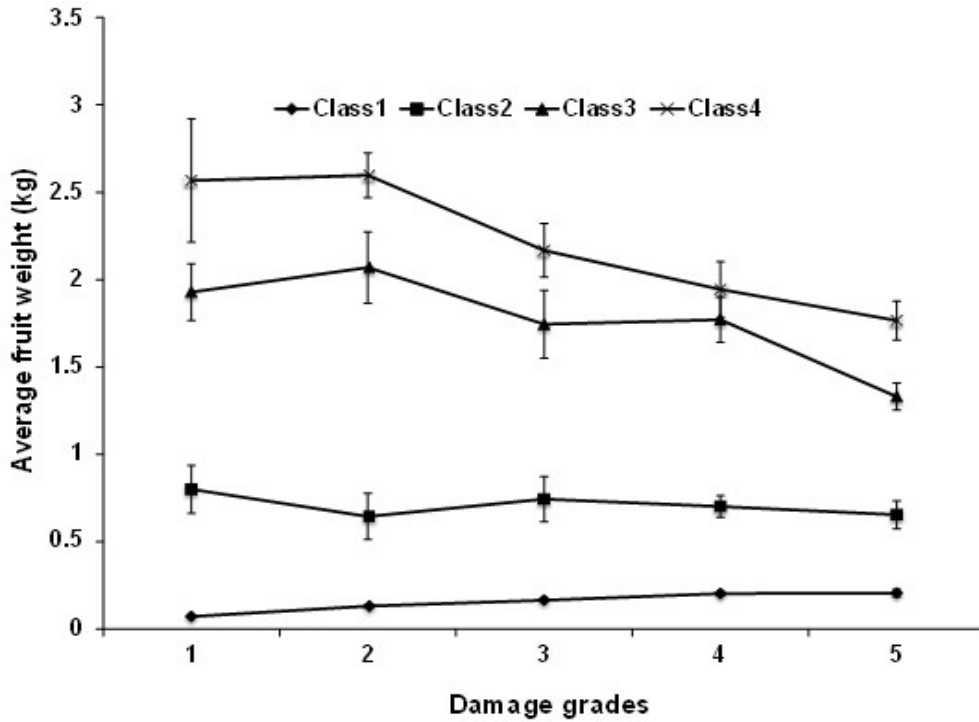


Figure 4: Weight of coconut fruits (mean \pm SE) in relation to damage severity grade in four fruit bunch age classes (FBA₁₋₄). FBA₁ (bunches 1 to 3); FBA₂ (bunches 4 to 6); FBA₃ (bunches 7 to 9) and FBA₄ (bunches 10 to 12). Grades correspond to % fruit surface damaged (Moore 1989) as follows: 1 (0%), 2 (1 to 10%), 3 (11 to 25%), 4 (26 to 50%), 5 (>50%).

Abundance of Aceria guerreronis

In Benin, the abundance of *A. guerreronis* varied from 0 to a maximum of 46,200 individuals per fruit, the latter observed on a 3 month-old fruit. Average density per fruit varied from 430 to 2900 individuals among Benin plantations (Fig. 5). In general, *A. guerreronis* population densities were higher in Benin than in Tanzania where densities varied from 97 to 1266 mites per fruit among plantations (Fig. 5). In Tanzania, the minimum and maximum counts were 0 and 22680, with the latter, as in Benin, observed on a 3 months old fruit.

Abundance of *A. guerreronis* varied greatly among fruit ages (Fig. 5) in both countries (ANOVA: $F_{11,734} = 13.32$, $P < 0.001$ for Benin and $F_{8,615} = 13.95$, $P < 0.001$ for Tanzania). There was an almost exponential increase in *A. guerreronis* densities from 1 to 3 or 4 months old fruits followed by a gradual decrease in abundance on 5 to 12 months old fruits (Fig. 5). The lowest average densities

were observed on 1 month old fruits followed by 9 or 12 months old fruits in Tanzania and Benin, respectively. Variability in *A. guerreronis* densities was the highest in FBA₁ (1 to 3 months old fruits) followed by FBA₄ (9 to 12 months old fruits). Their respective coefficients of variation (CV) were 0.61 and 0.42, while they were 0.27 for FBA₂ and 0.19 for FBA₃. Total densities were highest in FBA₂ and lowest in FBA₄ (10 to 12 months old fruits) (Fig. 5).

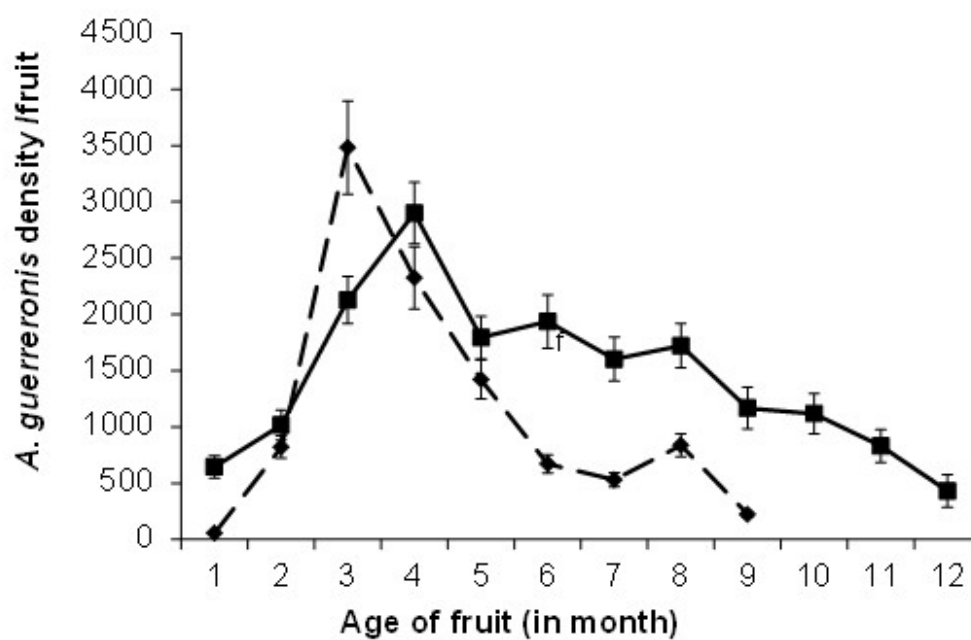


Figure 5: Population density of *Aceria guerreronis* (mean \pm SE per fruit) in relation to fruit age in Benin (solid line) and Tanzania (broken line).

Other mites found on coconut fruits and inflorescences

Mites other than *A. guerreronis* found on coconut fruits in Benin and Tanzania belonged to the families Phytoseiidae, Ascidae, Acaridae, Tarsonemidae, Bdellidae, Eupodidae, Tydeidae, Cunaxidae and Tenuipalpidae. They were encountered on the exocarp or beneath the floral bracts of the fruits, where *A. guerreronis* resides. Specimens of the family Ameroseiidae were found in very large numbers on inflorescences but not on fruits (Table 1).

In Tanzania, four phytoseiid species were identified: *Neoseiulus baraki* Athias-Henriot, *N. neobaraki* Zannou, Moraes & Oliveira, *N. paspalivorus* De Leon, and *Amblyseius largoensis* (Muma) (Table 1). The first three species were found in the micro-habitat beneath coconut bracts, closely associated with their prey *A. guerreronis*, while *A. largoensis* specimens were collected mostly from the fruit surface outside the bracts. *Neoseiulus neobaraki* was the most abundant and most frequently predator on coconut fruits in Tanzania (Table 1). It was present in 17 of the 18 plantations surveyed, while only 42 and 62 specimens of *N. paspalivorus* and *N. baraki*, respectively, were collected from only four and five plantations (Table 1). In one location, Ras Matuso, *N. neobaraki* and *N. paspalivorus* occurred together on two palms and one fruit. Other mites often collected from beneath the bracts were the tarsonemid *Steneotarsonemus furcatus* De Leon and the acarid *Tyrophagus putrescentiae* Schrank. Only one specimen of Ascidae, *Lasioseius* sp., was found on the fruit surface. Furthermore, the bdellids *Bdella distincta* Baker & Balock and *Spinibdella* sp. Thor and the eupodid *Eupodes* sp. Thor were found on the fruit surface. Ameroseiid specimens collected in extremely large numbers from the inflorescences belonged to the genus *Neocypholaelaps* Vitzthum. They were only scarcely found on the fruit exocarp. Larvae of the family Tenuipalpidae were collected from several fruits but no adult specimen were found.

In Benin, *N. paspalivorus* was by far the most abundant non-eriophyid mite collected from coconut fruits (Table 1). This predator was present in all surveyed plantations. *Neoseiulus baraki*, the second most abundant predatory mite on coconut fruits, was collected from five plantations in Mono province. In the same province only a few specimens of *N. neobaraki* were collected in two plantations. Like in Tanzania the two *Neoseiulus* species along with *T. putrescentiae* and *S. furcatus* were collected from beneath coconut bracts. *Steneotarsonemus furcatus* was far less abundant in Benin than in Tanzania. Other Phytoseiidae encountered in Benin on the fruit surface outside the bracts were

A. largoensis and *Iphiseius degenerans* (Berlese) (Table 1). Four genera of Ascidae were collected from the fruit surface: *Asca* sp. von Heyden, *Gamasellodes* sp. Athias-Henriot, *Lasioseius* sp. Berlese and *Proctolaelaps bickleyi* (Bram). Like in Tanzania thousands *Neocypholaelaps* sp. were collected from inflorescences (Table 1).

Discussion

Our surveys revealed that, on the basis of frequency and severity of coconut fruit infestations, the coconut mite *A. guerreronis* continues to be a serious pest of coconut in Benin and Tanzania. All surveyed coconut plantations in both countries were damaged by *A. guerreronis* and almost all palms in every plantation had damage symptoms. Such high levels of damage incidence and severity are probably due to the inability of existing natural enemies to sufficiently suppress *A. guerreronis* which inhabits the protected areas beneath the bracts. (Lawson-Balagbo et al. 2007a; Reis et al. 2008; Negloh et al. 2010).

The three predatory mites *Neoseiulus baraki*, *N. neobaraki* and *N. paspalivorus* were the three main predators found beneath the bracts but how many individuals and how early they reach the area beneath the bracts is of key importance to their effect on the pest (Lawson-Balagbo et al. 2007a; Negloh et al. 2010). Coconut mite damage levels recorded in our study were similar to the highest damage level reported from the Kalpitiya area in Sri Lanka (Fernando et al. 2002). In our study, at least 61% of fruits were damaged and more than 44% were at least severely damaged (>25% of surface damaged). In contrast, Ramaraju et al. (2002) observed only 5 to 48% damaged fruits in Pollachi and Udumaplet in India. In our studies, damage severity increased with fruit age probably as a consequence of the persistence of infestation by *A. guerreronis*. Indeed, *A. guerreronis* often colonizes the newly fertilized fruits (Moore et al. 1989; Fernando et al. 2003; Negloh et al. 2010) but visible injury to the fruits only appears a few weeks later. Population density of *A. guerreronis* increases progressively with fruit age, consequently increasing damage levels (Julia and Mariau 1979, Mariau 1986, Otterbein 1988).

Table 1: Numbers and percentages of mite specimens other than *A. guerreronis* collected on coconut fruits in Benin and Tanzania.

Taxa	Benin		Tanzania	
	Number	Percentage	Number	Percentage
Ascidae				
<i>Asca</i> sp. Von Heyden	2	0.08	0	0.00
<i>Gamasellodes</i> sp. Athias-Henrot	3	0.11	0	0.00
<i>Hoploseius</i> sp. Berlese	1	0.04	0	0.00
<i>Lasioseius</i> sp. Berlese	38	1.43	1	0.16
<i>Proctolaelaps bickleyi</i> Bram	25	0.94	0	0.00
Acaridae				
<i>Tyrophagus putrescentiae</i> Schrank	764	28.7	63	9.84
Bdellidae				
<i>Bdella distincta</i> Baker & Balock	39	1.47	117	18.3
<i>Spinibdella</i> sp. Thor	0	0.00	5	0.78
Eupodidae				
<i>Eupodes</i> sp. Martin	1	0.04	7	1.09
Heatherellidae				
<i>Heatherella</i> sp. Walter	6	0.23	0	0.00
Phytoseiidae				
<i>Amblyseius largoensis</i> Muma	18	0.68	8	1.25
<i>Galendromus</i> sp. Muma	1	0.04	0	0.00
<i>Iphiseius degenerans</i> Berlese	3	0.11	0	0.00
<i>Neoseiulus baraki</i> Athias-Henriot	200	7.52	62	9.69
<i>Neoseiulus neobaraki</i> Zannou, Moraes & Oliveira	26	0.98	161	25.2
<i>Neoseiulus paspalivorus</i> De Leon	1348	50.7	42	6.56
Tarsonemidae				
<i>Steneotarsonemus furcatus</i> De Leon	139	5.23	140	21.9
Tydeidae				
<i>Lorrya</i> sp. Oudemans	9	0.34	31	4.84
Ameroseiidae				
<i>Neocypholaelaps</i> spp. Vitzthum	>1000 (only collected from inflorescences in both countries)			

Peak densities of *A. guerreronis* were observed on 3 to 4 months old fruits (see also Negloh et al. 2010). In Sri Lanka, Fernando et al. (2003) found that *A. guerreronis* densities increased within the first 5 months and declined thereafter. Declining densities on older fruits were likely caused by dispersal of *A. guerreronis* from these fruits to younger ones probably due to intensified intraspecific competition, reduced nutritional quality of feeding sites, and increased access beneath the bracts by natural enemies (Aratchige 2007; Lawson-Balagbo et al. 2007a).

Fruit weight loss increased with damage severity in maturing fruits of FBA₃₋₄ (i.e. 7 to 12 months). Weight loss was not evident on fruits of FBA₁ (i.e. 1 to 3 months old fruits) probably because development outweighed the negative effects of *A. guerreronis* feeding. However, the increase in fruit tissue suitable for feeding on 1 to 3 month-old fruits fostered population growth of the pest, resulting in the highest densities on 3 and 4 month-old fruits, which can explain the high damage severity level and associated weight loss in FBA₃₋₄.

Various other mites, both herbivores and predators, were found on coconut fruits, some of which living in close association with *A. guerreronis* beneath the bracts. *Steneotarsonemus furcatus* was the second most frequently observed herbivorous mite, which was collected more frequently in Tanzania than in Benin (Table 1). *Steneotarsonemus furcatus* is, however, considerably less abundant in Benin and Tanzania than in Brazil (Navia et al. 2005; Lawson-Balagbo et al. 2008). Damage by *S. furcatus* was not observed in Benin and Tanzania, probably because of its very low density in these countries. Pollen feeding *Neocyphophlegma* sp. (Ameroseiidae) were abundant on coconut inflorescences in Benin and Tanzania as in India (Haq 2001) and Sri Lanka (Ramaraju et al. 2002) but was completely absent in Brazil (Lawson-Balagbo et al. 2008).

The most abundant predatory mite in Tanzania was *N. neobaraki*, consistently observed under the bracts in almost all plantations. Two closely related species, *N. paspalivorus* and *N. baraki*, were less abundant and found in only four plantations. In Benin, *N. paspalivorus* was the most frequent predator followed by *N. baraki*. The latter was found in only five plantations which were flooded during the rainy season or were bordering swamps, lakes or rivers. *Neoseiulus paspalivorus* and *N. baraki* also occur in Sri Lanka and Brazil (Fernando et al. 2003; Moraes et al. 2004; Lawson-Balagbo

et al. 2008). Only a few specimens of *N. neobaraki* were found in two Beninese plantations together with *N. baraki*. The differing distribution of the three species in Tanzania and Benin seems to reflect species-specific humidity requirements. In Brazil, *N. baraki* was collected in regions characterized by elevated humidity whereas *N. paspalivorus* was more abundant in drier regions (Lawson-Balagbo et al. 2008). Similarly, climatic differences between Tanzania and Benin could explain the observed differences in the occurrence of the three *Neoseiulus* species. Rainfall in coastal Tanzania is almost everywhere above 1000 mm and can exceed 1500 mm per year in some locations, while in Benin most places on the coast experience rainfall below 1000 mm (BBC 2010).

The three *Neoseiulus* species found on coconut fruits during this study are considered promising candidates for biological control of *A. guerreronis* (Moraes et al. 2002; Fernando et al. 2003; Lawson-Balagbo et al. 2007b; Negloh et al. 2008, 2010). All three species have elongated, flattened idiosomas and short legs, probably morphological adaptations to live in the tight areas similar to the areas beneath coconut bracts, and all three readily prey and reproduce on *A. guerreronis* (Lawson-Balagbo et al. 2007b; Negloh et al. 2008; Domingos et al. 2010; K. Negloh personal observations). *Amyloseius largoensis* was also frequently found on coconut palms, but unlike the three *Neoseiulus* species, it was mostly collected from the fruit surface outside the bracts. It has a larger idiosoma than the *Neoseiulus* spp., which hampers its ability to creep under the bracts. Other predatory mites encountered were *P. bickleyi* (uniquely from fallen fruits) and *Lasioseius* sp. of the family Ascidae, reported from Brazil (Lawson-Balago et al. 2008), and *Lasioseius phytoseiodes* Chan, reported from Columbia (Cardona and Potes 1971), and *B. distincta* reported from Brazil (Lawson-Balagbo et al. 2007a) and Benin (Mariau and Tchibozo 1973). The latter species preys on *A. guerreronis* but because of its large body size it rarely enters the area beneath the bracts as reported in this study and by Lawson-Balagbo et al. (2007a, 2008).

The present study focused on determining the diversity and distribution of the acarine fauna only on coconut fruits and inflorescences. Several additional studies are needed in order to obtain a comprehensive assessment of the diversity and distribution of the acarine fauna in the two targeted countries. First, similar future efforts should include other parts of the coconut palm and associated vegetation in order to get greater insight into the diversity and distribution of the acarine fauna. Of particular interest are the other host plants for the three *Neoseiulus* species found under coconut bracts,

and if these phytoseiids move between the coconut fruits and the ground vegetation. Such findings can have significant implications for promoting phytoseiid abundance to enhance biological control of *A. guerreronis*. Second, for Tanzania, where only one survey was conducted, additional surveys should provide further insights into the diversity and abundance of the acarine fauna at different climatic conditions (e.g., rainy season, end of dry season, etc.) than those occurring during the survey of the present study. Third, we found that coconut mite damage is widespread in both countries despite the presence of adapted predators under the coconut bracts where the pest resides and thrive. This indicates the need to conduct further studies on biology, ecology and interactions among all mites found beneath the bracts in order to determine the efficiency of associated predators as natural and biological control agents. Studies conducted in Benin (Negloh et al. 2010) showed that *A. guerreronis* colonizes the young fruits almost 1 month earlier than the predators. This gives *A. guerreronis* a head-start in population build-up and results in a delayed impact of the predators on the pest. In addition the rate of colonization by the predators is relatively slow. Recent studies demonstrated that *N. paspalivorus* and *N. baraki* performed the best on *A. guerreronis* as prey compared to other food sources (Lawson-Balagbo et al. 2007b; Negloh et al. 2008). Moreover, a single female of both predator species could kill over 50 individuals of *A. guerreronis* per day (Negloh et al. unpublished). These results suggest that the predators would be effective against the pest when it is reachable for them. Lastly, the presence of numerous other predators outside the coconut fruit bracts suggests that they may also play an important role in suppressing dispersing individuals of *A. guerreronis*. Their presence on other parts of the coconut palm and their role in the suppression of *A. guerreronis* merits consideration.

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CHAPTER 4

4. Season- and fruit age-dependent population dynamics of *Aceria guerreronis* and its associated predatory mite *Neoseiulus paspalivorus* on coconut in Benin

Season- and fruit age-dependent population dynamics of *Aceria guerreronis* and its associated predatory mite *Neoseiulus paspalivorus* on coconut in Benin

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Abstract

The coconut mite *Aceria guerreronis* Keifer resides beneath the perianth of coconut fruits where it feeds on the tender meristematic tissue. *Aceria guerreronis* is one of the most important coconut pests but knowledge of its population dynamics is scant. We quantified seasonal and fruit age-related population fluctuations of *A. guerreronis* and its predator *Neoseiulus paspalivorus* DeLeon in Benin. *Neoseiulus paspalivorus* was the most common and abundant beneath the bracts compared with other predators, which were very scarce and were largely found outside the bracts. Both percent fruit occupation and abundance of *A. guerreronis* and *N. paspalivorus* varied across sampling months as well as among coconut plantations and fruit age classes. Both parameters peaked in the middle of the rainy season and at the end of the dry season. Fruit age at which *A. guerreronis* and *N. paspalivorus* started to colonize the fruits was 0.9 and 1.2 months, respectively. The two species reached their peak abundance (1512 *A. guerreronis* and 2.3 *N. paspalivorus* per fruit) on 3 months old fruits. Peak percent fruit occupation by *A. guerreronis* (~70%) occurred after 4.3 months, which was ~0.7 months earlier than that by *N. paspalivorus* (~22%). Difficulties encountered by the predators in accessing the area beneath the perianth on the very young fruits allowed *A. guerreronis* a head-start in population build-up leading to strongly diverging population curves as a function of fruit age (higher population increase compared to *N. paspalivorus*). Protecting the very young fruits from *A. guerreronis* colonization should be a key issue for developing successful control strategies of this pest.

Keywords. Coconut mite; *Cocos nucifera*; Eriophyoid mites; Phytoseiidae; Population dynamics; Biological control.

Introduction

Knowledge of seasonal population dynamics and within-plant distribution of phytophagous pests and their natural enemies is essential for understanding predator-prey dynamics that are critical for natural enemy conservation and the development of sustainable pest management strategies. Spider mites (Acari: Tetranychidae) and eriophyoid mites (Acari: Eriophyidae) are the most important pests among phytophagous mites (Helle and Sabelis, 1985; Lindquist and Oldfield, 1996; Oldfield, 1996). Due to their small size and wormlike body shape eriophyoid mites have limited mobility and are highly vulnerable to predation. To escape from predation, many eriophyoid species have adapted to living in spatial refuges on their host plants. Such refuges may be constitutively present or induced by the mites (Sabelis and Bruin, 1996). It is well established that refuge use has important consequences for predator-prey dynamics (e.g., Hawkins et al., 1993; Berryman and Hawkins, 2006). Pest populations protected from predation may temporarily grow exponentially, interfere with the growth and development of the attacked plant part and cause enormous economic damage.

