BIOLOGICAL CONTROL

Temporal Variability and Progression of *Neozygites* sp. (Zygomycetes: Entomophthorales) in Populations of *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae)

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Variabilidade Temporal e Progressão de *Neozygites* sp. (Zygomycetes: Entomophthorales) em Populações de *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae)

RESUMO - O objetivo deste estudo foi caracterizar as epizootias de *Neozygites* sp. sobre alguns aspectos da interação entre este patógeno, o hospedeiro *Mononychellus tanajoa* (Bondar) (ácaro verde da mandioca) e fatores climáticos. Os estudos foram realizados em nove campos de mandioca em Piritiba, BA, Brasil, de março a outubro de 1994. Em todos os campos, o aparecimento do patógeno foi detectado quando a densidade do ácaro era moderada a alta. *M. tanajoa* infectados foram detectados primeiro nos campos localizados no noroeste, com uma aparente progressão para o sudeste. A defasagem entre a constatação do início da doença em um dos campos, até sua constatação em todos os campos foi de 23 dias. Durante a fase epizoótica da maioria dos campos a média diária de umidade relativa variou de 70% a 79% e a temperatura média diária entre 21,1°C e 24,3ºC. A progressão das epizootias foi documentada em dois dos nove campos. No campo onde uma dispersão mais lenta do patógeno foi observada a porcentagem da área com ácaros verdes infectados aumentou de 14% para 100% em 14 dias. O patógeno foi sempre observado em maiores proporcões em parcelas com maiores níveis de *M. tanajoa* e em ácaros coletados em folhas apicais comparado com folhas medianas. Considerável quantidade de *M. tanajoa* com esporos de resistência foi observada em junho e julho, mas no restante da epizootia, corpos hifais foi a única estrutura do fungo observada internamente nos ácaros infectados.

PALAVRAS-CHAVE: Ácaro verde da mandioca, fungo entomopatogênico, epizootiologia, Phytoseiidae.

ABSTRACT - The objective of this study was to characterize epizootics of *Neozygites* sp. by investigating the relationship between the pathogen, the host, *Mononychellus tanajoa* (Bondar) (Cassava green mite = CGM), and climatic

factors. Epizootics were studied from March through October 1994 in nine cassava fields at Piritiba, state of Bahia, Brazil. In all fields the pathogen appeared when CGM density was moderate to high. Infected CGM were first detected in northwestern fields, with an apparent progression to the southeast. The onset of the epizootic in the earliest field was observed 23 days prior to the onset of the epizootic in the latest field. During the epizootic phase of most fields (late May to late June), daily mean RH ranged from 70% to 79% and daily mean temperature ranged between 21.1°C and 24.3°C. Disease progression within CGM populations was documented in two of the nine fields. In the field where the slower spread of the pathogen was observed, the proportion of area with infected CGM increased from 14% to 100% in 14 days. *Neozygites* sp. was detected in higher proportions in plots with the highest levels of CGM and in greater abundance on mites collected from apical leaves as compared with median leaves. Resting spores of *Neozygites* sp. were recovered during June and July, but during the remaining period, infected mites contained only hyphal bodies.

KEY WORDS: Cassava green mite, entomopathogenic fungi, epizootiology, Phytoseiidae.

Neozygites sp. (Zygomycetes: Entomophthorales), a pathogenic fungus, is one of the most important natural enemies of the cassava green mite (CGM), *Mononychellus tanajoa* (Bondar), in the northeastern states of Brazil (Delalibera *et al.* 1992, Delalibera *et al.* 1999). At present, the specific name of this fungus remains unsettled: Keller (1997) has identified this species as *N. floridana* Weiser & Muma, while diverse lines of data gathered on this particular Brazilian *Neozygites* species suggest that it differs significantly from other spider mite pathogens identified as *N. floridana*. We are still gathering information to substantiate our intention to describe the Brazilian CGM pathogen as a new species of *Neozygites*. In the meantime, we will be conservative and refer to this CGM-pathogenic species from Brazil as *Neozygites* sp.

Previous observations indicate that epizootics of *Neozygites* sp. in Brazil tend to develop earlier in some fields than in others. Thus, one strategy for CGM control consists of opportune inoculations of native strains of *Neozygites* sp. to initiate epizootics in fields where disease development is slower. The technical viability of this strategy presupposes understanding of some aspects of epizootiology, reported here, which will indicate the appropriate time for pathogen inoculation. The factors necessary for development of fungal diseases have been identified for only a few pathogen-arthropod systems, making epizootics difficult to predict (Hajek *et al.* 1993). Understanding which factors determine the onset of infection and how development of epizootics occurs in the field will be essential to the successful utilization of *Neozygites* sp. for control of CGM.

The objective of this study was to assess temporal variability of the initial prevalence of *Neozygites* sp. and the disease progression through CGM-infested cassava fields. The dynamics of CGM, phytoseiid predatory mites and the pathogen, *Neozygites* sp., were also evaluated.

Materials and Methods

This study was conducted in nine cassava fields in the district of Sumaré, Piritiba county, state of Bahia, Brazil. All fields were planted

and maintained by local farmers and had variable proportions of the local cassava cultivars 'Pussi', 'Preta do Talo Vermelho' and 'Olho Roxo'. Spacing was approximately 1 x 1 m. No fertilizers or agrochemical inputs were used. Weeds were hoed periodically according to each farmers' judgement. Fields were located within an area having 5 km radius (Fig. 1). The approximate center was the community of Sumaré where equipments to measure rainfall, temperature and relative humidity were installed. Accumulated rainfall, daily mean temperature and relative humidity were summarized for the periods between sampling dates.

Temporal Variability of *Neozygites* sp. Epizootics. This study was conducted in eight cassava fields planted during January 1993. CGM population density and infection by *Neozygites* sp. were measured from March 2nd through November 1st, 1994. Evaluations were made biweekly during March, April, September and October, and when infected CGM were observed, from May through August, evaluations were done weekly. Twenty-five apical and 25 median leaves were randomly selected from each field, and the number of CGM present on each leaf was assessed visually in the field according to a scale proposed by Yaninek *et al*. (1989b): 0 = no CGM; 1 = 1 - 24 CGM; 2 = 25 - 200 CGM; 3 = >200 CGM. For phytoseiid mites, sampling was done by the presence-absence method (Lenis *et al*. 1993). Results were expressed as percentage of leaves with predators.