The refuge-bound predator-prey system investigated in the present study is composed of a phytophagous pest, the coconut mite *Aceria guerreronis* Keifer (Acari: Eriophidae), and its most common predator, *Neoseiulus paspalivorus* DeLeon (Acari: Phytoseiidae), on fruits of coconut *Cocos nucifera* L. (Arecaceae) in southern Benin. *Aceria guerreronis* had been first recorded from coconut in the 1960s in the state of Guerrero, Mexico, and was first described by Keifer in 1965 (Keifer, 1965). It is presently widespread in the tropics and sub-tropics causing important damage to coconut fruits (e.g. Mariau, 1969, 1986; Ramaraju et al., 2002; Fernando et al., 2002). It was also reported to cause significant damage to young queen palm seedlings, *Syagrus romanzoffiana* (Cham) Glassman in southern California (Ansolini, 2002 cited by Ansolini and Perring, 2004). *Aceria guerreronis* was reported from other Arecaceae (Flechtmann, 1989; Ramaraju et al., 2002; Navia et al., 2005) but damage and/or yield loss on those plants were not as important or widespread compared with those on coconut. The mite is likely native to Brazil and invasive in Africa and Asia (Navia et al., 2005). It lives beneath the perianth, i.e. the floral bracts, of coconut fruits where it feeds on the tender meristematic tissue, resulting in physical injuries that further develop into necrotic and suberized tissues on the fruit surface. The space beneath the perianth constitutes a spatial refuge for *A. guerreronis* and provides protection from environmental hazards and natural enemies and therefore interferes with natural,

biological and chemical control (Hernandez, 1977; Mariau, 1977; Julia and Mariau, 1979; Howard and Abreu-Rodriguez, 1991; Aratchige et al., 2007; Lawson-Balagbo et al., 2007a, 2008ab). Nonetheless, *A. guerreronis* has been found in association with a number of natural enemies, mainly predatory mites, in several countries in South America, Asia and Africa (Moraes and Zacarias, 2002; Fernando et al., 2003; Moraes et al., 2004; Lawson-Balagbo et al., 2007a, 2008a; Negloh et al., unpublished). Previous studies showed that *A. guerreronis* colonizes coconut fruits about four weeks after pollination (Julia and Mariau, 1979; Moore and Alexander, 1987; Fernando et al., 2003) but during this time the bracts adhere too tightly to the fruit surface to allow access for the predatory mites, which are much larger than *A. guerreronis* (Moore and Alexander, 1987; Howard and Abreu-Rodriguez, 1991; Aratchige et al., 2007; Lawson-Balagbo et al., 2007a). Aratchige et al. (2007) demonstrated that once *A. guerreronis* colonized the fruit, their injuries caused the space between the bracts and the fruit surface to increase leaving enough space for the predatory mite *Neoseiulus baraki* Athias-Henriot to move into the area under the bracts. As the fruits grow, the degree of adherence decreases (Julia and Mariau, 1979; Mariau, 1986; Otterbein, 1988; Negloh et al. unpublished data). Information on the fruit age at first infestation by both *A. guerreronis* and its associated predatory mites (mainly *N. paspalivorus* and *N. baraki*) and their respective progression within the palm canopy is crucial to get more insight in their predator-prey relationship. Such knowledge constitutes a cornerstone for implementation of sustainable control strategies. Several studies addressed population dynamics of eriophyoid mites in tropical regions (e.g. Muraleedharan et al., 1988; Varadarajan and David, 2002; Fournier et al., 2004), but detailed studies on multi-location seasonal dynamics of both prey and predator are very scarce (Fernando et al., 2003; Reis et al., 2008).

Due to their tiny size and wormlike body shape eriophyoid species have limited ambulatory dispersal abilities and therefore disperse mostly passively on air currents (Sabelis and Bruin, 1996) or through phoresy on winged insects such as honey bees (Waite and McAlpine, 1992; Waite, 1999). Nevertheless, even in tropical and subtropical regions with only moderate climatic fluctuations, the distribution patterns of eriophyoid populations between infested plant parts are likely to be non-random and heterogeneous over the seasons. For example, Fournier et al. (2004) found that populations of the eriophyoid *Calacarus flagelliset* Flechtmann, De Moraes & Barbosa peaked in summer and were more abundant in the middle and lower vertical strata of the papaya plant canopy and least abundant on the youngest leaves. Fernando et al. (2003) found that *A. guerreronis* and *N. baraki* had different

temporal and spatial distribution patterns in Sri Lanka, with *N. baraki* being mostly present on more mature fruits. Lawson-Balagbo et al. (2008a) and Reis et al. (2008) observed highest densities of *A. guerreronis* during the dry seasons in eastern and northeastern regions of Brazil. Overall, previous studies showed variable seasonal fluctuation patterns of *A. guerreronis* populations but details on the relationships with the associated predator dynamics and their within-plant distributions are scarce. Moreover, the most recent studies on *A. guerreronis* and its predators in West Africa date back to the late 1980s (Mariau, 1986) while information on the spatial and seasonal dynamics of both prey and predators are presently needed as an important step towards implementation of sustainable control strategies.

In the framework of a multi-institutional project, with the ultimate objective to develop a sustainable control strategy against *A. guerreronis* in sub-Saharan Africa and elsewhere in the world, we investigated in the present study colonization patterns, population dynamics and within-plant distribution of *A. guerreronis* and its associated predatory mite *N. paspalivorus* in southern Benin.

Materials and Methods

Study sites

The study was conducted in four coconut plantations in southern Benin, West Africa. Two of the plantations were located in the Atlantique Province (06°21'66N; 02°09'76E and 06°23'33N; 01°54'33E) while the other two were located in the Mono Province (06°21'73N; 01°55'08E and 06°15'20N; 01°42'54E). In each province, one plantation was inland (3.3 km and 8.5 km from the ocean in Atlantique and Mono, respectively) and one plantation was coastal (0.2 and 0.2 km from the ocean in both Atlantique and Mono). The two provinces are major coconut production areas in Benin and are located in the humid coastal Savannah Forest Mosaic (SFM) zone, which is characterized by a bimodal rainfall pattern in the rainy season that begins in late March and lasts until mid-November. The rainy season is interrupted by a short dry spell from mid-July to late-August.

The two inland plantations – 7 and 9 years old - contained two hybrid coconut cultivars (PB121 and PB111), while the coastal plantations - 12 and 15 years old - were planted with the cultivar PB121 only. The latter is at present replacing the old West African Tall (WAT) variety in Benin. PB121 is

produced by crossing WAT with Malayan Yellow Dwarf (MYD), while PB111 is obtained from crossing WAT and Cameroonian Red Dwarf (CRD) (Julia and Mariau, 1979). The two hybrids are smaller (2.5m on average at the beginning of their productive life) and produce fruits earlier - at 2 years of age - than the WAT variety. Inland plantations were younger than coastal ones but all trees were fully grown and had a good productivity at the time of sampling. The height of palms varied only slightly between and within plantations (~0.3 to 1m).

Sampling protocol

In each plantation, 30 coconut palms were randomly selected, marked and assigned to three groups of 10 palms each. In inland plantations, each group of 10 palms consisted of five palms of each variety, while in coastal plantations the groups consisted of a single palm variety. Each plantation was sampled at monthly intervals over a 12 months period. Within plantations, sampling was rotated among groups such that each group was sampled once every three months to avoid excessive removal of fruits.

Coconut palm canopy was divided into four strata, based on fruit bunch age (hereafter referred to as FBA_i where $i = 1-4$ is the fruit age class). Coconut fruits grow in bunches with all fruits within a given bunch being of about the same age. In the area where the study was conducted, a new coconut fruit bunch is produced approximately every month. Therefore, the position or the rank of a particular bunch on the palm corresponds to its age in months, the first just fertilized bunch being one month old. The 12 fruit-bunch ages normally found on a coconut palm were placed into four FBA classes: FBA_1 - first bunch (just fertilized fruits) to third bunch; FBA_2 - fourth to sixth bunch; FBA_3 - seventh to ninth bunch; and FBA_4 - tenth to twelfth bunch (mature fruits). Inflorescences were not included in the sampling. FBA_1 had significantly more fruits (up to ~40 per bunch) than FBA_2 to FBA_4 (down to 1 in some bunches, mainly because of fruit fall and delay in flowering probably caused by drought) (Negloh et. al, unpublished). Therefore, one fruit was sampled from each of the distal, middle and proximal section of each of the three bunches composing FBA_1 (nine fruits in total per palm per sampling date). In contrast, only one fruit was sampled from each FBA_{2-4} (one fruit per FBA per palm per sampling date) due to the fewer number of fruits on these FBAs. During the dry season, fewer fruits were sampled from single palms that did not harbor enough fruits for the above described

protocol; however, missing fruits were compensated for from other palms in the vicinity. Sampled fruits were examined with a hand-lens (x10) as soon as they were removed from the palm. Mites encountered on the fruit surface were collected and preserved in vials containing 75% ethanol. Each fruit was then labeled, placed in a paper bag and brought to the laboratory for further processing.

In the laboratory, the bracts (i.e. the perianth) of each fruit were sequentially and carefully removed to uncover the meristematic zone. Eggs and mobile stages of mites other than *A. guerreronis* present beneath the bracts were counted and collected in 75% ethanol. Mobile stages of those mites were further identified using a compound microscope after slide-mounting in Hoyer's medium. A total of 4933 phytoseiid specimens (0 to 160 individuals per fruit) were examined. The abundance of *A. guerreronis* was assessed using a methodology similar to the one developed by Siriwardena et al. (2005). Bracts as well as the meristematic zone of the fruit and some distance away on the exocarp (2-5 cm depending on the fruit size) were rinsed with 30 ml detergent solution into a container. The solution was vigorously shaken to distribute the mites evenly and immediately before one ml aliquot was drawn from the solution and placed in counting cells (Costar® Brand Cell Culture Clusters, 24 cells of 3.4 ml volume). All individuals of *A. guerreronis* present in the one ml aliquot were counted using a stereomicroscope. Eggs and mobile stages of other mites in the solution were counted and added to those previously collected in vials. Abundance of *A. guerreronis* per fruit was then estimated by multiplying the obtained values by 30 (the total volume of the rinse solution). The accuracy of this technique had been verified using 100 three to seven months-old fruits; the washing technique overestimated the actual *A. guerreronis* counts by ~13% (Negloh et al., unpublished).

Data analyses

All statistical analyses were performed in SAS 9.1 (SAS Institute, 2005). Data on mite, *A. guerreronis* and *N. paspalivorus*, occurrence (proportion of fruits occupied) and density per fruit (mobile stages for the former and eggs and mobile stages for the latter) were used separately as dependent variables in univariate analyses of variance with plantation, seasonal month and fruit bunch age class (FBA_i) as independent variables. Bonferroni post-hoc tests were used for mean separations. The analyses were conducted on log-transformed mite densities per fruit (x+1) and arcsine square root-transformed

proportions of mite occupied fruits, respectively. For analysis, the heterogeneous sample sizes among FBA_i were homogenized by using the average of all fruits sampled within each FBA_i per palm per date. Effects of rainfall on densities of *A. guerreronis* and *N. paspalivorus* were assessed using a separate linear regression for each plantation.

General linear model (GLM) analysis was used to compare within FBA₁ variation (age and position) in mite densities and proportion of fruits occupied by the mites. Data of FBA₁ was used in this analysis as it was the only FBA that contained fruits with proximal, middle, and distal positions within the bunch. Older bunches did not always have all three within-bunch fruit positions. Similarly, GLM analysis was used to compare mite densities and proportion of fruits occupied by mites among fruit ages (12 months). Means of the dependent variables were separated using Bonferroni post-hoc tests. All analyses were conducted on log-transformed densities per fruit (x+1) and arcsine square root-transformed proportions of mite occupied fruits.

Polynomial regression models along with linear interpolation on densities and proportion fruit occupation by *A. guerreronis* and *N. paspalivorus* were used to estimate the ages of first colonization of coconut fruits by the mites and subsequent progression of their density and proportion fruit infestation with fruit aging. The interpolation was based on the assumption of Thales' Theorem that the curves are linear between the upper and lower bound values of the age (<http://homeomath.imingo.net/interpol.htm>). Thales' Theorem is expressed as follows:

$$\frac{x - x_1}{x_2 - x_1} = \frac{y - y_1}{y_2 - y_1}$$

where $y = p(x)$, and x_1 and x_2 are the lower and upper bounds. The same method was used to estimate fruit ages at which either mite species reached 50 and 100% of their peak proportion fruit occupation. Moreover, we compared the slopes of the progression in proportion fruit occupation and densities of the two mites to determine divergence in trends. Polynomial regression analyses were used to obtain the parameters of the two functions as well as the standard errors of the slopes (E_1 and E_2). We then determined the slopes (S_1 and S_2 for *A. guerreronis* and *N. paspalivorus*, respectively) at every fruit age (month) using the derived functions. Student's *t* was used to compare the slopes of the two mite species. It was calculated as follows:

$$t = \frac{S_1 - S_2}{\sqrt{E_1^2 + E_2^2}}$$

The degree of freedom was n_1+n_2-4 .

Results

Seasonal predator-prey dynamics

The abundance of *A. guerreronis* varied among plantations, sampling dates and FBAs (Table 1, Fig. 1). Densities of *A. guerreronis* were lower in one coastal plantation (Fig. 1D) than in the two inland and the second coastal plantation (Bonferroni, $P < 0.05$). Fruits of FBA₁₋₂ had higher pest densities than fruits of FBA₃₋₄. FBA₄ fruits had a lower pest density than younger fruits (Bonferroni, $P < 0.05$). However, the two- and three way interactions (Table 1, Fig. 1) indicate that none of the main effects had similar patterns across plantations. The pattern of variation of the abundance of *A. guerreronis* during the observation period was about the same in the two inland plantations but different from that observed for the coastal plantations, which had similar *A. guerreronis* population levels.

In the inland plantations the abundance of *A. guerreronis* increased during the rainy season, then declined towards the end of the rainy season and increased again in the second half of the dry season (Fig. 1 and 3). The seasonal fluctuations in abundance differed among FBAs. The abundance of *A. guerreronis* gradually decreased in FBA₁ and FBA₃ over sampling dates but increased slightly in FBA₂ and remained almost constant in FBA₄. The difference in abundance among FBA₂ and the other FBAs was most evident between November and May (3 to 4 times higher in FBA₂). Furthermore, the 3-way interaction indicates that the differences in seasonal fluctuations among plantations varied with FBAs (Table 1). In FBA₁, the abundance of *A. guerreronis* differed among plantations mainly between June and September (~3-fold higher abundance in inland plantations than in coastal plantations) whereas in FBA₃ it differed among plantations through most of the year (~2-fold higher levels in inland plantations). Overall, *A. guerreronis* densities were positively influenced by rainfall in inland plantations but not in coastal plantations (Fig. 3 and 4).

In contrast to *A. guerreronis*, the abundance of *N. paspalivorus* was only influenced by plantation and FBA, but not by seasonal month. Similarly to *A. guerreronis*, *N. paspalivorus* abundance was lower in the coastal plantations compared with the inland plantations (Bonferroni, $P < 0.05$). Fruits of FBA₂ had the highest predator density, but due to high variability this density was not significantly different from that on fruits of FBA₁ and FBA₃ (Bonferroni, $P > 0.05$). Fruits of FBA₄ had a significantly or marginally significantly lower density than fruits of FBA₁₋₂ (Bonferroni, $P < 0.05$) and FBA₃ (Bonferroni, $P = 0.057$), respectively. However, the main effects were inconsistent. The differences in predator abundance among plantations and FBAs varied with the seasonal month (Table 1, Fig. 1). Towards the end of the observation period the abundance of *N. paspalivorus* slightly increased in one inland plantation (Fig. 1B) whereas it decreased in the three other plantations. The predator abundance fluctuated largely among months in FBA₁₋₃ - with 2 to 3 times higher levels in FBA_{1,2} than FBA_{3,4} from July to September and a 2 times higher level in FBA₃ than in the other FBAs in December and January - while it remained at a constant level throughout the year in FBA₄. There was no effect of rainfall on *N. paspalivorus* densities in any plantation (Fig. 3 and 4).

Percent fruit occupation by *A. guerreronis* was influenced by plantation, seasonal month and FBA (Table 2, Fig. 2). In one coastal plantation (Fig. 2D) a lower percentage of fruits was infested by *A. guerreronis* compared with the two inland and the second coastal plantation (Bonferroni, $P < 0.05$). Percent fruit occupation was lower in FBA₁ and FBA₄ than FBA₂₋₃ (Bonferroni, $P < 0.05$). It was higher in FBA₂ than all other FBAs (Bonferroni, $P < 0.05$). Nonetheless, similarly to abundance the main effects did not show similar patterns as indicated by the significant interactions (Table 2, Fig. 2). The significant 3-way interaction indicates that seasonal fluctuations in percent fruit occupation were more pronounced in inland than coastal plantations (Table 2, Fig. 2) and FBA₁ (see below). Percent fruit occupation followed a similar temporal trend in FBA₁ in all four plantations with the highest levels (40 to 80% depending on the plantation) occurring between July and September and in February.

Table 1. ANOVA for the effects of plantation, seasonal month and fruit-bunch age class (FBA) on the abundance of *Aceria guerreronis* and *Neoseiulus paspalivorus* per coconut fruit.

Source of variation	df	Mean squares	F	P value
<i>Aceria guerreronis</i>				
Plantation	3	36.2	24.21	<0.001
Month	11	13.1	8.78	<0.001
FBA	3	111.6	74.74	<0.001
Plantation x Month	33	4.42	2.96	<0.001
Plantation x FBA	9	4.58	3.06	0.001
Month x FBA	33	7.82	5.24	<0.001
Plantation x FBA x Month	99	2.25	1.50	0.001
Error	1620	1.50		
<i>Neoseiulus paspalivorus</i>				
Plantation	3	0.485	6.13	0.0004
Month	11	0.110	1.40	0.1685
FBA	3	0.353	4.47	0.0039
Plantation x Month	33	0.140	1.77	0.0046
Plantation x FBA	9	0.125	1.58	0.1166
Month x FBA	33	0.160	2.03	0.0005
Plantation x FBA x Month	99	0.085	1.07	0.3040
Error	1620	0.079		

Similarly to its abundance, the proportion of fruits occupied by *N. paspalivorus* was significantly influenced by plantation and FBA but not by seasonal month (Table 2, Fig. 2). Bonferroni post-hoc tests indicated that percent fruit occupation by the predators was higher in inland than coastal plantations and lower in FBA₁ than FBA₂₋₄ ($P < 0.05$). Fruits of FBA₂₋₄ were similarly often occupied. However, the significant 2-way interactions between plantation and month and FBA and month, respectively, indicate that the differences in proportion fruit occupation among plantations and FBAs varied with sampling dates (Table 2, Fig. 2). The highest difference between FBA₁ (< 0.05 fruits occupied) and FBA₂₋₄ (0.1 to 0.3 occupied) was observed between October and April. Proportion fruit

occupation by *N. paspalivorus* gradually decreased in FBA_{1,2} between July and January, but it increased gradually in FBA_{3,4}. Proportion fruit occupation by *N. paspalivorus* fluctuated less in one coastal plantation (Fig. 2D) than in the other three plantations with the largest difference between this plantation and the other three plantations occurring between October and February.

Table 2. ANOVA for the effects of plantation, seasonal month and fruit-bunch age class (FBA) on percent fruit occupation by *A. guerreronis* and *N. paspalivorus*.

Source of variation	df	Mean squares	F value	P value
<i>Aceria guerreronis</i>				
Plantation	3	7.93	19.7	<0.001
Month	11	3.74	9.28	<0.001
FBA	3	24.6	61.0	<0.001
Plantation x Month	33	1.00	2.49	<0.001
Plantation x FBA	9	0.982	2.44	0.009
Month x FBA	33	1.42	3.53	<0.001
Plantation x FBA x Month	99	0.601	1.49	0.002
Error	1620	0.403		
<i>Neoseiulus paspalivorus</i>				
Plantation	3	0.933	3.50	0.015
Month	11	0.432	1.62	0.087
FBA	3	3.46	12.96	<0.001
Plantation x Month	33	0.541	2.03	0.001
Plantation x FBA	9	0.251	0.94	0.486
Month x FBA	33	0.559	2.10	<0.001
Plantation x FBA x Month	99	0.328	1.23	0.065
Error	1620	0.226		

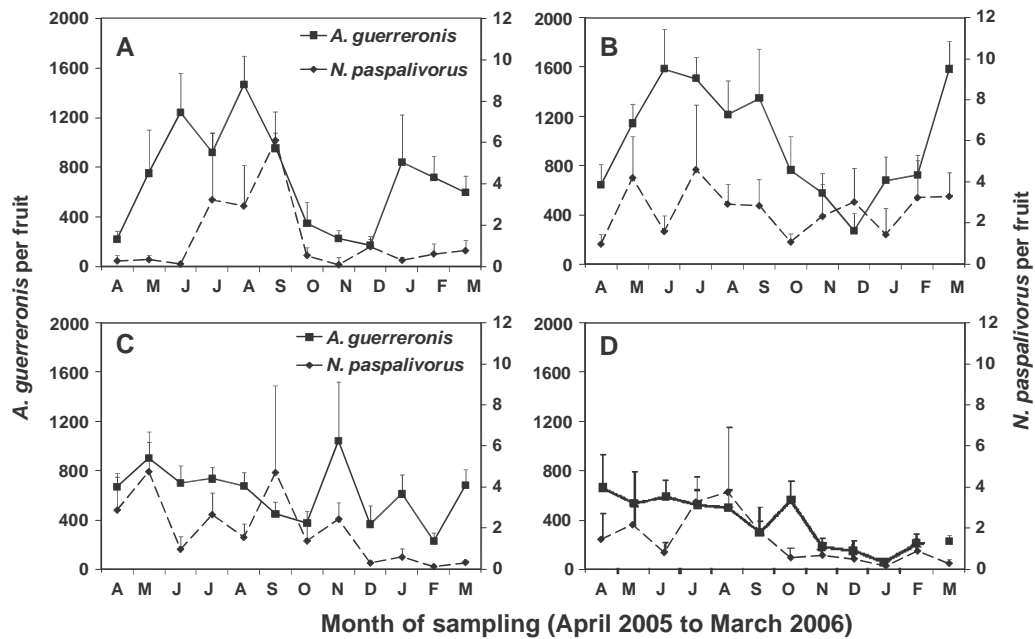


Figure 1: Abundance (mean \pm SE per fruit) of *Aceria guerreronis* (—■—) and *Neoseiulus paspalivorus* (—◆—) in four coconut plantations in Benin (A and B are inland plantations, C and D are coastal plantations) from April 2005 through March 2006.

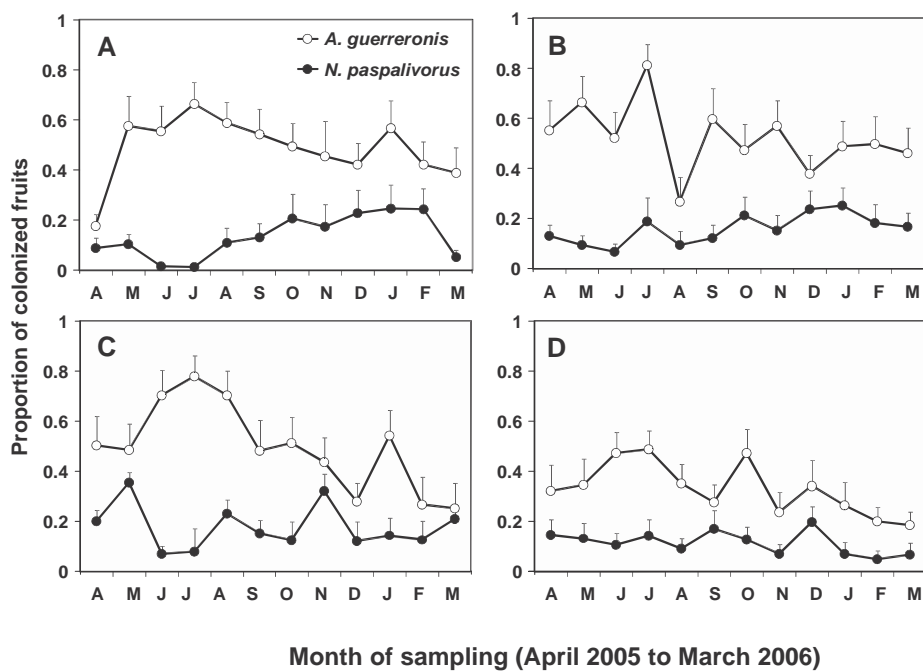
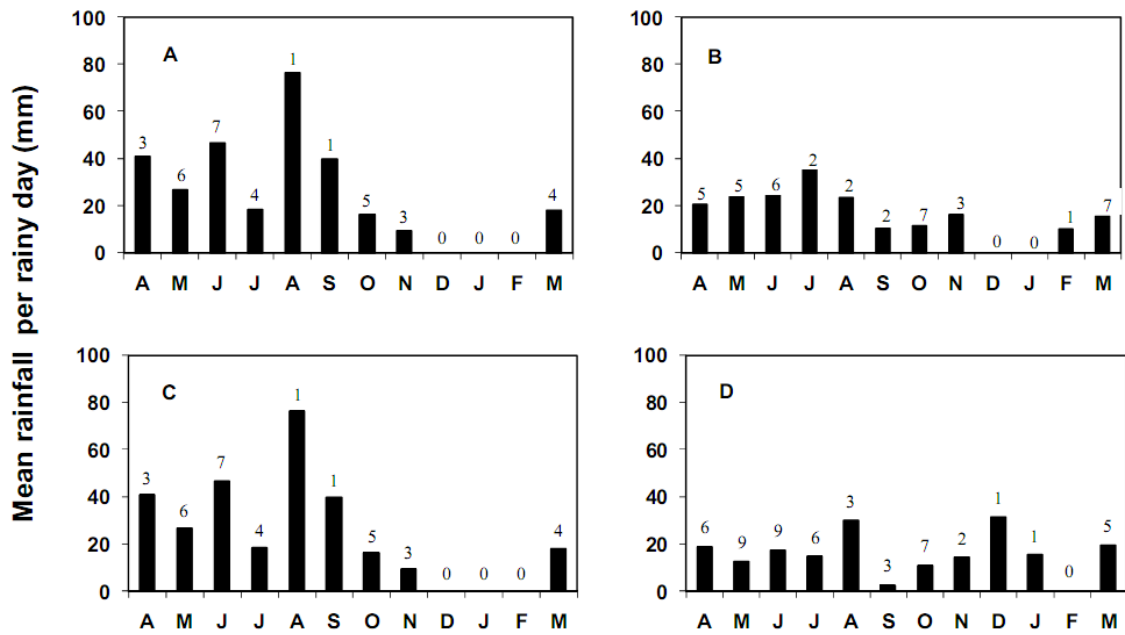


Figure 2: Proportion of coconut fruits (mean \pm SE) occupied by *Aceria guerreronis* (○) and *Neoseiulus paspalivorus* (●) in four coconut plantations in Benin (A and B are inland plantations, C and D are coastal plantations) from April 2005 through March 2006.



Month of sampling (April 2005 to March 2006)

Figure 3: Mean rainfall over the seasons in the four study plantations (A and B are inland plantations, C and D are coastal plantations). Numbers on top of rainfall bars refer to the number of rainy days per month.

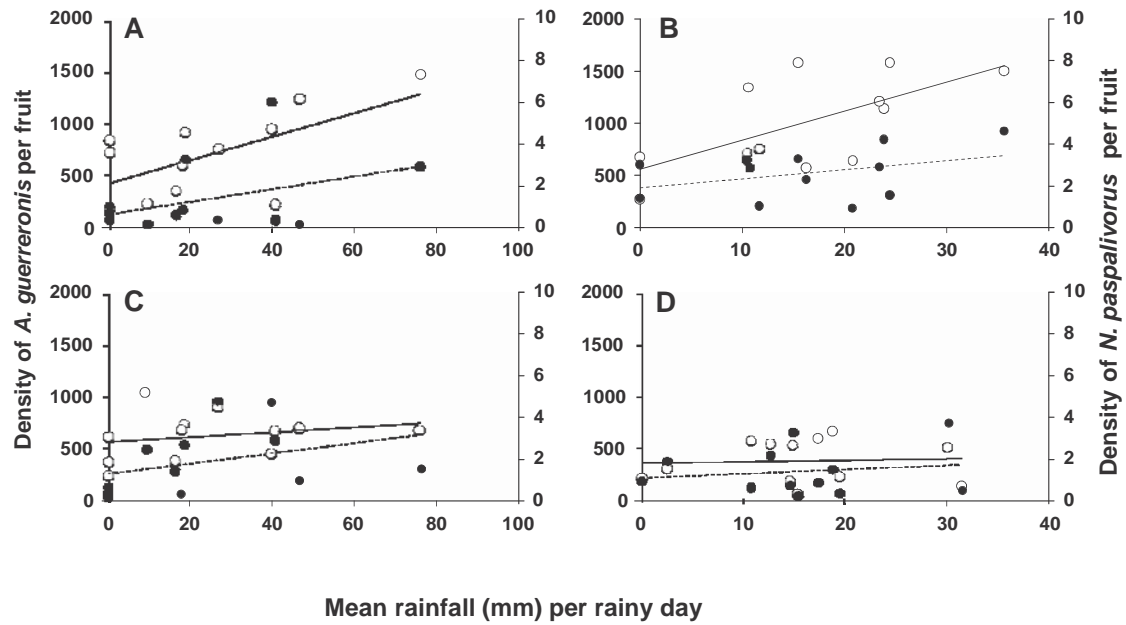


Figure 4: Linear regressions of mean rainfall against *Aceria guerreronis* (—○—) and *Neoseiulus paspalivorus* (-●-) densities in four coconut plantations in Benin (A and B are inland plantations; C and D are coastal plantations). Plantation A (*A. guerreronis*: $y = 11.4x + 427.0$, $r^2 = 0.40$, $P = 0.03$; *N. paspalivorus*: $y = 0.03x + 0.63$, $r^2 = 0.15$, $P = 0.21$). Plantation B (*A. guerreronis*: $y = 27.7x + 557.2$, $r^2 = 0.42$, $P = 0.02$; *N. paspalivorus*: $y = 0.04x + 1.93$, $r^2 = 0.14$, $P = 0.22$). Plantation C (*A. guerreronis*: $y = 2.36x + 561.6$, $r^2 = 0.06$, $P = 0.46$; *N. paspalivorus*: $y = 0.02x + 1.28$, $r^2 = 0.12$, $P = 0.27$). Plantation D (*A. guerreronis*: $y = 1.41x + 355.8$, $r^2 = 0.004$, $P = 0.85$; *N. paspalivorus*: $y = 0.02x + 1.06$, $r^2 = 0.02$, $P = 0.63$).

Fruit age-dependent variations

Results of the GLM analysis within FBA₁ showed that for each fruit age (1, 2 and 3 months) and both mite species (*A. guerreronis* or *N. paspalivorus*), the proportion of occupied fruits and mite densities did not significantly vary with the relative position of the sampled fruit (proximal, middle or distal) within a given fruit age (GLM: *A. guerreronis* density: $F_{2,2654} = 0.10$; $P = 0.903$; *N. paspalivorus* density: $F_{2,2654} = 0.16$, $P = 0.853$; *A. guerreronis* fruit occupation: $F_{2,2654} = 2.02$, $P = 0.133$; *N. paspalivorus* fruit occupation: $F_{2,2654} = 0.05$, $P = 0.949$) (Fig. 5). In contrast, the proportion of fruits occupied by either mite and mite densities were highly significantly different among fruit ages within FBA₁, with the highest proportion (GLM: $F_{2,2654} = 509.0$, $P < 0.001$; $F_{2,2654} = 91.7$, $P < 0.001$ for *A. guerreronis* and *N. paspalivorus*, respectively) and density (GLM: $F_{2,2654} = 212.3$, $P < 0.001$; $F_{2,2654} = 20.7$, $P < 0.001$ for *A. guerreronis* and *N. paspalivorus*, respectively) observed on 3 months old fruits followed by 2 months old fruits (Fig. 5).

GLM analyses were also used to compare percent fruit occupation and densities of both mites among all fruit ages (12 months). For both mites GLM revealed highly significant differences in fruit occupation among fruit ages ($F_{11,561} = 19.6$, $P < 0.001$ for *A. guerreronis* and $F_{11,561} = 4.19$, $P < 0.001$ for *N. paspalivorus*). The proportion of fruits occupied by *A. guerreronis* was the highest on 3 to 7 months old fruits. It was similar among these fruit ages (Fig. 6) but significantly different from younger and older fruits. Proportion fruit occupation by *N. paspalivorus* (Fig. 6) was similar on 3 to 12 months old fruits (Bonferroni, $P > 0.05$). Only 1 and 2 months old fruits were significantly less often occupied by *N. paspalivorus* (Bonferroni, $P < 0.05$) than the other age classes.

Densities of *A. guerreronis* and *N. paspalivorus* differed significantly among fruit ages (GLM: $F_{11,561} = 32.8$, $P < 0.001$ for *A. guerreronis* and $F_{11,561} = 6.80$, $P < 0.001$, respectively for *N. paspalivorus*) (Fig. 7). Mean separation by Bonferroni tests revealed that the densities of *A. guerreronis* were the highest on 3 months old fruits followed by 4, 5 and 7 months old fruits. Densities on 6 months old fruits were significantly lower than those on 3 months old fruits but similar to 4, 5 and 7 months old fruits. The lowest density was observed on 1 month old fruits, which was significantly different from the densities in all other fruit ages. Similarly, the highest density of *N. paspalivorus* was observed on 3 months old fruits, which was similar to densities on 4, 5 and 7 months old fruits (Fig. 7). The density on 6 months old fruits was significantly lower than that on 3 months old fruits but similar

to the other above-mentioned fruit ages. The lowest densities were found on 1, 2 and 9 to 12 months old fruits.

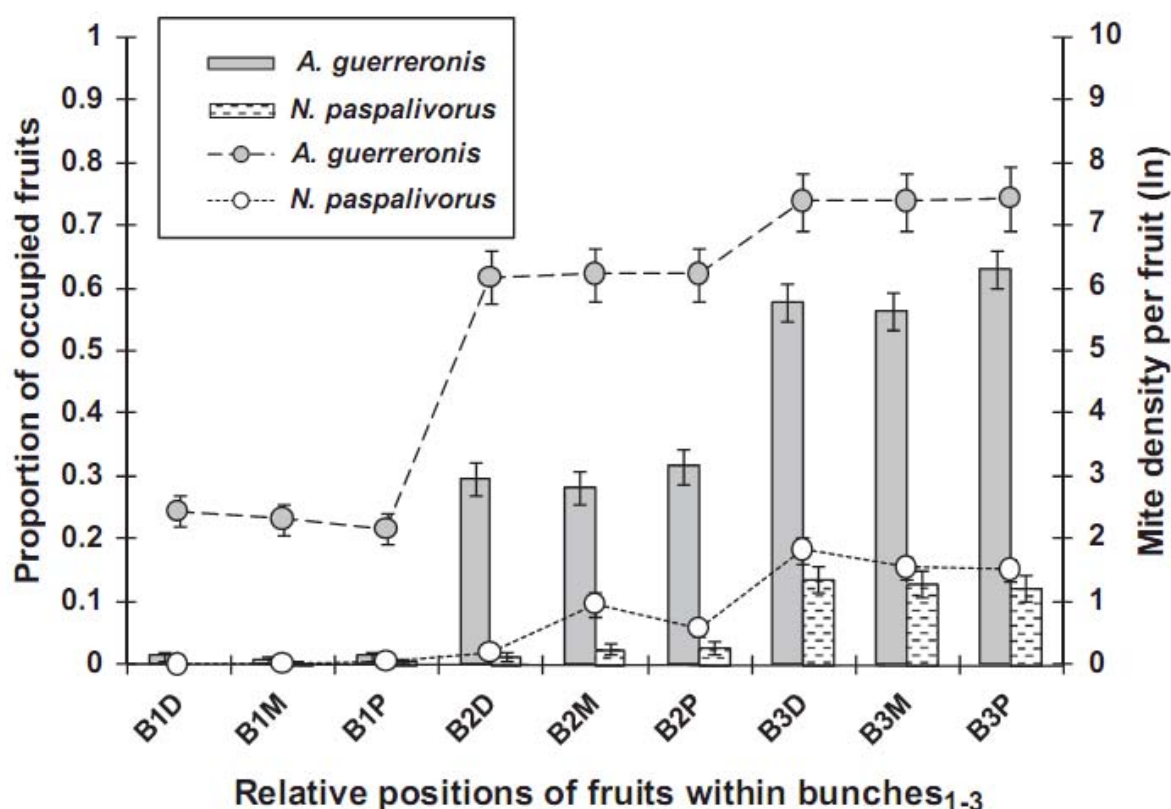


Figure 5: Variation in percent fruit occupation (bars; mean \pm SE) and abundance (lines; mean \pm SE) of *Aceria guerreronis* and *Neoseiulus paspalivorus* on 1 to 3 months old fruits. D, M and P are distal, middle and proximal positions, respectively, of the coconut fruits within a given bunch (B_{1-3}).

The estimated fruit ages at which the mites first appeared on the fruits was 0.9 and 1.2 months respectively for *A. guerreronis* and *N. paspalivorus*. However, only 6 out of 360 one month-old fruits were occupied by *N. paspalivorus*, whereas 13 out of 360 one month-old fruits were infested by *A. guerreronis*. *Aceria guerreronis* and *N. paspalivorus* reached 50% of their peak occupation after 2 and 3 months, respectively. The estimated peak occupation by *A. guerreronis* occurred after 4.3 months

which was 0.7 months earlier than that by *N. paspalivorus*. A comparison of the fruit occupation curves of both mites showed a significant difference at 1 month ($t_{20} = 4.32$, $P < 0.05$) and 2 months ($t_{20} = 2.88$, $P < 0.05$) old fruits but not in the following fruit ages (3: $t_{20} = 1.66$; 4: $t_{20} = 0.66$; 5: $t_{20} = -0.12$; 6: $t_{20} = -0.68$; 7: $t_{20} = -1.03$; 8: $t_{20} = -1.15$; 9: $t_{20} = -1.06$; 10: $t_{20} = -0.75$; 11: $t_{20} = -0.22$ and 12: $t_{20} = 0.53$; $P > 0.05$ for all these ages) (Fig. 6). However, the overall level of occupation by *A. guerreronis* was significantly higher than that by *N. paspalivorus*. The same comparisons performed on the density curves (Fig. 7) revealed similarity between the slopes at one month-old fruits ($t_{20} = 1.96$, $P < 0.05$), while the curves significantly diverged in subsequent fruit ages ($t_{20} < 5.81$, $P < 0.05$ for every month from month 2 to 12), density of *A. guerreronis* increasing more rapidly than that of *N. paspalivorus* (Fig. 7).

Discussion

We found that in Benin occurrence and abundance of *A. guerreronis* are more seasonally and spatially variable than those of its predator *N. paspalivorus*. Furthermore, our data indicated that *N. paspalivorus* is only sometimes able to numerically respond to fluctuations of the prey. The overall proportional distribution of *A. guerreronis* and *N. paspalivorus* among fruit ages was similar but predator fruit occupation and abundance increased less rapidly with fruit age compared to the pest. Fruit infestation occurred very early suggesting that a successful and sustainable control approach must focus on protecting the very young fruits. Our study reports the results of a single year but it is unlikely that the basic patterns in predator-prey dynamics would differ in other years because the sampled palms were fully grown and the climate in the sampling region varies little over years. Examination of 10-year average climate data from locations near the study sites indicated that the average temperature over 10 years was 26.9°C while it was 26.6°C and 26.7°C, respectively, in 2004 and 2005. Average relative humidity over the 10 year period was 77.4% while it was 78.5 and 80.4 in 2004 and 2005. It is unlikely that these small climatic differences between 2004/2005 and the 10 year average would affect the relative differences in predator and prey distribution within plantations.

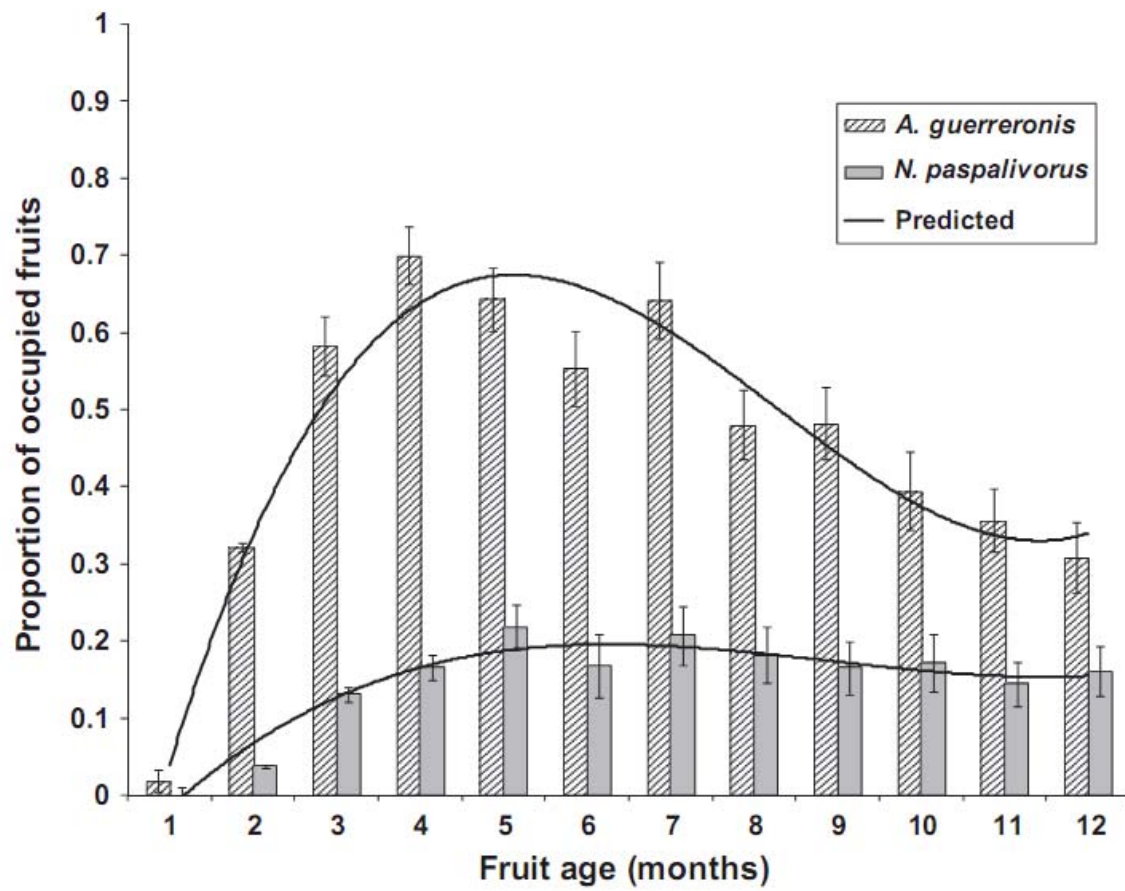


Figure 6: Observed and predicted proportions of coconut fruits (mean \pm SE) occupied by *Aceria guerreronis* and *N. paspalivorus* in dependence of fruit age ($y = 0.002x^3 - 0.068x^2 + 0.484x - 0.381$ and $y = 0.000x^3 - 0.015x^2 + 0.124x - 0.123$, respectively).

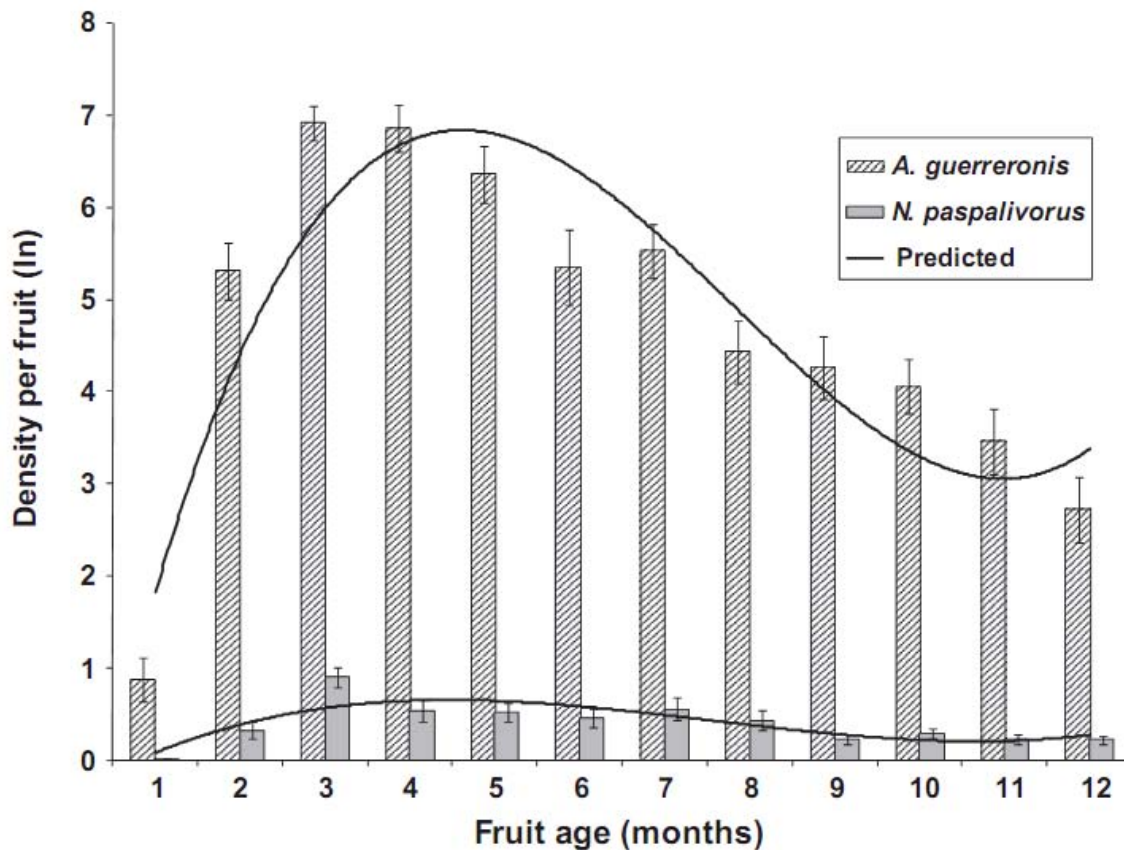


Figure 7: Observed and predicted densities (mean \pm SE per fruit) of *Aceria guerreronis* and *N. paspalivorus* on coconut fruits in dependence of fruit age ($y = 0.029x^3 - 0.686x^2 + 4.456x - 1.982$ and $y = 0.003x^3 - 0.085x^2 + 0.536x - 0.382$, respectively).

Seasonal and spatial variations

Variations of *A. guerreronis* densities between inland and coastal plantations were probably caused by sea winds, salty mist, very high humidity levels and other sea-related factors that hamper establishment of *A. guerreronis* on the fruits. In one coastal plantation we regularly observed that the rows of palms closest to the sea were the least damaged, which corroborates observations made in previous surveys (Negloh et al., unpublished). Seasonal variations of *A. guerreronis* infestation and densities in inland plantations corroborate findings by Julia and Mariau (1979) in Benin and Ivory Coast, and Otterbein (1988) in Costa Rica, who determined that the highest infestation levels of *A. guerreronis* occurred during the rainy season. Similarly, Howard et al. (1990) reported that *A. guerreronis* populations

increased immediately after periods of high rainfall in Puerto Rico and Florida. In contrast, Fernando and Aratchige (2006) observed the highest densities of *A. guerreronis* during the dry seasons in Sri Lanka. Particularly high densities of *A. guerreronis* were observed in regions with long drought periods in Brazil (eastern coast) (Lawson-Balagbo et al., 2008a). These discrepancies in the response of *A. guerreronis* in different regions could be due – among other factors - to region-specific climate characteristics (rainfall and temperature patterns). For example, in Sri Lanka (western coast) and Brazil (eastern coast) the overall yearly rainfall is higher and temperatures fluctuate less than in Benin (www.worldclimate.com; Reis et al., 2008). Relative humidity during the dry seasons may thus be high enough in Sri Lanka and Brazil to favor population growth of *A. guerreronis*, while excess water in the rainy seasons may either directly or indirectly decrease it. Another possible reason could be the difference in predator species composition and abundance in these countries; while *N. paspalivorus* is the most abundant in Benin (Negloh et al., unpublished), in Sri Lanka and Brazil the most abundant predator is *N. baraki* (Fernando et al., 2003; Lawson-Balagbo et al., 2007a, 2008b). In addition, recent studies showed that *N. baraki* is more voracious on *A. guerreronis* than *N. paspalivorus* (Negloh et al., unpublished); this could affect, in combination with other factors, the seasonal distribution of the pest in these countries. Fluctuations in the proportion of infested fruits and in the abundance of *A. guerreronis* in inland plantations were mainly due to fluctuations on fruits of FBA₁, whereas fruit infestation and abundance on medium aged and old fruits (FBA₂₋₄) were unaffected by season. In the two inland plantations increase of fruit infestation and abundance of *A. guerreronis* during the dry season, with a second peak at the end of the season, was similar to the findings by Reis et al. (2008) in Brazil. Possible explanations for the high population level of *A. guerreronis* during the rainy season are the favorable plant growth conditions and the resulting availability of fresh highly nutritive fruit tissues. The decline in proportion fruit infestation and population density of *A. guerreronis* at the end of the rainy season was probably due to dispersal from older heavily infested fruits and colonization of the high number of young fruits available during the rainy season. During dispersal, the mites are highly vulnerable to predator attacks and wash-down by rainfall. Increase in fruit infestation and density of *A. guerreronis* during the second half of the dry season was likely due to the overall reduction of the number of younger fruits (up to 7 months) from October to December (Negloh, personal observations) leading to aggregation on the remaining fruits. *Aceria guerreronis* tends to cluster on those fruits until fruit quality has declined to low levels or until more suitable becomes

available. Moreover, lower abundance of *N. paspalivorus* in the dry season was likely caused by negative effects of drought, thus releasing *A. guerreronis* from predation. Many phytoseiids are known to be negatively affected by drought (e.g. McMurtry and Croft, 1997; Hanna et al., 2005; Zundel et al., 2009). Numerous studies also reported negative effects of low relative humidity on egg viability, the most susceptible phytoseiid life stage (e.g. Schausberger, 1998; Vis et al., 2006; Walzer et al., 2007). In inland plantations populations of *N. paspalivorus* followed a similar seasonal pattern as *A. guerreronis* with up to 3 months lag. However, neither proportion fruit occupation nor abundance of the predators showed much seasonal variations.

Within-palm distribution

Aceria guerreronis starts colonizing coconut fruits just after fertilization (Moore and Alexander, 1987; Howard et al., 1990; Fernando et al., 2003; present study). Unfertilized flowers are free of *A. guerreronis* (Fernando et al., 2003; Negloh et al., unpublished); at this stage the bracts tightly cover the whole flower leaving no space for mites to move beneath. We therefore assumed that the first opportunity for *A. guerreronis* to colonize the developing fruits is the time of anthesis when pollination starts. Observations made in a manually pollinated plantation in Benin seem to corroborate this assumption. In fact, all fruits (young to mature) in that particular plantation were free of damage by *A. guerreronis* (Negloh, personal observation). The process of manual pollination probably hampers fruit colonization by *A. guerreronis*. Male flowers are removed before they open, while female flowers are covered with paper bags to prevent natural pollination.

Fruit colonization by *A. guerreronis* may occur by active ambulatory dispersal and/or through phoresy on pollinators visiting the flowers as reported for other eriophyoid mites (Waite and McAlpine, 1992; Waite, 1999). Recent studies, however, failed to show any phoresy by *A. guerreronis* (Negloh et al., unpublished data). Populations of *A. guerreronis* increase rapidly as it benefits from the highly nutritional value of the young fruit tissue (Moore and Alexander, 1987; Fernando et al., 2003; present study). Due to limited active dispersal abilities - as observed in eriophyoid mites in general (Sabelis and Bruin, 1996) – it is likely that *A. guerreronis* usually remains on the first infested fruits until the nutritional value of the tissue beneath the bracts is depleted. Thus, preventing or delaying colonization of

the fruits as long as possible or providing means to reduce pest population growth during the first 3 months could prevent damage to the fruits or at least substantially reduce damage levels.

Only a few *N. paspalivorus* individuals were able to colonize the fruits at the same time as *A. guerreronis* and the predator's rate of colonization was dramatically lower than that of *A. guerreronis*. This was probably due to the high degree of adherence of the bracts to the surface of young fruits (Lawson-Balagbo et al., 2007a; Negloh et al., unpublished data), which hampers colonization by *N. paspalivorus*. The predators' difficulty in colonizing the very young fruits gives *A. guerreronis* a head-start in population build-up leading to heavy damage on more mature fruits. The decrease in fruit occupation and densities of *A. guerreronis* observed on older fruits can be attributed to the following effects: (1) natural and/or *A. guerreronis*-caused drying-out of the fruit tissue, (2) overexploitation of the fruits by *A. guerreronis* intensified intraspecific competition and resulting population crashes, and (3) predation by *N. paspalivorus* or (4) most likely a combination of these factors. The first two scenarios force *A. guerreronis* to search for younger and more nutritious fruits. Fruit occupation by *N. paspalivorus* was constant on old fruits, which was probably due to easier access beneath the bracts following the relaxed adherence of the bracts to the fruit surface. Knowledge of seasonal and within-plant distribution of *A. guerreronis* constitutes an important step towards developing a sampling plan for the pest and its associated predatory mites.

Implications for biological control

Our study clearly underscores that *A. guerreronis* control strategies must focus on protection of the very young fruits. Julia and Mariau (1979), Mariau (1986) and Otterbein (1988) observed that the earlier the attack the greater the damage severity on older fruits. *N. paspalivorus* seems to be able sometimes to numerically respond to *A. guerreronis*. This phytoseiid predator has greater ability - than other predators present on coconut - to access the refuge of *A. guerreronis* because of its flat and elongated body shape (Moraes et al., 2004; Lawson-Balagbo et al., 2007a, 2008b). It shares this specific morphology with the closely related *N. baraki* which can also be commonly found under coconut bracts (Aratchige et al., 2007; Lawson-Balagbo et al., 2007a, 2008a; Negloh et al., 2008). Recent studies demonstrated that both predator species performed best when feeding on *A. guerreronis* in comparison with other food sources

(Lawson-Balagbo et al., 2007b; Negloh et al., 2008). These results suggest that *N. paspalivorus* as well as *N. baraki* would be effective against the pest when it is accessible to the predators, but there is still much to learn about how to break the refuge barrier of the bracts. An augmentative biological control strategy could be envisioned so as to increase the density of the predators and consequently enhance the suppressive effect on *A. guerreronis* (Lawson-Balagbo et al., 2007a; Fernando et al. 2010).

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CHAPTER 5

5. Comparative demography and diet breadth of Brazilian and African populations of the predatory mite *Neoseiulus baraki*, a candidate for biological control of coconut mite

Comparative demography and diet breadth of Brazilian and African populations of the predatory mite *Neoseiulus baraki*, a candidate for biological control of coconut mite

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Abstract

Neoseiulus baraki Athias-Henriot (Phytoseiidae) is one of the few predators associated with the coconut mite *Aceria guerreronis* Keifer (Eriophyidae), the most damaging pest of coconut fruits in the Americas, Africa and more recently in Oman, Sri Lanka and parts of India. As Brazil is presently considered the putative origin of *A. guerreronis*, a large effort is presently underway to develop a classical biological control strategy for this pest in Africa and Asia. In this study, we investigated the life history of a Brazilian (NbBr) and a Beninese (NbBe) population of *N. baraki* on prey and non-prey diets under laboratory conditions ($25 \pm 1^\circ\text{C}$ 70-90% RH and 12:12 h L:D). Both populations were able to complete juvenile development and reproduce when feeding on *A. guerreronis*, *Tetranychus urticae* Koch eggs – a prey commonly used in the maintenance of phytoseiid mite colonies – and maize pollen. The two predators developed faster on *A. guerreronis* than on any other diet. The longest developmental time was recorded for NbBe on castor bean pollen (12.3 days), which also was not suitable at all for the development of NbBr. The longest developmental time of NbBr was 8.94 days on *T. urticae* eggs, whereas NbBe needed only 5.86 days to develop from eggs to adult stage on the same diet. For both populations, oviposition rate and longevity as well as demographic parameters were most favorable on *A. guerreronis*, the target prey. Intrinsic rate of natural increase (r_m) and net reproductive rate (R_o) were significantly higher for NbBr (0.19 and 24.9) than for NbBe (0.16 and 18.0). Taken together, the life history data from this study predict that NbBr is a more specialized and efficient predator of *A. guerreronis* compared with NbBe. The ability of the latter to utilize alternative food types, however, predicts that it would be able to persist longer in coconut habitat in the absence of its primary prey *A. guerreronis*. Implications for the implementation of a sustainable control strategy against *A. guerreronis* are discussed.

Keywords: *Cocos nucifera*, *Aceria guerreronis*, Eriophyidae, Phytoseiidae, life history, classical biological control

Introduction

Phytoseiid mites are well studied plant-inhabiting predators because of their established usefulness in the biological control of a wide range of phytophagous arthropods on agricultural crops (e.g., Helle and Sabelis, 1985, McMurtry and Croft, 1997). Among other traits, the diet breadth of a phytoseiid predator is considered an important factor determining its suitability and efficacy in the control of a given pest and in its ability to persist in a crop when the primary prey pest is scarce (McMurtry 1982; McMurtry and Croft, 1997).

Two phytoseiid mites, *Neoseiulus baraki* Athias-Henriot and *N. paspalivorus* DeLeon, were recently found associated with the coconut pest *Aceria guerreronis* Keifer (Acari: Eriophyidae) during surveys conducted in Sri Lanka, Brazil, Benin, and Tanzania (Fernando et al., 2003; de Moraes et al., 2004; Lawson-Balagbo et al., 2007ab; Negloh et al., unpublished). The two species were the most abundant predatory mites found in association with *A. guerreronis*; preliminary observations showed that both readily feed and reproduce on *A. guerreronis*. They were therefore suggested as possible natural enemies in a classical or augmentative biological control program for *A. guerreronis* (Sabelis, 1996; Moraes and Zacarias, 2002). This approach has recently gained support due to the finding that *A. guerreronis* might be native to Brazil (or elsewhere in South America) and is definitely invasive in Africa and Asia (Navia et al., 2005).

Aceria guerreronis was first recorded from coconut in the 1960's in the state of Guerrero, Mexico, and was subsequently found in Central America and the Caribbean, as well as in Brazil and several other countries in South America (Cartujano, 1963 cited by Mariau, 1986) and Africa (Mariau, 1969). This species recently invaded Sri Lanka and India, two major coconut production countries (Fernando et al., 2002; Ramaraju et al., 2002) and is likely to continue its devastating expansion into south Asia and the Pacific where coconut palm is native and where much of the world's coconut is produced.

The tiny wormlike *A. guerreronis* exclusively lives beneath the perianth of the coconut fruits where it feeds on the tender meristematic tissues resulting in physical injuries that develop into necrotic and suberized scars on the fruit surface from the perianth down to the bottom part of the fruit. The space beneath the perianth provides a shelter for *A. guerreronis*, protecting it from environmental

hazards and natural enemies and therefore interferes with natural, biological and chemical control (Hernandez, 1977; Mariau, 1977; Julia and Mariau, 1979; Aratchige et al., 2007; Lawson-Balagbo et al., 2007ab). The species is responsible for considerable coconut yield losses worldwide (Hernandez, 1977; Julia and Mariau, 1979; Moore et al., 1989).

To determine the potential of *N. baraki* and *N. papalivorus* as biological control agents of *A. guerreronis*, studies are required to provide insight into the relationships of the predators to the target pest in term of their ability to feed and multiply on *A. guerreronis* and other food sources available in coconut habitat. The ability of phytoseiid mites to feed and survive on various food sources is well documented (e.g., Klein, 1990; Bruce-Oliver et al., 1996; Toko et al., 1995; Gnanvossou et al., 2003, 2005), and life history studies of a Brazilian population of *N. paspalivorus* have been recently completed on various food types (Lawson-Balagbo et al., 2007b); but to our knowledge studies on life history and diet breadth of *N. baraki* are non-existent.

In the present study we investigated under laboratory conditions the life history of two populations of *N. baraki* - from Benin and Brazil - on diets of all life stages of *A. guerreronis*, eggs and larvae of *Tetranychus urticae* (Koch), pollens of maize (*Zea mays* (L.)), coconut (*Cocos nucifera* L.) and castor bean (*Ricinus communis* L.). Pollens were included to determine if they can be used by the predator as food source in the absence of mite prey. *Tetranychus urticae* was included to determine its potential as alternative food for mass-rearing of *N. baraki* populations, and as proxy for other tetranychid species found in the coconut habitat. *Tetranychus urticae* has been shown to be a suitable diet for the maintenance of several phytoseiid mite species worldwide (see Helle and Sabelis, 1985 for review).

Materials and methods

Origin and colony maintenance of predatory mites

The Beninese population of *N. baraki* was collected from coconut fruits in the village of Gbéhoué (06°21'73 N; 01°55'08 E) in southern Benin, while the Brazilian population originated from Itamaraca (07°46'S, 34°52'W) in northeastern Brazil. Both populations were maintained in the laboratory on a diet of eggs and mobile juveniles of *T. urticae* for at least one year prior to the start of the experiments. Each rearing unit consisted of a Petri dish (14.5 cm diameter and 1 cm high) filled with water-saturated

cotton wool. A foam pad (4cm x 4 cm x 1 cm) carrying a black PVC tile (4cm x 4 cm x 0.1 cm) rested on top of the cotton. The edges of the tile were covered with moist tissue paper reaching down into the water-saturated cotton to prevent the mites from escaping. A tuft of hydrophobic cotton wool covered by a piece of transparent plastic was placed in the center of each rearing arena and served as oviposition site for the predators.

Experimental procedures

One hundred to 150 gravid *N. baraki* females of each population were placed on separate arenas and fed eggs and larvae of *T. urticae* for 24 h. Eggs of each population, 70 and 100 respectively for NbBr and NbBe for each treatment, were collected and placed singly on arenas of 2 cm dia, constructed as described above. Experimental units were placed in a large plastic tray and kept in an incubator at $25 \pm 1^{\circ}\text{C}$, 70-90% RH and 12:12 h L:D photoperiod.

Life history experiments consisted of the following food type treatments: (1) All stages of *A. guerreronis* collected from meristematic tissue and bracts of coconut fruits, (2) eggs of *T. urticae* obtained using a method described by Megevand et al. (1993), (3) coconut pollen, (4) castor bean pollen, and (5) maize pollen. All pollens were collected just prior to their use from plants present on the IITA-Benin campus. Each food type was provided in surplus quantities and replenished every 48 hours.

Mite development was monitored at 12-hr intervals until all individuals reached adult stage; and subsequently at 24-hr intervals until the last individual died. One adult male of the respective population was added to each arena soon after the protonymph molt. Males were removed immediately after the initiation of oviposition. Males that died before the initiation of oviposition were replaced. Eggs laid by females were removed daily and kept separately for each female for sex determination once they reached deutonymph stage.

Statistical analyses

Life history data were summarized as follows: (1) duration of development of each juvenile stage and total juvenile development time (days), (2) juvenile survival rate (%), (3) pre- and post-oviposition periods (days), (4) oviposition rate (eggs per female per day), and (5) adult longevity (days). Development period was determined only for individuals that reached the adult stage. Sex ratio was calculated on the basis of proportion of female progeny. A single-factor ANOVA (using General Linear Model) was used to test the effects of food types on each of the life history parameters except juvenile survival. When treatment F-test was significant ($P < 0.05$), all parameter means were compared within and between predator populations using Student-Newman-Keuls multiple range-tests (SNK). Juvenile survival was tested with overall chi square and post-hoc chi square tests with Bonferroni's corrections. Data on adult longevity and duration of oviposition, pre- and post-oviposition periods were log-transformed [$\ln(x+1)$] prior to the analyses. Juvenile survivorship was compared among food types with chi-square tests, and LIFETEST with Wilcoxon Statistic (SAS, 2005) was used to compare survivorship curves of adult stages of each predator population on the various food types. Sex ratio data were arcsine-transformed prior to statistical comparisons. A program developed by Maia et al. (2000) was used to calculate and compare, within and between predator populations, the demographic parameters (net reproductive rate R_0 , mean generation time T , intrinsic rate of natural increase, r_m , doubling time Dt , and finite rate of increase λ). For details on these parameters, see Carey (1993). All analyses were performed in SAS 9.1 TS Level 1M0, XP Pro platform.

Results

Juvenile development and survival

Food types significantly influenced stage-specific and total juvenile developmental times, which ranged from 5.64 days (on *A. guerreronis*) to 12.3 days (on castor bean pollen) for NbBe, and from 6.56 days (on *A. guerreronis*) to 8.94 days (on *T. urticae* eggs) for NbBr (Table 1). NbBe developed faster than NbBr on *A. guerreronis* and on *T. urticae* eggs ($F_{1,137} = 97.03$, $P < 0.0001$; $F_{1,167} = 1054.5$, $P < 0.0001$) but slower on pollen diets (Table 1). For both predator populations, development was

faster on *A. guerreronis* than on any other food type ($F_{4,319} = 648.10$, $P < 0.0001$ for NbBe; $F_{3,250} = 151.2$, $P < 0.0001$ for NbBr; Table 1).

Juvenile survival of NbBe and NbBr was highest when fed *A. guerreronis* or *T. urticae* eggs (Table 2). Juveniles of both populations survived at higher levels on mite diets than on all pollen diets, except for NbBr which survived at the same rate on maize pollen as on mite diets (Table 2). Indeed, maize pollen was superior to the other pollen types for the development of both predator populations (Table 1). Castor bean pollen was excluded from further analysis since NbBr failed to reach adulthood on this pollen.

Table 1

Developmental times in days (mean \pm SE ^a) of the Beninese and Brazilian populations of *Neoseiulus baraki* fed with different food types at $25 \pm 1^\circ\text{C}$, 70-90% RH and 12 h photophase

Predator stages	Food types				
	<i>A. guerreronis</i>	<i>T. urticae</i> eggs	Maize pollen	Coconut pollen	Castor bean pollen
<i>N. baraki</i> Benin					
Egg	2.45 \pm 0.06	1.43 \pm 0.06	2.13 \pm 0.06	2.97 \pm 0.10	2.72 \pm 0.08
Larva	0.81 \pm 0.03	1.15 \pm 0.02	1.17 \pm 0.03	1.33 \pm 0.10	1.50 \pm 0.08
Protonymph	1.56 \pm 0.04	1.82 \pm 0.04	3.08 \pm 0.09	2.25 \pm 0.12	4.51 \pm 0.29
Deutonymph	1.31 \pm 0.04	1.46 \pm 0.04	2.00 \pm 0.08	1.93 \pm 0.10	3.52 \pm 0.18
Egg to Adult	5.64 \pm 0.06a	5.86 \pm 0.08b	8.38 \pm 0.08c	8.47 \pm 0.09c	12.2 \pm 0.27d
<i>N. baraki</i> Brazil					
Egg	2.28 \pm 0.07	2.28 \pm 0.05	2.07 \pm 0.04	1.88 \pm 0.05	---
Larva	1.21 \pm 0.03	2.30 \pm 0.13	1.41 \pm 0.03	1.36 \pm 0.05	---
Protonymph	1.62 \pm 0.05	2.35 \pm 0.08	2.56 \pm 0.06	2.62 \pm 0.10	---
Deutonymph	1.46 \pm 0.06	2.00 \pm 0.05	1.39 \pm 0.05	1.75 \pm 0.07	---
Egg to Adult	6.56 \pm 0.07a	8.94 \pm 0.09b	7.42 \pm 0.07c	7.58 \pm 0.11c	---

Eggs to Adult values followed by the same letter within a population are not significantly different (SNK tests, $P > 0.05$)

--- indicates no data.

^a Number of individuals ranged from 100 to 29 in the Beninese population and 70 to 50 in the Brazilian population.

Table 2

Juvenile survival (% individuals reaching adulthood) of the Beninese and Brazilian populations of *Neoseiulus baraki* fed with different food types at $25 \pm 1^\circ\text{C}$, 70-90% RH and 12 h photophase.

<i>N. baraki</i> population				
Food types	Benin		Brazil	
	N ^a	Survival (%) ^b	N ^a	Survival (%) ^b
<i>A. guerreronis</i>	100	100a	70	98.6a
<i>T. urticae</i> eggs	100	100a	70	98.6a
Maize pollen	100	89b	70	94.3a
Coconut pollen	100	36c	70	71.4b
Castor bean pollen	100	29c	70	0.00c
χ^2_4		241.6		249.4
<i>P</i>		< 0.0001		< 0.0001

^a N is the initial number of mites in the cohort.

^b Means within a column followed by the same letter are not significantly different (Overall chi square and post hoc follow-up chi-square tests ,with Bonferroni's correction, $P > 0.044$).

Adult longevity and reproductive traits

Survival of NbBe was significantly higher on *A. guerreronis* than that of NbBr, whereas both populations survived at similar levels on the other diets (Figure 1). Adult age-specific survival curves (Figure1) were influenced by food type in both populations (Wilcoxon: $\chi^2_4 = 38.4$, $P < 0.0001$ for NbBe and $\chi^2_3 = 45.8$, $P < 0.0001$ for the NbBr), as was adult life span of the two predator populations ($F_{4,201} = 20.9$ $P < 0.0001$ for NbBe and $F_{3,167} = 34.9$, $P < 0.0001$ for NbBr; table 3). The predators lived longest on *A. guerreronis* diet (39.8 days for NbBe and 30.2 days for NbBr), followed by *T. urticae* eggs (Table 3). NbBe lived longer than NbBr on either *A. guerreronis* or *T. urticae* eggs ($F_{1,87} = 4.89$, $P = 0.03$; $F_{1,120} = 8.71$, $P = 0.003$). Adult longevity was similar among the three pollen diets for NbBe (Table 3), and was least when the predators were maintained on castor bean pollen (8.4 days) and coconut pollen (8.9 days) for NbBe and NbBr respectively.

NbBe successfully reproduced on all diets except on castor bean pollen, while NbBr reproduced only on *A. guerreronis*, *T. urticae* eggs and maize pollen. Maximum daily oviposition was reached at 3

to 5 and 4 to 8 days after the onset of oviposition in NbBe and NbBr, respectively. NbBe oviposition rate (eggs per female per day) was higher on mite prey than any of the pollen diets ($F_{3,187} = 8.83$, $P < 0.0001$), with the highest rate being on *A. guerreronis* (1.20), which was similar to the rate achieved on *T. urticae* eggs (1.06). Similarly, NbBr had the highest oviposition rate on *A. guerreronis* (2.22), which was significantly higher than that of NbBe ($F_{1,87} = 154$, $P < 0.0001$) and was higher than the rate reached on *T. urticae* eggs and maize pollen ($F_{2,68} = 102$, $P < 0.0001$). NbBe oviposition rate was higher on *T. urticae* eggs than that of NbBr ($F_{1,104} = 9.84$, $P = 0.002$). Pollen diets resulted in similar oviposition rates for NbBe (Table 3).

In both *N. baraki* populations, food type had a significant effect on pre-oviposition and oviposition periods. Oviposition started earlier on *A. guerreronis* and *T. urticae* eggs than on the pollen diets (Tables 3). NbBe sex ratio differed significantly among food types ($F_{3,91} = 12.1$, $P < 0.0001$) (Table 3) but was similar for NbBr. On *A. guerreronis*, NbBe sex ratio was higher than that of NbBr ($F_{1,70} = 6.85$, $P = 0.01$).

Table 3

Adult longevity (days), oviposition rate (eggs/female/day), oviposition periods (days), and offspring sex ratio^a (% female) (\pm SE^b) of the Beninese and Brazilian populations of *Neoseiulus baraki* fed with different food types at $25 \pm 1^\circ\text{C}$, 70-90% RH and 12 h photophase.

Adult parameters	Food types				
	<i>A. guerreronis</i>				
Adult longevity	---	---	---	---	---
Oviposition rate	$1.20 \pm 0.05\text{a}$	$1.06 \pm 0.04\text{a}$	$0.78 \pm 0.05\text{b}$	$0.75 \pm 0.09\text{b}$	---
Pre-oviposition period	$3.20 \pm 0.12\text{a}$	$4.62 \pm 0.13\text{b}$	$8.45 \pm 0.34\text{c}$	$5.27 \pm 0.55\text{b}$	---
Oviposition period	$21.3 \pm 1.31\text{a}$	$12.9 \pm 0.81\text{b}$	$6.71 \pm 0.64\text{c}$	$6.18 \pm 0.94\text{c}$	---
Post-oviposition	$15.3 \pm 2.33\text{a}$	$6.10 \pm 1.02\text{b}$	$4.24 \pm 0.69\text{b}$	$2.36 \pm 1.13\text{b}$	---
Sex ratio (%)	$74.1 \pm 0.01\text{ab}$	$63.2 \pm 0.03\text{a}$	$76.8 \pm 0.04\text{b}$	$35.7 \pm 0.09\text{c}$	---
<i>N. baraki</i> Brazil					
Adult longevity	$30.2 \pm 2.53\text{a}$	$16.3 \pm 1.60\text{b}$	$15.9 \pm 1.54\text{b}$	$8.90 \pm 0.97\text{c}$	---
Oviposition rate	$2.22 \pm 0.07\text{a}$	$0.83 \pm 0.06\text{b}$	$1.09 \pm 0.17\text{b}$	---	---
Pre-oviposition period	$3.86 \pm 0.21\text{a}$	$6.96 \pm 1.11\text{a}$	$13.8 \pm 2.79\text{b}$	---	---
Oviposition period	$18.4 \pm 1.20\text{a}$	$6.75 \pm 1.25\text{b}$	$2.71 \pm 0.61\text{b}$	---	---
Post-oviposition	$7.89 \pm 1.79\text{a}$	$4.54 \pm 1.23\text{a}$	$8.29 \pm 2.33\text{a}$	---	---
Sex ratio (%) ^a	$63.4 \pm 0.02\text{a}$	$50.8 \pm 0.07\text{a}$	$50.0 \pm 0.14\text{a}$	---	---

Means within the same row and population followed by the same letter are not significantly different (SNK tests, $P > 0.05$).

--- indicates no data

^a Sex ratio was subjected to arcsine transformation before analyses.

^b SE, standard error

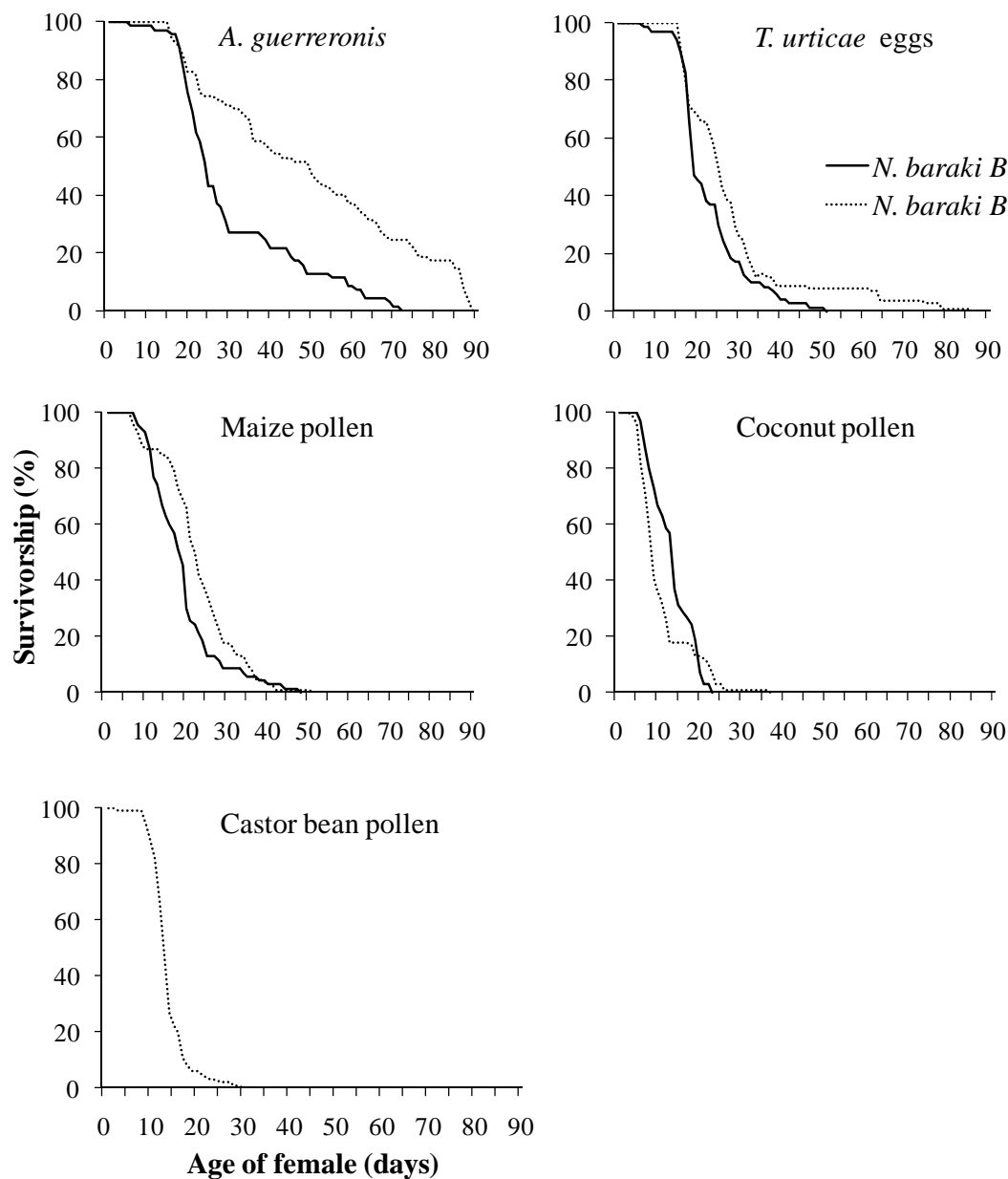


Figure 1. Influence of food type on age-specific survival of Beninese and Brazilian populations of *Neoseiulus baraki*. The Brazilian *N. baraki* did not reach adulthood when fed with castor bean pollen.

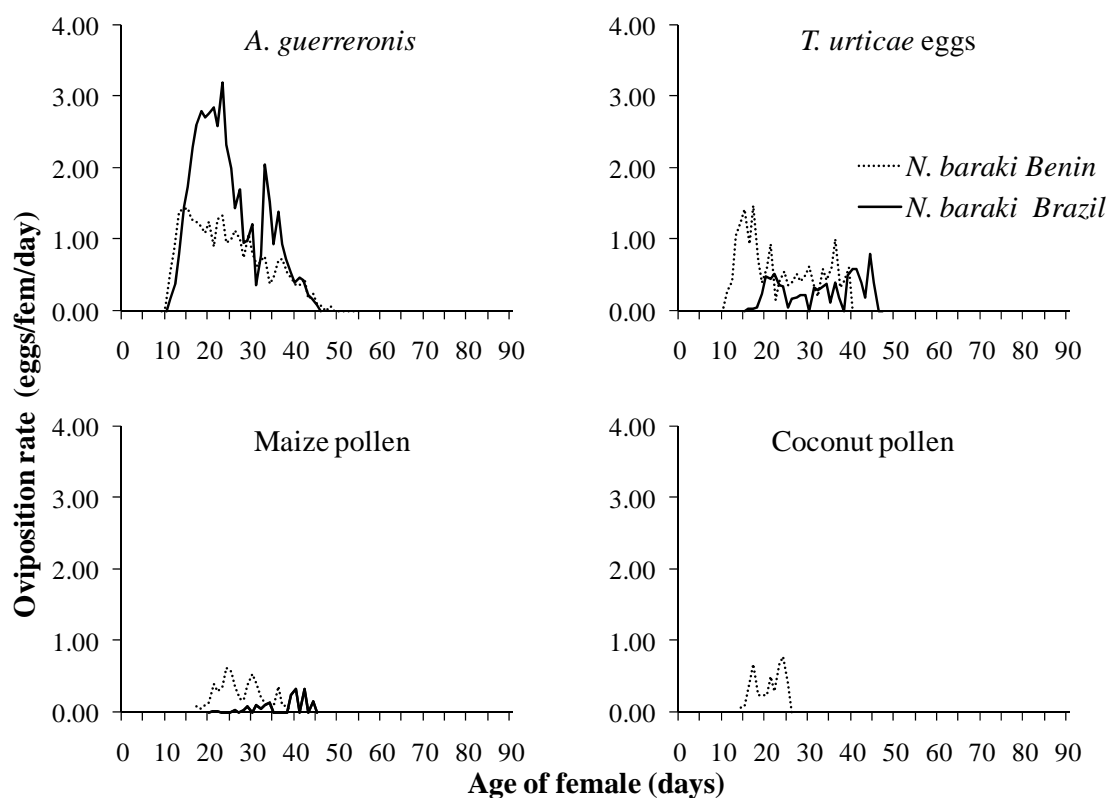


Figure 2. Influence of food type on age-specific fecundity (On the x-axis, the period between 0 and the start of oviposition includes the duration of immature development and adult female pre-oviposition period) of Beninese and Brazilian populations of *Neoseiulus baraki*. Brazilian *N. baraki* did not reproduce when fed with coconut pollen.

Demographic parameters

Demographic parameters of both predator populations were higher when they were maintained on *A. guerreronis* than on any other diet (T-test; $P < 0.0001$) (Table 4). Respectively for NbBe and NbBr, intrinsic rates of natural increase (r_m) on *A. guerreronis* were 0.16 and 0.19 per female/day, and the net reproductive rates (R_o) were 18.0 and 24.9. The two parameters were significantly higher for NbBr compared with NbBe (T-test; $P < 0.0001$). The rank-order of food type suitability was *A. guerreronis* > *T. urticae* eggs > maize pollen > coconut pollen > castor bean pollen for NbBe, and *A. guerreronis* > *T. urticae* eggs > maize pollen for NbBr (Table 4). Development and reproduction of NbBr were inhibited on coconut and castor bean pollens (Table 4).

Table 4

Demographic parameters (\pm SE) of the Brazilian and Beninese *Neoseiulus baraki* fed with different food types at $25 \pm 1^\circ\text{C}$, 70-90% RH and 12 h photophase.

Food types	Demographic parameters				
	R_o	r_m	λ	T	DT
<i>N. baraki</i> Benin					
<i>A. guerreronis</i>	$18.0 \pm 1.14\text{a}$	$0.16 \pm 0.00\text{a}$	$1.17 \pm 0.00\text{a}$	$18.4 \pm 0.44\text{a}$	$4.40 \pm 0.09\text{a}$
<i>T. urticae</i> eggs	$7.87 \pm 0.44\text{b}$	$0.13 \pm 0.00\text{b}$	$1.14 \pm 0.00\text{b}$	$15.7 \pm 0.35\text{b}$	$5.26 \pm 0.12\text{b}$
Maize pollen	$2.50 \pm 0.23\text{c}$	$0.05 \pm 0.01\text{c}$	$1.05 \pm 0.01\text{c}$	$19.2 \pm 0.56\text{a}$	$14.5 \pm 1.44\text{c}$
Coconut pollen	$0.39 \pm 0.09\text{d}$	$(0.04) \pm 0.01\text{d}$	$0.96 \pm 0.01\text{d}$	$26.0 \pm 0.35\text{c}$	$(19.1) \pm 4.84\text{d}$
<i>N. baraki</i> Brazil					
<i>A. guerreronis</i>	$24.9 \pm 1.76\text{a}$	$0.19 \pm 0.00\text{a}$	$1.21 \pm 0.01\text{a}$	$16.6 \pm 0.45\text{a}$	$3.59 \pm 0.08\text{a}$
<i>T. urticae</i> eggs	$1.51 \pm 0.32\text{b}$	$0.02 \pm 0.01\text{b}$	$1.02 \pm 0.01\text{b}$	$18.6 \pm 1.75\text{a}$	$19.3 \pm 19.4\text{ab}$
Maize pollen	$0.18 \pm 0.07\text{c}$	$(0.06) \pm 0.02\text{c}$	$0.94 \pm 0.02\text{c}$	$30.1 \pm 3.52\text{b}$	$(12.1) \pm 3.26\text{b}$

Means within the same column and population followed by the same letter are not significantly different (Pairwise student's *t*-tests; $P > 0.05$). Values in parentheses are negative.

Discussion

This study was conducted as part of an intercontinental effort to develop a suitable biological or integrated control strategy for *A. guerreronis*. The Beninese and Brazilian populations of *N. baraki* used in this study shared some similar life history features and differed in others.

Aceria guerreronis was by far the best food for both *N. baraki* populations, indicating that *A. guerreronis* may very well be the primary prey for this predator on coconut palm in Benin (Africa) and Brazil (South America). Moreover, demographic parameters show that, compared with NbBe, NbBr performs better on *A. guerreronis*. This finding provides the basis for further evaluation of potential field introductions of NbBr into Benin (and elsewhere in Africa and possibly Asia) to enhance the biological control of *A. guerreronis*.

The phytoseiid mite *N. paspalivorus* was also found in association with *A. guerreronis* in Brazil (Lawson-Balagbo et al., 2008) and Africa (Negloh et al., unpublished data), but information on life history and diet breadth are only available for the Brazilian population of *N. paspalivorus* (Lawson-Balagbo et al., 2007b). Similar to our results, Lawson-Balagbo et al. (2007b) found that *A. guerreronis* was the most suitable food for population growth of *N. paspalivorus*. Further studies are needed to determine if the Brazilian and Beninese populations of *N. paspalivorus* also differ in their predation potential, life history characteristics and diet breadth. In addition, information on the climatic requirements of the two populations and their interactions with *N. baraki* are also needed to determine their suitability for an eventual field introduction into Africa and Asia.

Our study also showed that larvae of *N. baraki* were obligatory feeders and could not molt to protonymph stage in the absence of food, similar to larvae of *N. paspalivorus*, *Galendromus* (*Syn. Metaseiulus*) *occidentalis* (Nesbit), *Euseius finlandicus* Oudemans and *E. hibisci* (Chant) (Zhang and Croft 1994; Schausberger and Croft 1999; Lawson-Balagbo et al., 2007b).

The two *N. baraki* populations were able to utilize *T. urticae* eggs, but this prey was more suitable for NbBe than for NbBr (Tables 1-4); intrinsic rate of natural increase of NbBe was 6.5 fold higher than that of NbBr on *T. urticae* diet. Notwithstanding these differences in r_m , colonies of the two populations were successfully maintained on a diet of *T. urticae* eggs collected with a washing technique (McMurtry and Scriven, 1965) from a colony of this mite maintained in a screenhouse on

peanut plants, *Arachis hypogaeae* L. It is possible that the Brazilian population may have acquired the ability to utilize *T. urticae* egg through its maintenance on this prey; however, the value of *T. urticae* to *N. baraki* in coconut habitat is not known.

The results of the present study on *N. baraki* and that on its sister species *N. paspalivorus* (Lawson-Balagbo et al., 2007b) indicate that these two phytoseiid species may prefer eriophyid mites as the primary prey. These findings are in line of results from numerous other studies that have shown the suitability of eriophyid mites as prey for phytoseiid mites (reviewed by Sabelis, 1996).

The diet of the two *N. baraki* populations also includes plant pollens, but for both populations, pollens were far less suitable for population growth. Moreover, NbBe may be able to use more pollen types than NbBr. While juveniles of the two predator populations developed, survived to adulthood, and reproduced at about the same rate on maize pollen (Table 1-3), the intrinsic rate of increase of NbBe on this pollen was higher (31-35% of *T. urticae* and *A. guerreronis*, respectively) than that of NbBr (which was negative). Negative r_m values mean that maize pollen is unsuitable for NbBr population growth. It is the consequence of the production of less than 1 female per female ($R_0 < 1$) resulting in lack of recruitment. The population may go extinct if it doesn't switch to or complement its diet with a more suitable food. In comparison to maize pollen, coconut pollen was intermediate in nutritional value for juveniles of both populations. NbBe and NbBr juveniles developed to adulthood at about the same rate on coconut pollen, but NbBr survived at nearly twice the rate of that of NbBe on this diet. Interestingly, adult females of NbBe survived for nearly the same period as the latter on coconut pollen, but NbBr did not reproduce, while the net reproductive rate of NbBe was very low (Table 3 and 4). NbBe was able to make use of castor bean pollen for juvenile development and survival, while NbBr was not. Gnanvossou et al. (2003, 2005) also showed that castor bean pollen was the least suitable diet for the development of *Amblydromalus* (Syn. *Typhlodromalus*) *manihoti* Moraes and *Neoseiulus idaeus* Denmark in comparison with pollens of maize and *Leuceuna leucophela* (Lam.) De Wit.

It is somewhat surprising that coconut pollen was not suitable for *N. baraki* reproduction despite being abundantly available on coconut palm. *Neoseiulus baraki* (and several other *Neoseiulus* species that are adapted to inhabiting tight spaces on plants) (Zannou et al., 2006) may have evolved on grasses (Poaceae) on which they are commonly found, which might explain the higher performance of *N.*

baraki on maize pollen (Poaceae) compared with castor bean (Euphorbiaceae) and coconut (Arecaceae) pollens. Additional life history studies with NbBr and NbBe on other pollens are clearly needed to determine if the pollens used in our study can be considered as proxy for pollens within their respective families.

Numerous predatory mites readily accept and reproduce on pollen of various plant species (Overmeer, 1985; McMurtry and Croft, 1997; van Rijn and Tanigoshi, 1999a; Nomikou et al., 2003). For many species, pollen is a more suitable food than arthropod prey, albeit different pollen species largely differ in their accessibility to and nutritional value for predatory mites (Abou-Setta and Childers, 1989; Broufas and Koveos, 2000). For example, many *Euseius* species have a lower immature mortality and a higher reproductive capacity when reared on certain pollens compared with arthropod prey (Tanigoshi et al., 1983; Abou-Setta and Childers, 1989; Zhao and McMurtry, 1990; Schausberger, 1992). In our study, maize pollen was superior to the other pollens tested. This pollen is a carbohydrate-rich food source known to supplement mite diet and sustain predators during prey scarcity (Tanigoshi et al., 1993; Toko et al., 1994; Hanna et al., 2005). Maize pollen is also a suitable food for the reproduction of *Typhlodromalus aripo* DeLeon, the effective biological control agent of the spider mite *Mononychellus tanajoa* (Bondar) in Africa (Gnanvossou et al., 2005; Hanna et al., 2005).

Several studies have shown life history differences among populations of the same phytoseiid species of different origins. Croft (1971) (cited in Galazzi and Nicoli, 1996) found significant differences in the critical photo-phase prior to diapause in four populations of *Typhlodromus occidentalis* Nesbit. Moraes and McMurtry (1985) reported different oviposition rates in *Phytoseiulus persimilis* Athias-Henriot from different geographic regions. Likewise, Galazzi and Nicoli (1994) cited in Galazzi and Nicoli (1996) and Galazzi and Nicoli (1996) reported differences in the performances and reproductive traits of different populations of the same species.

Our findings indicate that *N. baraki* from either Benin or Brazil could be a suitable candidate for biological control of *A. guerreronis*. While the Brazilian population appears to have a narrower diet breadth, the two populations should also be able to temporarily persist on alternative food when the target prey, *A. guerreronis*, is scarce. The higher r_m of the Brazilian population on *A. guerreronis* could be an indicator that this population is a more efficient predator on *A. guerreronis* than the Beninese population. Other factors must be considered, however, to determine the validity of this assumption, as

a predator's higher intrinsic rate of increase does not always predict greater predator efficiency in suppressing the target prey. On cassava in Africa, although the predatory mite *Amblydromalus* (syn. *Typhlodromalus*) *manihoti* had higher r_m than *Typhlodromalus aripo*, the latter species proved to be a better biological control agent of cassava green because of its ability to persist at low prey densities, its ability to disperse rapidly (and prior to depletion of prey), and its tolerance to a wider range of environmental conditions (Gnanvossou et al., 2003; Onzo et al., 2003; Yaninek and Hanna 2003).

Finally, it is possible that food sources other than those tested in this study and present in the coconut environment may be suitable for *N. baraki*. In addition to considering other pollen types, future studies should assess the effect of combined food types on the life history of *N. baraki*, the response of this predator to varying temperature and relative humidity, and the possible interactions among co-occurring biological control agents of the coconut palm as well as the behavioral and reproductive compatibility of the two populations tested here. Small scale releases of *N. baraki* should also be carried out to test their potential in controlling *A. guerreronis* under field conditions.

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CHAPTER 6

6. Intraguild predation and cannibalism between the predatory mites *Neoseiulus neobaraki* and *N. paspalivorus*, natural enemies of the coconut mite *Aceria guerreronis*.

Intraguild predation and cannibalism between the predatory mites

Neoseiulus neobaraki and *N. paspalivorus*, natural enemies

of the coconut mite *Aceria guerreronis*.

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Abstract

Neoseiulus neobaraki and *N. paspalivorus* are amongst the most common phytoseiid predators of coconut mite, *A. guerreronis*, found in the spatial niche beneath coconut fruit bracts. Both predators occasionally co-occur on coconut palms or fruits in Benin and Tanzania and are therefore likely to interact with each other. Here, we assessed cannibalism and intraguild predation of the two predators in absence and presence of their primary prey *A. guerreronis*. In absence of the shared extra-guild prey *A. guerreronis*, *N. neobaraki* killed 19 larvae of *N. paspalivorus* per day and produced 0.36 eggs/female/day, while the latter species killed only 7 larvae of the former and produced 0.35 eggs/female/day. Presence of the herbivorous prey *A. guerreronis* only slightly decreased IGP by *N. neobaraki* but strongly decreased IGP by *N. paspalivorus*, which consumed 4 to 7 times less IG prey than *N. neobaraki*. Resulting offspring-to-IG prey ratios were however 4 to 5 times higher in *N. paspalivorus* than *N. neobaraki*. Overall, additional provision with *A. guerreronis* increased oviposition in both species. In the cannibalism tests, in absence of *A. guerreronis*, *N. neobaraki* and *N. paspalivorus* consumed 1.8 and 1.2 conspecific larvae and produced almost no eggs. In the presence of abundant herbivorous prey, cannibalism dramatically decreased but oviposition steeply increased in both *N. neobaraki* and *N. paspalivorus*. In summary, we conclude that (1) *N. neobaraki* is a much stronger intraguild predator than *N. paspalivorus*, (2) cannibalism is very limited in both species, and (3) both IGP and cannibalism are reduced in the presence of the common herbivorous prey with the exception of IGP by *N. neobaraki*, which remained at high levels despite presence of herbivorous prey. We discuss the implications of cannibalism and IGP on the population dynamics of *A. guerreronis* and the predators in view of their geographic and within-palm distribution patterns.

Keywords : Trophic interactions, Eriophyidae, Phytoseiidae, biological control, coconut

Introduction

Knowledge of trophic interactions among predator species is important to understand predator-prey and predator-predator dynamics, and to predict the impact of predators in natural and biological control of herbivorous arthropod pests (Polis et al., 1989; Soluk, 1993; Sih et al., 1998; Losey and Denno, 1999). Two common trophic interactions among predators are intraguild predation (IGP), the killing and consumption of heterospecific food competitors (Polis et al., 1989), and cannibalism, the killing and consumption of conspecifics (Elgar & Crespi, 1992). Both phenomena are widespread in the animal kingdom (Fox, 1975; Polis, 1981; Polis et al., 1989, Elgar & Crespi, 1992; Polis and Holt 1992; Rosenheim et al., 1995), and often intensify when heterospecific prey (for cannibalism) and extraguild prey (for IGP) are scarce (Polis, 1981; Polis et al., 1989; MacRae and Croft, 1997; Schausberger, 1997; but see Hironori and Katsuhiko, 1997 and Lucas et al., 1998). Both IGP and cannibalism not only provide additional food to the predators but also relax inter- or intraspecific competition and predation risk in the case of mutual predation. Both types of predation may affect the distribution and abundance of populations and species, and, for example, result in spatial and temporal segregation of predators and prey, completely displacing the prey or restricting it to less suitable habitats or time periods (Sih et al., 1985; Hurd and Eisenberg, 1990; Moran et al., 1996; Walzer et al., 2001, 2010).

Prevalence of both phenomena in predator communities of the family Phytoseiidae has received considerable attention in recent years (Yao and Chant 1989; Croft and Croft 1993; Croft and Zhang 1994; Schausberger, 1997; Schausberger and Croft, 2000a, b; Schausberger 2003 for a review of cannibalism). Most studies to date focused on phytoseiid species important in natural or biological control of tetranychid mites or thrips in the USA, Australia or Europe. Analogous studies on IGP within phytoseiid communities occurring in Africa are scarce and dealt exclusively with IGP among native and exotic phytoseiid mites of the cassava agroecosystem, in the framework of classical biological control of the cassava green mite *Mononychellus tanajoa* (Onzo et al., 2004; Onzo et al., 2005, Zannou et al., 2005).

In the course of developing a sound biological control program against the coconut mite, *A. guerreronis*, a complex of predatory mite species was found associated with the pest on coconut fruits in Benin, Brazil, Ghana, Sri Lanka, and Tanzania. In all surveyed countries, predatory mites of the family Phytoseiidae were the most common, namely *Neoseiulus paspalivorus* DeLeon, *N. baraki*

Athias-Henriot and *N. neobaraki* Zannou, Moraes de, Oliveira (Fernando et al., 2003; Lawson-Balagbo et al., 2008a; Negloh et al., 2008; Negloh et al., 2011). These predators are adapted to living beneath the coconut bracts where *A. guerreronis* lives. Laboratory observations showed that all three species readily prey on coconut mites and particularly *N. baraki* and *N. paspalivorus* appear to have a preference for *A. guerreronis* as prey (Lawson-Balagbo et al., 2007; Negloh et al., 2008; Domingos et al. 2010). Results of surveys conducted in Benin and Tanzania indicated that *N. baraki*, *N. neobaraki* and *N. paspalivorus* may share palm trees, and even coconut fruits, albeit to a lesser extent (Negloh et al., 2011). The three predatory mites exhibit different distribution patterns in both countries. While in Benin *N. paspalivorus* was the most frequently found, followed by *N. baraki* and *N. neobaraki*, the frequency order in Tanzania was *N. neobaraki*, *N. baraki*, and *N. paspalivorus*. The way and extent to which these predators interact may be, among other factors, one of the reasons for these distribution patterns. Therefore, insight in the potential intraguild interactions of these predators is important in developing a sound biological control program involving one or more of these species. So far only one topically relevant study has been undertaken for a predatory mite community in Brazil (Lawson-Balagbo et al 2008b) but none for Africa. In the present study, we investigated cannibalism and IGP, in absence and presence of *A. guerreronis* as extra-conspecific or extra-guild prey, of the two phytoseiid mites *N. neobaraki* and *N. paspalivorus*.

Materials and Methods

Predators and prey origins and maintenance

Experimental phytoseiids originated from laboratory populations established from specimens collected on coconut fruits in southern Benin in 2004 and 2005. Specimens of *N. paspalivorus* were collected in Fonsa (06°21'66" N, 02°09'76" E), a village located near Ouidah, department of Atlantique, while specimens of *N. neobaraki* were collected in Gbehoue (06°21'45" N, 01°55'05" E), in the department of Mono.

In the laboratory, both predators were reared on separate rearing units on eggs and larvae of *Tetranychus urticae* (Koch) prior to the initiation of the experiments presented here (eggs were provided that often hatched into larvae within few days; the larvae never molted to the nymphal stage).

Each rearing unit consisted of a Petri dish (14.5 cm diameter and 1 cm high) filled with water-saturated cotton wool. A foam pad ($4 \times 4 \times 1$ cm) carrying a black PVC tile ($4 \times 4 \times 0.1$ cm) rested on top of the cotton. The edges of the tile were covered with moist tissue paper reaching down into the water-saturated cotton to prevent the mites from escaping. A tuft of hydrophobic cotton wool covered by a piece of transparent plastic was placed in the center of each rearing arena and served as oviposition site for the predators. To obtain the larvae used as prey in the experiments, cohorts of gravid females were set up. Eggs were collected from these cohorts and transferred to experimental discs 24 h prior to the experiment. The larvae obtained during this period were then adjusted to the desired number on each experimental disc. *Aceria guerreronis* was obtained from freshly collected coconut fruits. Climatic conditions in the maintenance room were 27 ± 1 °C, 70 to 80% RH and 12:12 h light:dark photophase.

Experimental procedures

We assessed predation on con- and hetero-specific larvae by adult females of *N. neobaraki* and *N. paspalivorus*, in absence and presence of their primary prey *A. guerreronis*. Each predator-predator species combination was subjected to three treatments: (1) 20 predator larvae, (2) 20 predator larvae plus 20 *A. guerreronis*, and (3) 20 predator larvae plus 100 *A. guerreronis*. Each treatment was replicated 70 to 100 times. To start the experiment, eggs of predators were collected from established colonies of 100 to 150 gravid females and placed on a new arena. After 24 h, 20 freshly hatched larvae were placed on each experimental unit with or without *A. guerreronis* collected from young coconut fruits. Female predators were randomly withdrawn from the rearing units, starved for 24 h prior to testing, and then singly introduced onto arenas. Occurrence (yes/no) and predation rates in cannibalism and IGP, predator female survival and oviposition were assessed every 24 h over 10 days. All prey items were replenished daily. Evidence of the consumption of larvae was determined when the dead larvae were shriveled. Additionally, longevities and oviposition of *N. paspalivorous* and *N. neobaraki* were compared when held without prey, Experimental units were stored at 25 ± 1 °C, 70-80 % RH, and 12:12 h light: dark photophase.

Data analyses

All analyses were performed in SAS 9.2 TS Level 1MO, W32_VSPRO Platform (SAS, 2008). Occurrence (yes/no) of cannibalism and IGP in absence or presence of *A. guerreronis* were tested within each predator species with overall chi-square and post-hoc chi-square tests with Bonferroni's correction ($\alpha = 0.048$). Predation rates and oviposition rates were separately compared between species and among prey treatments using two-way repeated measures analysis of variance (ANOVA). Post-hoc means separations were performed using least square means with Bonferroni adjustment. Longevities were compared within each species among treatments with the lifetest procedure. The same procedure was used to compare longevities of starved predators.

Results

The occurrence of cannibalism and IGP was calculated as the percentage of predation occurrence (yes/no) on con- or hetero-specific larvae over 24 h. Occurrence of cannibalism varied with absence/presence of *A. guerreronis* in both predator species. In absence of *A. guerreronis*, cannibalism reached ~80% in both *N. neobaraki* and *N. paspalivorus*, while IGP was 100% in both species (Tables 1, 2). The occurrence of cannibalism by *N. neobaraki* females was significantly reduced when they were additionally provided with *A. guerreronis*. In contrast, the occurrence of cannibalism by *N. paspalivorus* decreased only slightly when 20 *A. guerreronis* were provided but was negligible when 100 *A. guerreronis* were provided (Table 1). The occurrence of IGP was less affected by the addition of *A. guerreronis* prey than the occurrence of cannibalism. IGP occurrence deviated from 100% only in *N. paspalivorus* provided with 100 *A. guerreronis* (Table 2).

The consumption rate of both predator species was much higher with hetero-specific than con-specific larvae regardless of the absence/presence of *A. guerreronis* prey (Tables 1, 2). Consumption decreased significantly over time in the cannibalism experiments ($F_{9, 530} = 19.64$, $P < 0.0001$), with the highest consumption (1.52 larvae) on the first day and the lowest (0.22) on the thenth day. In contrast, consumption was not influenced by time in the IGP experiments ($F_{9, 474} = 0.99$, $P = 0.4509$). *N. neobaraki* cannibalized significantly more larvae in absence than presence of *A. guerreronis* (no matter the *A. guerreronis* density), whereas the cannibalism rate of *N. paspalivorus* was only reduced when they were additionally provided with 100 *A. guerreronis* (Table 1). *N. neobaraki* cannibalized more

larvae in absence of *A. guerreronis* than *N. paspalivorus* did ($t_{584} = 4.61$, $P < 0.0001$) but cannibalism by *N. neobaraki* was dramatically reduced (about 3 times) in the presence of 20 *A. guerreronis*, in contrast to *N. paspalivorus* (Table 1).

In IGP, *N. neobaraki* consumed slightly more *N. paspalivorus* larvae when offered alone compared to when offered with 20 *A. guerreronis* but consumed much less when offered with 100 *A. guerreronis* (Table 2). Comparison between both treatments with *A. guerreronis* revealed that *N. neobaraki* consumed significantly less *N. paspalivorus* larvae in presence of 100 *A. guerreronis* than in presence of 20 *A. guerreronis* ($t_{528} = -5.35$, $P < 0.0001$). *N. paspalivorus* females consumed less *N. neobaraki* larvae when additionally offered either 20 or 100 *A. guerreronis* (Table 2).

Oviposition of cannibalizing predators increased with increasing provision of herbivorous prey (Table 1). Both predator species produced less eggs with only conspecific larvae as prey than when additionally provided with 20 or 100 *A. guerreronis*. Similarly to consumption, oviposition varied significantly over time in cannibalism ($F_{9, 530} = 32.24$, $P < 0.0001$) while it remained similar over time in IGP ($F_{9, 472} = 0.14$, $P = 0.9985$). In cannibalism, oviposition showed an overall increase from day 1 to day 4 then decreased towards day 10. With only conspecific larvae as prey, both species produced similar number of eggs ($t_{584} = 0.41$, $P = 0.68$). In IGP, oviposition was lowest when both *N. neobaraki* and *N. paspalivorus* were only offered heterospecific larvae. Both species produced similar number of eggs within each treatment (least square means: $t_1 = -0.02$, $P = 0.99$ for larvae alone; $t_1 = 5.27$, $P = 0.12$ for larvae with 100 *A. guerreronis* and $t_1 = -0.00$, $P = 0.99$ for larvae with 20 *A. guerreronis*). The oviposition rates increased significantly with the addition of *A. guerreronis* ($F_{2, 499} = 72.91$, $P < 0.0001$) (Table 2).

In the cannibalism experiment, all *N. paspalivorus* survived throughout the 10 d experiment regardless of *A. guerreronis* addition or not. Similarly, all *N. neobaraki* survived the 10 d experiment when fed with conspecific larvae plus 100 *A. guerreronis*. Their survival time decreased negligibly to 9.5 and 9.8 days, respectively, in the other treatments (Table 1). In the IGP experiment, all individuals of both species survived the 10 d experiment regardless of the treatment.

Starved *N. neobaraki* and *N. paspalivorus* survived 2.0 and 4.8 days, respectively ($F_{1, 584} = 25.57$, $P < 0.0001$). Neither species produced eggs during the starvation experiment.

Discussion

In the present study, we determined whether and to which extent IGP and cannibalism occur in *N. neobaraki* and *N. paspalivorus*, two phytoseiid species commonly found in association with *A. guerreronis* beneath the bracts of coconut fruits in Benin, Tanzania, Brazil and Sri Lanka (Fernando et al., 2003; Lawson-Balagbo et al., 2007; Negloh et al., 2011). Both species engaged in cannibalism and IGP, both trophic interactions were modulated by absence/presence of the preferred herbivorous prey *A. guerreronis*. Overall, both species refrained from cannibalism in the presence of abundant *A. guerreronis*. *Neoseiulus neobaraki* was much more prone to IGP than was *N. paspalivorus*.

Propensity to cannibalism and IGP

Occurrence and levels of cannibalism and IGP observed in the present study were largely consistent with initial predictions. Both trophic interactions occurred at relatively high levels in the absence of herbivorous prey in both species. Cannibalism decreased substantially with increasing levels of herbivorous prey, while IGP by both species remained high even in the presence of extraguild prey. These findings suggest that cannibalism by both species, as in several other systems (e.g., Rosenheim et al., 1995; Onzo et al., 2005; Zannou et al., 2005), is an adaptive survival strategy during periods of absence or scarcity of heterospecific prey whereas IGP has additional functions such as elimination of competitors and predators.

Our results also show that *N. neobaraki* and *N. paspalivorus* feed more on hetero- than con-specific larvae, especially in absence of the common herbivorous prey *A. guerreronis*. This likely indicates the ability of *N. paspalivorus* and *N. neobaraki* to discriminate between con – and heterospecific larvae and feed preferentially on the latter (Schausberger and Croft, 1999, 2000a; Schausberger, 2003; Montserrat et al., 2006). The two predator species live gregariously beneath the bracts of coconut fruits (Lawson-Balagbo et al., 2007b; Negloh et al., 2010, 2011) and such a life style may promote the evolution of species and kin recognition abilities (Schausberger and Croft, 2001; Schausberger, 2003; Montserrat et al., 2006), eventually allowing them to feed less on conspecific than heterospecific larvae. Species discrimination and preferential consumption of heterospecific phytoseiids when given a choice appears to be a common trait in generalist phytoseiid predators (Schausberger, 1999; Schausberger and Croft, 1999, 2000b). Zannou et al. (2005) suggested that the low cannibalism

observed in *Typhlodromalus aripo* DeLeon was a consequence of the gregarious life style of that species, which lives most of its life in the small apex of cassava plants.

The presence of herbivorous prey decreased cannibalism substantially, to almost nil, while the decrease in IGP was much less pronounced in the presence of increasing extraguild prey densities, as compared with cannibalism. Such a pattern was also observed for another phytoseiid guild on cassava (Zannou et al., 2005). In that study, the three phytoseiid species in the cassava agro-ecosystem considerably reduced predation on con- and heterospecific larvae when alternative food was added. Herbivorous prey tends to be more easily accessible, because of being less mobile when feeding, and often has higher nutritional value (Onzo et al., 2005; Zannou et al., 2005) than intraguild prey. When present in abundant quantity the predator would be expected to spend more time and energy on this primary prey, thereby decreasing the use of con- or heterospecific larvae in the current case (Schausberger, 2003; Zannou et al., 2005). In addition, the predators run risk of being injured or killed when attacking other predators even if they are juveniles (Montserrat et al., 2006). Presence of abundant extraguild prey often substantially reduces IGP (Zannou et al., 2005), but in our study *N. neobaraki* was only little affected by the presence of extraguild prey, contrary to *N. paspalivorus*. Adult and juvenile *N. neobaraki* are much larger and more voracious than *N. paspalivorus* (Negloh, personal observations) and would therefore need more food to meet their nutritional needs. Functional response tests (Negloh, unpublished) indicated that gravid *N. neobaraki* females can consume more than 200 *A. guerreronis* per day, leaving room to additionally consume *N. paspalivorus* larvae on top of the 100 *A. guerreronis* offered in the IGP experiment of the present study. Due to its high nutritional needs, *N. neobaraki* could tend to protect its food sources against competitors, hence the great propensity to kill *N. paspalivorus* larvae. In addition, the body size ratio between predator and prey is a common determinant in IGP and cannibalism (Polis et al. 1989; Croft et al. 1996; Schausberger 1997, 2003; Walzer and Schausberger 1999; Zannou et al. 2005). Because of their smaller body size, *N. paspalivorus* larvae were probably easier to capture and overwhelm for adult *N. neobaraki* females than *N. neobaraki* larvae for adult *N. paspalivorus* females. The body size of *N. neobaraki* larvae approaches that of *N. paspalivorus* protonymphs (Negloh, personal observation).

In all IGP treatments, *N. neobaraki* killed substantially more heterospecific predator larvae than *N. paspalivorus* did. *N. neobaraki* seems therefore able to simultaneously suppress *N. paspalivorus* and

the extraguild prey *A. guerreronis*. Due to *N. neobaraki* being larger, more voracious and stronger in IGP, *N. paspalivorus* runs risk of being suppressed by *N. neobaraki*, which could contribute to the separation in the distribution of these two species in the field. *N. paspalivorus* could have evolved avoidance behaviors regarding *N. neobaraki* (Janssen et al., 1997; Magalhães et al., 2004, Cakmak et al., 2006; Walzer et al., 2009, Walzer and Schausberger 2011). This argument is supported by the current occurrence of both species in Benin and Tanzania. *N. neobaraki* is more frequently found and more abundant than *N. paspalivorus* in Tanzania while in Benin *N. paspalivorus* is the most frequent and abundant (Negloh et al., 2011). Though both species can occasionally be found on the same palm trees, they usually do not co-occur on the same fruits (Negloh et al., unpublished). Further observations indicate that, while *N. paspalivorus* is widespread in Benin and encountered throughout the year, *N. neobaraki* is found in very few locations mainly in swampy areas, and difficult to find through most part of the year (Negloh, personal observations).

Survival and reproduction

Both predator species had very low oviposition rates when they consumed only conspecific larvae but oviposition increased in presence of *A. guerreronis*. Avoidance to feed on conspecific larvae leading to little biomass ingested probably contributed to the observed low oviposition rate by cannibalism. Reproduction with heterospecific larvae alone was higher than with cannibalism but still low when compared with treatments where *A. guerreronis* was present. These observations are similar to those made in other phytoseiid guilds (e.g., Yao and Chant, 1989; Walzer and Schausberger, 1999; Schausberger and Croft, 2000b; Zannou et al., 2005) and true for many other generalist phytoseiids that benefit more from IGP than from cannibalism (Schausberger, 1999; Walzer and Schausberger, 1999; Schausberger and Croft, 2000a). Whether higher reproduction with *A. guerreronis* was the result of more and/or qualitatively superior nutrients or less energy investment in handling needs further investigations. Survival was similar for both species in either cannibalism or IGP tests, regardless of the predator-predator combination, suggesting that the nutrients gained from addition of herbivorous prey did not translate in enhanced survival, at least not for the 10 d experimental period tested. Both predators, however, survived much longer than when confined without any food. Both species therefore likely use con- or heterospecific juvenile prey to survive prey scarcity. Other studies showed variations in survival between cannibalism and IGP or between the two phenomena and combinations with herbivorous or alternative prey (Zannou et al., 2005). The duration of our experiments (10 d) was

probably too short to show the effect of additional herbivorous prey provision on survival of these predators by cannibalism and IGP.

Implications to natural and biological control of A. guerreronis

Three possible directions can result from interactions among predators regarding their impact on the pest population (Loosey and Denno, 1999). First, two predators may induce complementary effects thus increasing the risk posed to the prey (Heinz and Nelson, 1996; Riechert and Lawrence, 1997; Loosey and Denno, 1998; Onzo et al. 2004; Onzo et al., 2005; Walzer et al., 2009). Second, the two predators may interfere with each other such that the predation risk to the shared prey is decreased (Spiller, 1986; Finke and Denno, 2003; Cakmak et al. 2006; Abad-Moyano et al., 2010). Third and largely theoretical, the interactions of both species could have no effect on the prey. Besides, density-mediated and trait-mediated effects can arise as lethal and non-lethal, respectively, consequences of the interactions between both predator species (e.g. Preisser et al. 2005). Density-mediated effects result from true predation while trait-mediated effects result from predator-induced changes in the behavioral, morphological or life history traits of prey. In the case of IGP these effects may cascade down to the extraguild prey and further to the plant level (e.g. Walzer and Schausberger 2009). Due to differences in temporal and spatial distribution of both species in the present case (Negloh, personal observations) it is unlikely that IGP would induce an immediate negative effect on natural or biological control of *A. guerreronis*. One would however expect increased control of the pest in the presence of both species since they tend to refrain from IGP when extraguild prey is present. It is also possible that the intraguild prey *N. paspalivorus* develop an antipredator behavior (Magalhães et al. 2002) to *N. neobaraki* thus avoiding niches occupied by the latter species. Previous observations actually showed that both species were rarely found on the same fruit, although they could be found on the same palm (Negloh et al. unpublished data). Mutual predator avoidance behaviors may reduce the enemy free space for the shared prey and enhance control (Walzer et al. 2009).

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Table 1: Cannibalism on larvae by adult females of *Neoseiulus neobaraki* and *N. paspalivorus* in absence or presence of *A. guerreronis* prey.

Prey type ¹	N	Occurrence (%) ²	Larvae killed/day ²	Oviposition/day ²
<i>N. neobaraki</i>				
Larvae	93	81.7a	1.78 ± 0.09a	0.06 ± 0.08a
Larvae+20Ag	97	43.3b	0.60 ± 0.08b	0.52 ± 0.08b
Larvae+100Ag	100	2.00c	0.03 ± 0.02c	1.38 ± 0.08c
χ^2 ; F _{2, 260}		126.5	32.46	27.45
P		<0.0001	<0.0001	<0.0001
<i>N. paspalivorus</i>				
Larvae	100	80.0a	1.23 ± 0.08a	0.02 ± 0.08a
Larvae+20Ag	100	71.0a	1.10 ± 0.08a	0.93 ± 0.08b
Larvae+100Ag	100	2.00b	0.02 ± 0.01b	1.29 ± 0.08c
χ^2 ; F _{2, 270}		145.7	126.07	153.42
P		<0.0001	<0.0001	<0.0001

¹Ag stands for *A. guerreronis*.

²Values within a column, under a given species, followed by the same letter are not significantly different (Overall chi-square and post hoc follow-up chi-square tests with Bonferroni's correction $P > 0.048$ for occurrence, two-way repeated measures analysis of variance (ANOVA) followed by least square means separations with Bonferroni adjustment, $P \leq 0.05$).

Table 2: Intraguild predation on larvae by adult females of *Neoseiulus neobaraki* and *N. paspalivorus* in absence or presence of *A. guerreronis* prey.

Prey type ¹	N	Occurrence (%) ²	Larvae killed/day ²	Oviposition/day ²
<i>N. neobaraki</i>				
Larvae	95	100a	18.27 ± 0.28a	0.35 ± 0.09a
Larvae+20Ag	85	100a	17.45 ± 0.30a	1.05 ± 0.09b
Larvae+100Ag	100	100a	15.29 ± 0.27b	1.80 ± 0.09c
χ^2 ; F _{2, 268}		-	21.31	81.40
P		-	<0.0001	<0.0001
<i>N. paspalivorus</i>				
Larvae	70	100a	7.47 ± 0.33a	0.36 ± 0.10a
Larvae+20Ag	84	100a	3.99 ± 0.30b	1.05 ± 0.09b
Larvae+100Ag	100	78b	2.15 ± 0.27c	1.16 ± 0.09b
χ^2 ; F _{2, 242}		37.1	146.97	15.92
P		<0.0001	<0.0001	<0.0001

¹Ag stands for *A. guerreronis*.

²Values within a column, under a given species, followed by the same letter are not significantly different (Overall chi-square and post hoc follow-up chi-square tests with Bonferroni's correction $P > 0.048$ for occurrence; two-way repeated measures analysis of variance (ANOVA) followed by least square means separations with Bonferroni adjustment, $P \leq 0.05$).

CHAPTER 7

7. Morphological variation and reproductive incompatibility of three coconut-mite-associated populations of predatory mites identified as *Neoseiulus paspalivorus* (Acari: Phytoseiidae)

Morphological variation and reproductive incompatibility of three coconut-mite-associated populations of predatory mites identified as *Neoseiulus paspalivorus* (Acari: Phytoseiidae)

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Abstract

Predatory mites identified as *Neoseiulus paspalivorus* DeLeon (Phytoseiidae) have been considered as agents for classical biological control of the coconut mite, *Aceria guerreronis* Keifer (Eriophyidae), in Africa and elsewhere. Preliminary identification of geographically distinct populations as belonging to the same species (*N. paspalivorus*) was based on their morphological similarity. However, laboratory studies recently conducted have shown large differences in feeding behaviors and biological characteristics among individuals collected from three geographic origins: Brazil (South America), Benin and Ghana (West Africa). As morphologically similar specimens do not necessarily belong to the same species, we evaluated under laboratory conditions, reproductive compatibility between the specimens from three geographic locations to ascertain their con-specificity. Morphological measurements were also made to determine whether there is a means of discriminating between them. Inter-population crosses showed complete reproductive isolation between the three geographic populations, but inter-population discontinuities in morphometric characters were absent. These results indicate that the tested specimens are distinct biological entities despite morphological similarity. Further molecular genetic studies are therefore proposed, including screening for endosymbionts and assessment of genetic differentiation, to determine the cause of reproductive incompatibility and to clarify the taxonomic relationship between those populations.

Keywords Morphometrics, Reproductive isolation, Conspecificity, Biological species

Introduction

Coconut, *Cocos nucifera* L. is one of the main crops in the Tropics. The coconut mite, *Aceria guerreronis* Keifer, has become one of the most important arthropod pests of this crop around the world. It causes severe damage to the crop by attacking the meristematic tissues underneath the bracts. According to recent assessments the abundance of the coconut mite and its damage to the crop may be lower, and the predator fauna associated with coconut mite may be richer in certain areas of Brazil than in Africa (Benin, Ghana and Tanzania) and Sri Lanka (Fernando et al. 2003; Lawson-Balagbo et al. 2008; Reis et al. 2008; Negloh et al. 2010; Fernando et al. unpublished). Over the past two decades, attempts were made to control the pest through the use of chemicals and biopesticides. These control measures, however, have proven to be costly, often ineffective and ecologically undesirable (Moore and Alexander 1987; Moore et al. 1989). Because of the exotic nature of the coconut mite in Africa (originated from South America) (Navia et al. 2005), classical biological control, i.e., the intentional importation and release of natural enemies in the native range of the target pest, where the pest and its natural enemies coevolved, for permanent establishment and self-sustained control of the target pest, is thought to be a reasonable approach to combat the coconut mites.

Mites in the family Phytoseiidae are mostly predators that are well-known to play a significant role in the biological control of arthropod pests such as mites and small insects (McMurtry et al. 1970; Helle and Sabelis 1985; Lindquist et al. 1996; Sabelis and Van Rijn 1997; Gerson et al. 2003). The predatory mite *Neoseiulus paspalivorus* (DeLeon) (Acari: Phytoseiidae) is the most common natural enemy associated with the coconut mite in Brazil (Lawson-Balagbo et al. 2008; Reis et al. 2008). In Benin and Ghana, West Africa, *N. paspalivorus* was also frequently reported on infested coconuts (Negloh et al. 2010). However, recent laboratory experiments by Famah Sourassou et al. (in prep.) showed large differences in feeding behaviors and biological characteristics among specimens from the three geographic locations: Benin, Ghana (West Africa) and Brazil (Itamaraca, State of Pernambuco). All three geographic populations developed and reproduced on coconut mite prey, but their biological parameters differed. Furthermore, the specimens from Beninese and Brazilian populations were able to develop and reproduce on *Tetranychus urticae* Koch (Acari: Tetranychidae), whereas the Ghanaian ones were able to develop on this prey, yet unable to convert the food obtained from this prey into eggs. Moreover, the specimens from Brazilian and Ghanaian populations were able to develop on

coconut pollen, whereas those from Beninese populations were not. All three geographic populations of *N. paspalivorus* did not produce any eggs on coconut pollen. Contrasting results with our data were previously reported by Lawson-Balagbo et al. (2007), showing that *N. paspalivorus* specimens collected in Acarau (Ceara State, Brazil) could not feed and develop on *T. urticae*, whereas they were able to develop and reproduce on coconut pollen. Taken together, there are clear indications that the three *N. paspalivorus* geographic populations are biologically different despite their morphological similarity. Such biological differences between morphologically similar populations may indicate cryptic species (Muma and Denmark 1969; Monetti and Croft 1997; Tixier et al. 2003, 2004, 2006a, 2008). To test whether the three allopatric populations are ‘potentially able to interbreed and produce viable progeny’ (biological species sensu Mayr 1940) it is necessary to understand the evident biological differences between the three geographic populations.

The study reported in this article aimed to evaluate reproductive compatibility among three geographic populations of predatory mites (Brazil, Benin, and Ghana) that have been identified as *N. paspalivorus* based on morphological similarity only. We also conducted morphological comparison to determine if there are morphological traits other than those used for their identification that may serve to distinguish these populations.

Materials and methods

Source populations and rearing techniques

Specimens of *N. paspalivorus* were collected from coconut in October 2005 in Ouidah (06° 21' N; 02° 09' E), Southern Benin, in September 2006 in Itamaraca (07°46' S; 34°52' W), State of Pernambuco, Northeastern Brazil and in December 2008 near Winneba (05°22' 907" N; 00°38' 685" W), Southern Ghana. These three samples were used for propagation in a rearing unit consisting of a black PVC tile (4 x 4x 0.1 cm) placed on top of a foam pad (4 x 4x 0.1 cm) resting in a Petri dish (14.5 cm in diameter and 1 cm in height). The edges of the tile were covered with a band of tissue paper that also contacted the foam pad. To prevent mites from escaping, distilled water was supplied to the Petri dish on a daily basis to keep wet the foam pad and the tissue paper. A tuft of hydrophobic cotton wool covered by a piece of transparent plastic was placed in the center of each rearing unit to serve as an oviposition site

for the predators. Colonies of all populations were maintained in a climate controlled room at 25–27°C, 70–90% relative humidity and a 12–12 h light–dark cycle. The colonies were provided with a new supply of immature stages of *T. urticae* at 3 day intervals.

Crossing experiments

Each experiment started with a cohort of 1-day old eggs obtained from gravid females of each of the three populations. To obtain them, one hundred females were confined to rearing units similar to that described above and offered eggs of *T. urticae* as prey. After 24 h, each egg laid by the predator was transferred to a new unit, again similar to that previously described above, except that the PVC was 2.5 cm diameter black PVC disk and had a hole of 2 mm diameter in the center to serve as an oviposition site. The nymphal stages of the three populations were reared on all stages of *A. guerreronis* supplied *ad libitum*. When reaching adulthood, predators were sexed and females and males of each population were kept apart for the subsequent crosses.

Mating of all possible combinations of recently moulted females and males of the three populations were considered to determine reproductive compatibilities. All crosses were set up as a single pair mating between virgin females and males. Additionally, 10 virgin females of each population were kept in isolation, to ascertain that mating is necessary for oviposition to take place. Each pair was observed daily and the number of eggs laid was recorded for a period of 10 days. Eggs found in the arenas of a given male–female combination of geographic strains were pooled in a unit similar to that previously described (8.5 cm in diameter and 1.5 cm in height), and reared to adulthood to determine egg viability, post-embryonic survivorship and sex-ratio. Offsprings of each cross were backcrossed to assess hybrid fertility. All crossing experiments were carried out simultaneously under the same environmental conditions as described previously (25–27°C, 70–90% relative humidity and a light–dark cycle of 12–12 h). At the end of the observation period, females and males of each population were preserved in 70% alcohol for morphological analysis.

Morphological analysis

Twenty adult females and ten adult males of each population were slide-mounted in Hoyer's medium for examination under a phase-contrast microscope. Measurements were done with an ocular

micrometer, at 4009 magnification to measure body size and shield dimensions and at 1,0009 magnification to measure the length of setae, spermathecal calyx (spermatodactyl for male) and cheliceral digits. Females and males were characterized based on 32 morphological traits (Tables 2, 3) that are used commonly for the identification of phytoseiid mites (i.e., Chant and McMurtry 1994, 2005; Moraes et al. 2004; Zannou et al. 2006). Setal nomenclature follows that of Lindquist and Evans (1965), as applied to the phytoseiids by Rowell et al. (1978) and Chant and Yoshida-Shaul (1991). For measurement of cheliceral digits and spermatodactyl, another set of at least 20 females and 20 males of each population were slide-mounted separately. The gnathosoma was cut from the rest of the body and a slight pressure was applied to the mount by touching the slide coverslip with the rear end of a small brush to help in distinguishing the two cheliceral digits. Holotype measurements, taken from the original description (De Leon 1957), were also included in the results for comparison.

Data analysis

Crossing data

A single-factor ANOVA was used to test the effects of crossing types on fecundity and duration of the pre-oviposition period. Data of the latter were log-transformed [$\ln(x + 1)$] for ANOVA. Means were compared using the Student–Newman–Keuls multiple range test (SNK) at $\alpha = 0.05$.

Morphological data

Morphological characters of females and males were analyzed separately with SAS software (SAS Institute 2003). For each population and each character examined, descriptive statistics (mean, standard error, maximum, minimum) were calculated using the MEANS procedure (PROC MEANS). Differences among geographic populations were tested by one-way analysis of variance (PROC ANOVA) followed by a Newman-Keuls multiple comparison test at $\alpha = 0.05$. Next, a multifactorial analysis (Principal Component Analysis) and a Canonical Discriminant Analysis were performed to assess patterns of morphological variation and to identify morphological characters that contribute most to morphological differentiation among the three geographic populations. Prior to multifactorial analysis, a discriminant analysis was carried out to examine how many specimens were correctly classified into their original populations.

Results

Cross-breeding experiments

All mated females of intra-population crosses laid eggs, but the proportion of ovipositing females from inter-population crosses ranged between 80 and 100%. Unmated females did not oviposit (Table 1). The pre-oviposition period of offspring from each of the inter-population crosses (ranging between 2.9 and 5.4 days) was longer than that of offspring from the intra-population crosses (<2.0 days) ($P < 0.001$; Table 1).

Average fecundity was about two- to three-fold higher in intra-population than in interpopulation crosses ($P < 0.001$; Table 1). All eggs obtained from intra-population crosses were viable, with 71% producing females. All eggs produced by inter-population crosses were malformed and non-viable, similar to crosses between females from the Beninese population and males from the Brazilian population. In the crossings between females from Brazil and males from Benin, for which nearly 14% of the eggs produced (13 eggs out of 92) had a normal shape, only 7.6% of the eggs (7 eggs) hatched giving rise to male progeny only. Eggs were not produced by females of any of the backcrosses involving hybrid males from Brazilian females and Beninese males or Beninese males and Brazilian females ($n = 3$ for each combination), although mating was observed. In the control backcrosses ($n = 3$ for each combination), average of 11.3 and 11.0 eggs per female were recorded for Brazilian and Beninese populations respectively.

Table 1 Outcome of crosses involving three geographic populations identified as *Neoseiulus paspalivorus*

Cross	<i>N</i>	% ovipositing females	Pre-oviposition period (±SE)	No. eggs/female (±SE)	% deformed eggs/female	% egg hatchability	Immature survivorship (±SE)	Sex ratio (% female)
<i>Female × male</i>								
<i>Intra-population crosses</i>								
Brazil × Brazil	20	100	1.6 ± 0.19 c	12.8 ± 0.64 a	0	100	99.0 ± 0.65	71.9 ± 3.92
Benin × Benin	20	100	1.8 ± 0.26 c	12.2 ± 0.48 a	0	100	99.4 ± 0.51	71.8 ± 1.11
Ghana × Ghana	15	100	1.9 ± 0.19 c	10.1 ± 0.55 b	0	100	100	74.1 ± 4.01
<i>Inter-population crosses</i>								
Brazil × Benin	30	83.3	3.8 ± 0.31 b	5.2 ± 0.33 c	86	7.6	100	0.0*
Benin × Brazil	30	93.3	2.9 ± 0.25 b	5.7 ± 0.55 c	100	0	—	—
Brazil × Ghana	15	100	5.4 ± 0.13 a	3.8 ± 0.29 c	100	0	—	—
Ghana × Brazil	15	93.3	5.2 ± 0.22 a	4.2 ± 0.41 c	100	0	—	—
Benin × Ghana	10	100	3.1 ± 0.10 b	5.8 ± 0.66 c	100	0	—	—
Ghana × Benin	10	80	5.1 ± 0.54 a	3.9 ± 0.84 c	100	0	—	—

Means followed by the same letter in a column are not significantly different (SNK test, $P > 0.05$)

N number of pair crosses

* All progeny are males

Table 2 Mean, standard error and range of 32 morphometric variables measured on adult females of three geographic populations identified as *Neoseiulus paspalivorus*

Morphological characters	Benin		Ghana		Brazil		Holotype (De Leon 1957)
	Mean \pm SE	Min–Max	Mean \pm SE	Min–Max	Mean \pm SE	Min–Max	
Length of dorsal shield	343.1 \pm 0.99 a	336–352	338.2 \pm 0.73 c	333–342	340.6 \pm 0.74 b	336–349	340
Width of dorsal shield	137.9 \pm 0.96 a	130–146	138.8 \pm 0.78 a	130–146	138.3 \pm 0.98 a	127–143	140
j1	11.4 \pm 0.13 a	10–12	11.5 \pm 0.15 a	10–12	10.3 \pm 0.12 b	10–11	11
j3	10.5 \pm 0.14 b	10–12	11.4 \pm 0.18 a	10–12	10.1 \pm 0.22 b	8–11	11
j4	9.3 \pm 0.16 a	8–10	9.4 \pm 0.23 a	8–11	9.0 \pm 0.17 a	10–12	9
j5	9.0 \pm 0.13 a	8–10	9.5 \pm 0.24 a	8–11	9.2 \pm 0.16 a	8–10	10
j6	9.8 \pm 0.12 b	9–11	10.4 \pm 0.18 a	9–11	9.6 \pm 0.17 b	8–10	11
J2	10.9 \pm 0.17 a	9–12	11.1 \pm 0.16 a	10–12	10.4 \pm 0.13 b	10–11	11
J5	9.0 \pm 0.12 b	8–10	9.9 \pm 0.23 a	8–11	9.3 \pm 0.14 b	9–10	9
z2	9.3 \pm 0.19 b	8–10	10.7 \pm 0.19 a	9–12	9.6 \pm 0.13 b	9–10	11
z4	10.3 \pm 0.17 b	9–11	11.0 \pm 0.17 a	9–12	10.8 \pm 0.14 b	10–11	11
z5	9.2 \pm 0.13 b	9–10	10.0 \pm 0.12 a	9–11	8.8 \pm 0.17 b	7–10	9
Z1	11.1 \pm 0.20 a	10–14	11.4 \pm 0.20 a	9–12	10.1 \pm 0.21 a	9–12	11
Z4	17.0 \pm 0.21 a	15–18	17.0 \pm 0.19 a	15–19	16.0 \pm 0.17 b	15–17	17
Z5	54.6 \pm 0.36 a	51–56	50.2 \pm 0.40 a	48–52	52.5 \pm 0.27 b	51–55	52
s4	12.5 \pm 0.15 a	11–14	12.4 \pm 0.16 a	11–15	11.8 \pm 0.19 b	10–12	12
S2	13.6 \pm 0.16 a	12–15	13.9 \pm 0.20 a	12–15	12.8 \pm 0.17 b	11–14	14
S4	14.5 \pm 0.19 b	14–16	15.3 \pm 0.15 a	14–16	14.6 \pm 0.18 b	14–16	15
S5	15.8 \pm 0.18 b	15–18	18.7 \pm 0.16 a	17–20	19.0 \pm 0.17 a	18–20	19
r3	11.7 \pm 0.22 b	10–14	13.8 \pm 0.14 a	12–15	11.8 \pm 0.19 b	11–14	11
R	11.0 \pm 0.23 a	9–12	11.4 \pm 0.16 a	10–12	10.1 \pm 0.12 b	9–11	10
St IV	17.6 \pm 0.20 b	16–19	18.4 \pm 0.22 a	16–20	17.5 \pm 0.19 b	16–19	–
ST1-ST3	80.8 \pm 0.43 a	76–82	80.5 \pm 0.42 a	79–86	80.7 \pm 0.36 a	79–82	–
ST2-ST2	51.9 \pm 0.42 ab	47–54	50.8 \pm 0.48 b	47–54	52.6 \pm 0.42 a	51–57	–
ST5-ST5	59.6 \pm 0.37 a	57–63	58.3 \pm 0.35 b	57–60	57.4 \pm 0.39 b	54–60	–
VSW-ANT	81.4 \pm 0.33 a	79–82	81.1 \pm 0.48 a	79–85	82.0 \pm 0.47 a	79–86	–
VSW-POST	73.5 \pm 0.49 a	70–76	60.8 \pm 1.11 b	54–73	73.8 \pm 0.40 a	70–76	–
Length of ventrianal shield	103.6 \pm 0.56 a	98–108	107.8 \pm 0.57 a	101–111	102.2 \pm 0.75 b	95–108	–
Length of fixed digit	17.8 \pm 0.19 a	17–19	18.0 \pm 0.27 a	16–19	18.3 \pm 0.19 a	17–19	–
Length of movable digit	23.7 \pm 0.19 a	23–25	23.1 \pm 0.38 a	22–25	23.3 \pm 0.27 a	21–25	–
Length of calyx	7.8 \pm 0.18 a	6–9	8.2 \pm 0.17 a	6–9	8.0 \pm 0.16 a	6–9	–
Diameter of calyx	6.8 \pm 0.14 a	6–7	6.6 \pm 0.18 a	5–7	6.3 \pm 0.10 a	6–7	–

All measurements in micrometers

Means with same letter in a row are not significantly different (SNK test, $P > 0.05$)

VSW-ANT width of ventrianal shield at level of ZV2, VSW-POST width of ventrianal shield at anus

Table 3 Mean, standard error and range of 32 morphometric variables measured on adult males of three geographic populations identified as *Neoseiulus paspalivorus*

Morphological characters	Benin		Ghana		Brazil		Holotype (De Leon 1957)
	Mean \pm SE	Min–Max	Mean \pm SE	Min–Max	Mean \pm SE	Min–Max	
Length of dorsal shield	257.1 \pm 1.73 a	250–269	257.7 \pm 1.16 a	253–263	256.8 \pm 2.11 a	241–263	260
Width of dorsal shield	124.5 \pm 0.67 a	120–127	117.6 \pm 0.87 b	114–120	124.0 \pm 0.56 a	120–127	120
j1	9.6 \pm 0.19 a	8–10	9.8 \pm 0.25 a	9–11	10.0 \pm 0.12 a	9–10	–
j3	10.0 \pm 0.26 a	8–11	10.2 \pm 0.12 a	9–11	10.3 \pm 0.16 a	9–11	–
j4	7.8 \pm 0.25 b	6–9	7.6 \pm 0.22 b	6–9	8.5 \pm 0.16 a	7–9	–
j5	7.8 \pm 0.26 a	6–9	8.1 \pm 0.21 a	7–9	8.4 \pm 0.19 a	7–9	–
j6	8.6 \pm 0.22 a	7–10	9.4 \pm 0.27 a	8–11	9.0 \pm 0.36 a	7–10	–
J2	9.2 \pm 0.33 a	8–11	9.6 \pm 0.19 a	8–10	10.0 \pm 0.26 a	9–11	–
J5	7.0 \pm 0.20 b	6–8	6.6 \pm 0.19 b	6–8	7.6 \pm 0.29 a	6–9	–
z2	8.2 \pm 0.27 b	6–9	9.4 \pm 0.20 a	8–10	8.6 \pm 0.22 b	7–10	–
z4	9.1 \pm 0.26 b	8–10	10.0 \pm 0.18 a	9–11	9.8 \pm 0.22 a	9–11	–
z5	7.3 \pm 0.22 a	6–9	8.0 \pm 0.20 a	7–9	7.8 \pm 0.19 a	7–9	–
Z1	9.6 \pm 0.19 a	8–10	9.2 \pm 0.42 a	7–11	9.5 \pm 0.33 a	9–10	–
Z4	14.4 \pm 0.27 ab	12–15	14.0 \pm 0.36 b	13–16	15.2 \pm 0.31 a	14–16	–
Z5	40.4 \pm 0.64 a	36–44	41.1 \pm 0.65 a	38–44	40.0 \pm 0.69 a	37–45	–
s4	10.5 \pm 0.27 a	9–11	11.2 \pm 0.18 a	10–12	10.8 \pm 0.19 a	10–11	–
S2	11.2 \pm 0.18 b	10–12	12.1 \pm 0.19 a	11–13	11.4 \pm 0.22 b	10–12	–
S4	12.2 \pm 0.31 b	11–14	13.1 \pm 0.33 a	11–15	11.8 \pm 0.20 a	11–13	–
S5	12.7 \pm 0.29 b	11–14	14.8 \pm 0.34 a	13–16	15.0 \pm 0.32 a	13–16	–
r3	9.6 \pm 0.19 b	9–10	10.2 \pm 0.25 ab	9–11	10.8 \pm 0.26 a	10–12	–
R	9.1 \pm 0.32 b	8–10	9.5 \pm 0.20 ab	8–10	10.2 \pm 0.25 a	9–11	–
St IV	15.0 \pm 0.18 b	14–16	16.1 \pm 0.22 a	15–17	15.3 \pm 0.25 b	14–16	–
ST1-ST3	67.2 \pm 0.63 a	63–70	66.2 \pm 0.56 a	63–70	65.9 \pm 0.42 a	63–66	–
ST2-ST2	40.2 \pm 0.48 ab	38–41	40.0 \pm 0.51 b	38–41	41.5 \pm 0.31 a	41–44	–

Table 3 continued

Morphological characters	Benin		Ghana		Brazil		Holotype (De Leon 1957)
	Mean \pm SE	Min–Max	Mean \pm SE	Min–Max	Mean \pm SE	Min–Max	
ST5-ST5	33.6 \pm 0.51 a	32–35	30.7 \pm 0.67 b	28–35	33.0 \pm 0.51 a	31–35	–
VSW-ANT	118.0 \pm 1.62 a	111–127	117.0 \pm 1.31 a	111–123	116.6 \pm 1.31 a	108–120	–
VSW-POST	76.7 \pm 1.55 a	70–82	75.1 \pm 1.06 a	70–79	78.6 \pm 0.92 a	73–82	–
Length of ventrianal shield	92.5 \pm 0.92 a	89–98	94.4 \pm 0.63 a	92–98	92.0 \pm 1.05 a	85–95	–
Length of fixed digit	12.0 \pm 0.20 b	11–12	12.8 \pm 0.26 a	11–14	12.3 \pm 0.25 ab	11–14	–
Length of movable digit	17.6 \pm 0.30 a	16–19	17.2 \pm 0.25 a	16–19	17.7 \pm 0.22 a	16–19	–
Length of spermatod. shaft	11.7 \pm 0.20 a	11–12	12.0 \pm 0.20 a	11–12	11.8 \pm 0.21 a	11–12	–
Length of spermatod. foot	4.6 \pm 0.32 a	4–6	4.8 \pm 0.25 a	4–6	4.5 \pm 0.27 a	4–6	–

All measurements in micrometers

Means with same letter in a row are not significantly different (SNK test, $P > 0.05$)

Spermatod Spermatodactyl

Table 4 Classification based on discriminant analysis of 32 morphological characters of three geographic populations of *Neoseiulus paspalivorus* females and males

	Female				Male			
	% well-classified ^a	Benin	Ghana	Brazil	% well-classified ^a	Benin	Ghana	Brazil
Benin	90	18	0	2	60	6	2	2
Ghana	85	0	17	3	80	0	8	2
Brazil	85	2	1	17	50	0	5	5
Total	86.7	20	18	22	63.33	6	15	9

^a Percentage of well-classified individuals in their original populations

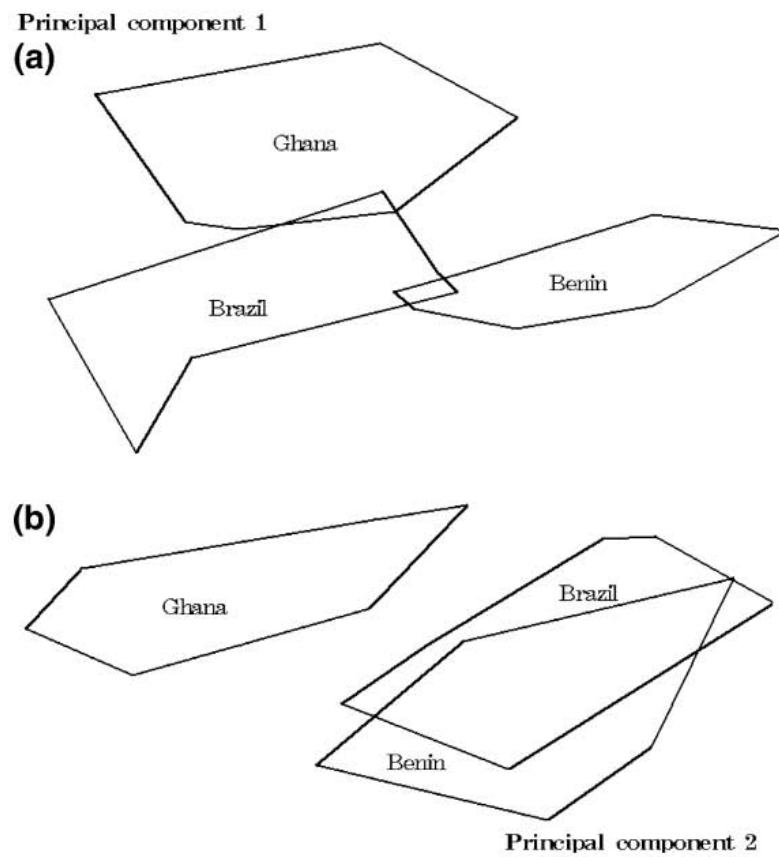


Fig. 1 Principal component analysis based on 32 morphometric characters on adult females **(a)** and males **(b)** from three geographic specimens identified as *Neoseiulus paspalivorus*: Polygons formed based on the projection of individuals of each population onto the principal components 1 and 2

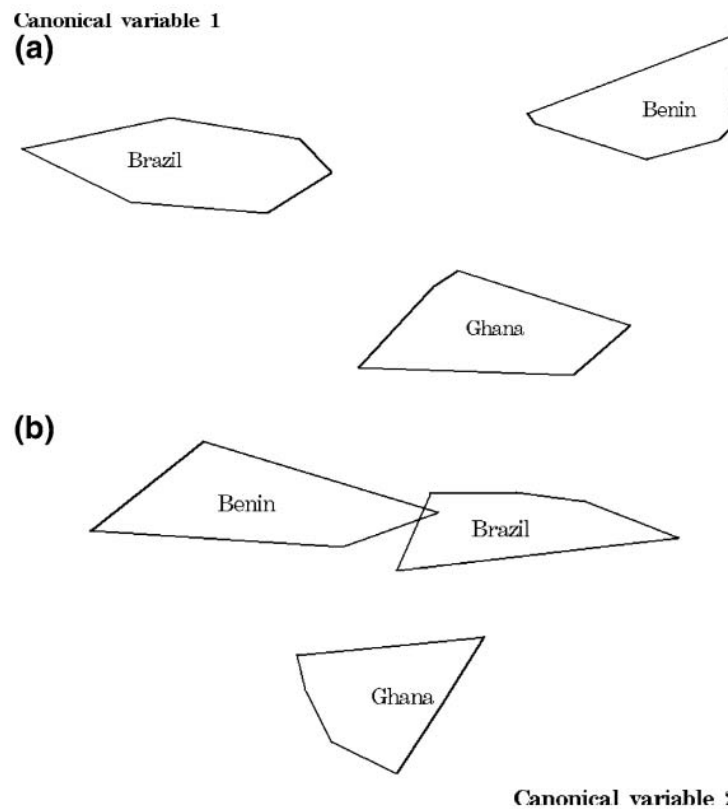


Fig. 2 Canonical discrimination analysis of 32 morphological characters of adult females (a) and males (b) from three geographic populations of *Neoseiulus paspalivorus*: Polygons formed based on the projection of individuals of each population onto the canonical variable 1 and 2

Morphological analysis

Mean, standard errors and range of the 32 characters are shown in the Tables 2 and 3, respectively for female and male populations. Significant differences were observed for 22 and 15 out of the 32 characters measured, respectively among female and male populations. However, these differences were very small, except for the width of the ventrianal shield at anus level (POST-WVS), which was smaller for the Ghanaian females and the length of seta S5, which was slightly shorter for Beninese females. The standard errors within populations were low and the differences between the minimal and the maximal values for each of the measured morphological variables (except for POST-WVS of the Ghanaian females) were very small.

Predicted classification of specimens based on discriminant analysis is shown in Table 4. Overall, 86.7% of female specimens and 63.3% of male specimens were classified in the population of origin. The majority of misclassifications for males concerned the Ghanaian and Brazilian specimens (7 of 11, or 63.6%), whereas for females 50% of the misclassifications concerned the Beninese and Brazilian populations, and the Ghanaian and the Brazilian populations. The results of discriminant analysis were corroborated by the principal component analysis (Fig. 1); substantial overlaps of measurements of females occurred between the Brazilian and Beninese specimens, and between the Brazilian and the Ghanaian specimens. Overlaps were observed between the Ghanaian and Beninese female specimens (Fig. 1a). In relation to male specimens, considerable level of overlap was found between the measurements on the Brazilian and Beninese specimens (Fig. 1b). The canonical discrimination analysis allowed a clear distinction between the females of the three populations (Fig. 2a). The Brazilian and Beninese specimens are the most distant, while the Ghanaian specimens were intermediate between latter two. However, in males, only the Ghanaian specimens could be clearly distinguished from the two others (Fig. 2b).

The posterior width of the ventrianal shield (VSW_POST) and the lengths of j1, Z5, S5 and r3 contributed the most to the morphological differentiation between females of the three geographic populations, whereas the width of the dorsal and genital shields and length of seta S5 contributed the most to the morphological differentiation of males from the three geographic populations.

N. paspalivorus females from Ghana had a slightly narrower dorsal shield and relatively longer ventrianal shield with a reduced width at the level of the anal shield (posterior width of ventrianal shield) and slightly shorter seta Z5, while males of the same population had slightly narrower dorsal and genital shields. The two sexes of Beninese specimens differed from those of Brazil and Ghana by having slightly shorter seta S5. Specimens from Brazil appeared to be intermediate, but much closer to Beninese specimens by having a similar size of ventrianal shield (for female) and genital shield (for male) with the Beninese specimens and similar length of setae S5 with the Ghanaian specimens.

Discussion

The results of univariate and multivariate analyses are consistent with the previous identification of all three populations - from Benin, Ghana and Brazil - as belonging to the same species. Overlapping morphometric values indicate great morphological similarity among the geographic populations tested. The measurements of the specimens examined were similar to the holotype measurements of *N. paspalivorus* (De Leon 1957) and measurements of females for the same species from Sri Lanka (Moraes et al. 2004).

Differences in setal measurements were small, and the discrimination between the geographic populations by multifactorial analyses was based on only some characters that have low weight in species differentiation. McMurtry (1980) indicated that caution should be exercised in using relatively small differences in setal length to differentiate species. Tixier et al. (2006b) reported large differences in setal lengths between *Kampimodromus hmiminai* (McMurtry and Bounfour) from France and Morocco and *K. adrianae* (Ferragut and Pena-Estevez) from the Canary Islands, but molecular analysis indicated that they are synonyms. However, the results of cross-breeding indicate reproductive isolation between the populations investigated, which, should therefore be considered as separate species (Mayr 1940). The absence of oviposition in unmated *N. paspalivorus* females observed in this study was in agreement with what is known to date for the majority of phytoseiids (Croft 1970; McMurtry et al. 1976; McMurtry 1980; Moraes and McMurtry 1981; Noronha and Moraes 2002, 2004). Thus, the high proportion of ovipositing females observed in inter-population crosses imply that mating had occurred, therefore indicating the absence of pre-mating reproductive barriers between the

populations under test. The complete bidirectional post-mating reproductive incompatibility observed between the geographic populations was expressed in the form of reduced fecundity, zygotic mortality and a male-biased sex ratio among the few sterile offspring.

Bidirectional incompatibility is a rare phenomenon, as unidirectional incompatibilities are the most common in phytoseiid and tetranychid mite populations (Hoy and Cave 1988; Gotoh and Nugochi 1989; Gotoh et al. 1995; Breeuwer 1997; Johanowicz and Hoy 1998; Vala et al. 2000, 2002; Noronha and Moraes 2002, 2004). Our results of bidirectional incompatibility among geographic populations of *N. paspalivorus* are therefore quite exceptional, but are similar to those reported by Monetti and Croft (1997) with respect to crosses between the morphologically similar phytoseiid species *Neoseiulus californicus* (McGregor) and *N. fallacis* (Garman). Moreover, Klimov et al. (2004) observed postzygotic reproductive isolation between two cryptic species of *Sancassania* mites, *Sancassania salasi* and *S. ochoai* (Klimov, Lekveishvili and OConnor), and molecular analysis of these populations showed that they represent distinct species.

The causes of reproductive incompatibility are poorly studied in mites, particularly in phytoseiids. Only in the phytoseiid *Galendromus occidentalis* (Nesbitt) and in some spider mites, the endosymbionts *Wolbachia* and more recently *Cardinium* have been demonstrated to mediate unidirectional reproductive incompatibility (Hess and Hoy 1982; Gotoh et al. 1995; Johanowicz and Hoy 1998; Breeuwer 1997; Vala et al. 2000, 2002, 2003; Gotoh et al. 2006; Ros and Breeuwer 2009). Bidirectional incompatibility is assumed to be caused by either negative nuclear-nuclear genes interactions, as has been reported in the spider mite *Panonychus mori* Yokoyama (Gotoh et al. 2005), or infection by different strains of *Wolbachia*, as is well documented for insects (Laven 1959, 1967; Mercot et al. 1995; O'Neill and Karr 1990; Clancy and Hoffmann 1996). Although males and females from different geographic origins were able to mate, we did not obtain F1 hybrids (except in the cross involving Beninese females and Brazilian males where very few sterile males were produced). This apparent lack of gene exchange among the three geographically isolated populations revealed a pattern of reproductive isolation among them. Moreover, the crossing data appeared to corroborate the previously known biological differences observed among these three geographic populations (Famah et al. in prep).

Taken together, we expect the reproductive incompatibility observed in this study, to be the result of genetic divergence due to allopatric differentiation among the populations investigated (Hurt and Hedrick 2003; Dettman et al. 2008) rather than to be caused by *Wolbachia*. Even if endosymbionts would be present, there is no guarantee that they were the cause of reproductive isolation because some *Wolbachia* strains are incapable of inducing reproductive incompatibility in their host (Giodarno et al. 1995; Turelli and Hoffmann 1995; Gotoh et al. 2005). For example, Gotoh et al. (2005) observed bidirectional reproductive incompatibility between two Japanese populations of *P. mori* (from Hanayama and Toyama) although harbouring the same *Wolbachia* strain. In this case, the procedure suggested by Breeuwer (1997) i.e. crossing experiments in combination with antibiotic treatment should be used to demonstrate whether they are involved in the reproductive incompatibility. Reproductive isolation is generally thought to develop by the gradual accumulation of genetic differences between populations as a by-product of other adaptive or neutral genetic changes that take place in allopatry (Mayr 1963; Charlesworth et al. 1987; Coyne 1992, 1993; Wu and Davis 1993; Dre's and Mallet 2002; Gavrillets 2003; Coyne and Orr 2005; Dettman et al. 2008). Moreover, reproductive isolation has been shown to represent an initial step in the speciation process by preventing or greatly reducing gene flow between populations (Laven 1959, 1967; Conner and Saul 1986; Thompson 1987, 1995; Dettman et al. 2008), and our data are therefore consistent with incipient allopatric speciation (Dre's and Mallet 2002; Gavrillets 2003; Coyne and Orr 2005) among these three geographically isolated specimens identified as *N. paspalivorus*.

In conclusion, we found that three geographically isolated populations of predatory mites preliminarily identified as *N. paspalivorus*, show reproductive isolation indicating that they are distinct biological species despite morphological similarity. Based on the crossing data reported in this study, we propose that there is every reason to screen for the presence of endosymbionts and to perform molecular characterization in order to test the hypothesis on allopatric speciation. It would be also informative to use molecular methods for determining the origin and invasion route of these predatory mites in comparison with what is already known for *A. guerreronis* (Navia et al. 2005), i.e. the prey of these predatory mites and the pest of coconut palms. By including more geographic populations, our work may provide a basis for inferring the intercontinental and between country invasion of these predatory mites and creates a solid basis for finding the best strains for developing biological control of coconut mites.

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Summary

Coconut, *Cocos nucifera* L., a perennial tree crop, is the third most produced tree crop of the world, and widespread through the sub-tropical regions of America, Asia and Africa. It constitutes an important food and income source for hundred thousands people around the world. For decades in America and Africa, and more recently in Asia, one of the major biotic constraints to coconut production has been and continues to be a tiny worm-like mite of the family Eriophyidae, the coconut-mite, *Aceria guerreronis* Keifer. *A. guerreronis* leaves beneath the perianth of coconut fruits and causes there heavy damage to the developing tissues. To mitigate the damaging effects of the pest on growth of coconut fruits many control attempts have been made in Africa and America from the 1960s until the 1980s, without substantial success. The scientific researches, however, provided valuable insights in the intractable nature and importance of the pest. Several control strategies have been tested among which biological control became and remains the most promising to date. The overall objective of the present thesis, as a part of a larger program initiated in 2004, was to help to develop and possibly implement sound and sustainable biocontrol strategies against the coconut mite in Africa and elsewhere in the world through studies on its occurrence, ecology and natural enemies.

The five core studies of the thesis first included surveys in major coconut growing areas of Benin and Tanzania to ascertain the nature and pest status of the coconut mite and its distribution in Africa, with emphasis on its abundance, the severity of damages induced, potential natural enemies and other associated acarine fauna. In both countries not a single coconut plantation was free of damage. The damage incidence was more than 80% at the palm level and the severity of damage increased with fruit age. The density of coconut mites was the highest on 3 to 4 months old fruits. The phytoseiid predatory mites *Neoseiulus paspalivorus*, *N. baraki* and *N. neobaraki* were the most often associated with the pest in both countries, though they showed reverse patterns of occurrence in these countries. *N. paspalivorus* was the most abundant in Benin, in contrast to Tanzania where the most abundant was *N. neobaraki*. Additionally, several other herbivorous, fungivorous and predatory mites of different taxonomic groups were found on the coconut fruit.

To characterize the interactions between the pest and its most common predators, the population dynamics of *A. guerreronis* and *N. paspalivorus* were studied in four coconut plantations of two areas,

coastal and inland, in Southern Benin. The seasonal and fruit age dependent fluctuations of both mite populations were assessed and the within-plant and within-bunch distribution of both organisms were examined in terms of their population density and proportions of infested fruits. *N. paspalivorus* was only occasionally able to follow the fluctuations of its prey but with a time lag that allowed the pest to thrive. In addition, the predatory mites arrived on the fruits and started to colonize them with almost one month delay after the first arrival of the pest.

In the laboratory, the life history traits of *N. baraki* originating from Benin and Brazil were compared on five food sources, one of which was *A. guerreronis*. Individuals of both origins were able to complete development on *A. guerreronis*, the two-spotted spider mite *Tetranychus urticae* and maize pollen. Both origins achieved the most favorable demographic parameters on *A. guerreronis* but the Brazilian population was slightly superior to the Beninese population in its population growth parameters. The Brazilian population was, however, less well able than the Beninese population in utilizing *T. urticae*. The other food sources, coconut pollen and castor bean pollen, were not suitable to sustain growth of any population.

The most commonly found predators occurred sometimes in the same area or on the same palm. Thus, we found it necessary to investigate possible predator-predator interactions and examined cannibalism and intraguild predation of *N. paspalivorus* and *N. neobaraki*. The latter species was previously found to be a far more voracious predator of *A. guerreronis* than the former. In presence of coconut mite prey, both species refrained from cannibalism but *N. neobaraki* continued to engage in intraguild predation. *N. neobaraki* was superior in intraguild predation to *N. paspalivorus* but was less efficient in converting food into offspring, compared to *N. paspalivorus*. In complete absence of food, *N. paspalivorus* survived longer than *N. neobaraki* but none of them produced eggs.

Although morphologically identified as *N. paspalivorus*, three populations of this species originating from Brazil, Ghana and Benin, appeared to have substantial biological differences. Therefore, detailed morphometric characterizations and reproductive compatibility studies were conducted to ascertain the con-specificity of these three allopatric populations. The results of the inter-population crosses showed that the three populations were completely isolated from one another, although morphological measurements showed no differences. The three populations are therefore distinct biological entities despite their morphological similarities.

Zusammenfassung

Kokospalmen, *Cocos nucifera* L., sind in den sub-tropischen Regionen von Amerika, Asien und Afrika weit verbreitet und weltweit die dritt-häufigste holzige Nutzpflanze. Kokospalmen sind eine bedeutende Nahrungs- und Einkommensquelle für Hunderttausende Menschen auf der ganzen Welt. Der wichtigste Schadorganismus der Früchte der Kokospalmen ist - seit mehreren Jahrzehnten in Amerika and Afrika, und seit einigen Jahren auch in Asien - eine winzige wurmförmige Milbe der Familie Eriophyidae (Rost- und Gallmilben), die Kokosnußmilbe *Aceria guerreronis* Keifer. *A. guerreronis* lebt unter dem Perianth der Kokosfrüchte und verursacht dort durch ihre Saugtätigkeit gravierende Schäden am jungen, sich entwickelnden Gewebe. Zur Verringerung der durch die Kokosnußmilben verursachten Schäden, wurden zwischen 1960 und 1980 in Afrika und Amerika zahlreiche Kontrollmethoden getestet, jedoch ohne substanziellen nachhaltigen Erfolg zu erzielen. Diese Arbeiten lieferten jedoch wertvolle Erkenntnisse über die Bedeutung und Hartnäckigkeit dieses Schädling. Im Vergleich der untersuchten Methoden stellte sich bald die Biologische Kontrolle, i.e. der Einsatz natürlicher Gegenspieler, als die vielversprechendste Kontrollstrategie heraus. Diese Doktorarbeit war in ein umfangreiches, multinationales, im Jahr 2004 initiiertes, Projekt über die Kontrolle der Kokosnußmilben eingebunden. Generelles Ziel der Doktorarbeit war es, zur Entwicklung und potentiellen Implementierung von fundierten und nachhaltigen biologischen Kontrollstrategien gegen die Kokosnußmilbe in Afrika und anderswo in der Welt durch eingehende Studien über ihr Vorkommen, ihre Ökologie und ihre natürlichen Gegenspieler, beizutragen. Die Doktorarbeit besteht aus fünf separaten, aber thematisch zusammenhängenden Studien.

Die erste Studie befasste sich primär mit faunistischen Erhebungen in den wichtigsten Kokosanbaugebieten in Benin und Tansania. Der aktuelle Status der Kokosnußmilbe als Schädling, ihr Vorkommen und ihre Verbreitung, ihre Abundanz, das Ausmaß der verursachten Schäden, das Vorkommen potentieller natürlicher Gegenspieler and anderer assoziierter Milbenarten wurden eruiert. Jede Kokosplantage in beiden Ländern war von der Kokosnußmilbe befallen, wobei mehr als 80% aller untersuchten Palmen Schadsymptome aufwiesen. Das Schadensausmaß verstärkte sich mit dem Alter der Früchte. Die höchste Abundanz der Kokosnußmilben wurde an 3 bis 4 Monate alten Früchten beobachtet. In beiden Ländern waren die Raubmilben *Neoseiulus paspalivorus*, *N. baraki* and *N. neobaraki* (alle Familie Phytoseiidae) die am häufigsten mit der Kokosnußmilbe assoziierten

natürlichen Gegenspieler. Diese drei Räuberarten hatten aber unterschiedliche Verbreitungsmuster in Benin und Tansania. *N. paspalivorus* war die häufigste Art in Benin, *N. neobaraki* war die häufigste Art in Tansania. Einige weitere herbivore, fungivore und räuberisch lebende Milben verschiedener taxonomischer Gruppen wurden auf den Kokosfrüchten gefunden.

Zur Charakterisierung der Interaktionen zwischen dem Schädling und ihrem häufigsten natürlichen Gegenspieler, wurde die Populationsdynamik von *A. guerreronis* und *N. paspalivorus* in vier Kokosplantagen in zwei Gebieten, an der Küste und im Inland, im Süden von Benin untersucht. Die saisonalen Fluktuationen beider Milbenpopulationen wurden beobachtet und in Relation zum Alter der Früchte gesetzt. Die Verteilung der Milben innerhalb der Palmen und Fruchtbündel wurde untersucht, ihre Populationsdichten gemessen und der proportionale Anteil der befallenen Früchten eruiert. *N. paspalivorus* konnte numerisch nur gelegentlich den Populationsfluktuationen ihrer Beute folgen. Die numerische Reaktion erfolgte nur mit starker Zeitverzögerung und erlaubte der Beutepopulation zeitweilig starkes ungehindertes Populationswachstum. Darüber hinaus besiedelten die Raubmilben die jungen Früchte erst ein Monat nach Erstbefall durch die Schädlinge.

Im Labor wurden die Lebenszyklus-Charakteristika von *N. baraki* aus Benin und Brasilien bei fünf verschiedenen Nahrungsquellen, darunter *A. guerreronis*, miteinander verglichen. Beide Herkünfte konnten die Juvenilentwicklung mit *A. guerreronis*, der Bohnenspinnmilbe *Tetranychus urticae* und Maispollen als Nahrung abschließen. Beide Herkünfte erzielten die günstigsten demographischen Werte mit *A. guerreronis* als Beute, wobei die brasilianische Population günstigere Populationswachstumsparameter aufwies als jene aus Benin. Die brasilianische Population konnte hingegen *T. urticae* weniger gut als Beute nutzen als dies die Population aus Benin konnte. Die anderen beiden Nahrungsquellen, Pollen von Kokospalmen und Rizinus, waren nicht zur Aufrechterhaltung der Populationen geeignet.

Die am häufigsten auftretenden Räuberarten wurden zum Teil im gleichen Gebiet und auf denselben Palmen gefunden. Es war daher wichtig, mögliche Räuber-Räuber Interaktionen in Betracht zu ziehen und Kannibalismus und Intragilden Prädation innerhalb und zwischen *N. paspalivorus* und *N. neobaraki* zu untersuchen. Frühere Beobachtungen hatten gezeigt, dass *N. neobaraki* ein weitaus gefräßigerer Räuber von *A. guerreronis* ist als *N. paspalivorus*. Bei zusätzlichem Angebot von Kokosnußmilben als Beute, war Kannibalismus bei beiden Arten vernachlässigbar, *N. neobaraki*

betrieb aber weiterhin Intragilden Prädation. *N. neobaraki* war der überlegene Intragildenräuber aber weniger effizient als *N. paspalivorus* in der Umwandlung aufgenommener Beute in die Produktion von Nachkommen. Bei völligem Fehlen von Nahrung, überlebte *N. paspalivorus* länger als *N. neobaraki* aber keine der beiden Arten produzierte Eier.

Die fünfte Studie basierte auf der Beobachtung, dass morphologisch ähnliche Individuen dreier Populationen von *N. paspalivorus*, aus Brasilien, Ghana und Benin, substantielle biologische Unterschiede aufwiesen. Es wurden daher detaillierte morphometrische Charakterisierungen und Reproduktionskompatibilitätsstudien durchgeführt, um die Artenzugehörigkeit dieser drei Populationen zu überprüfen. Reziproke Kreuzungsstudien ergaben, dass die drei Populationen inkompatibel sind, wohingegen die morphometrischen Messungen keine Unterschiede zeigten. Diese drei Populationen müssen daher als getrennte biologische Arten, trotz großer morphologischer Ähnlichkeit, angesehen werden.

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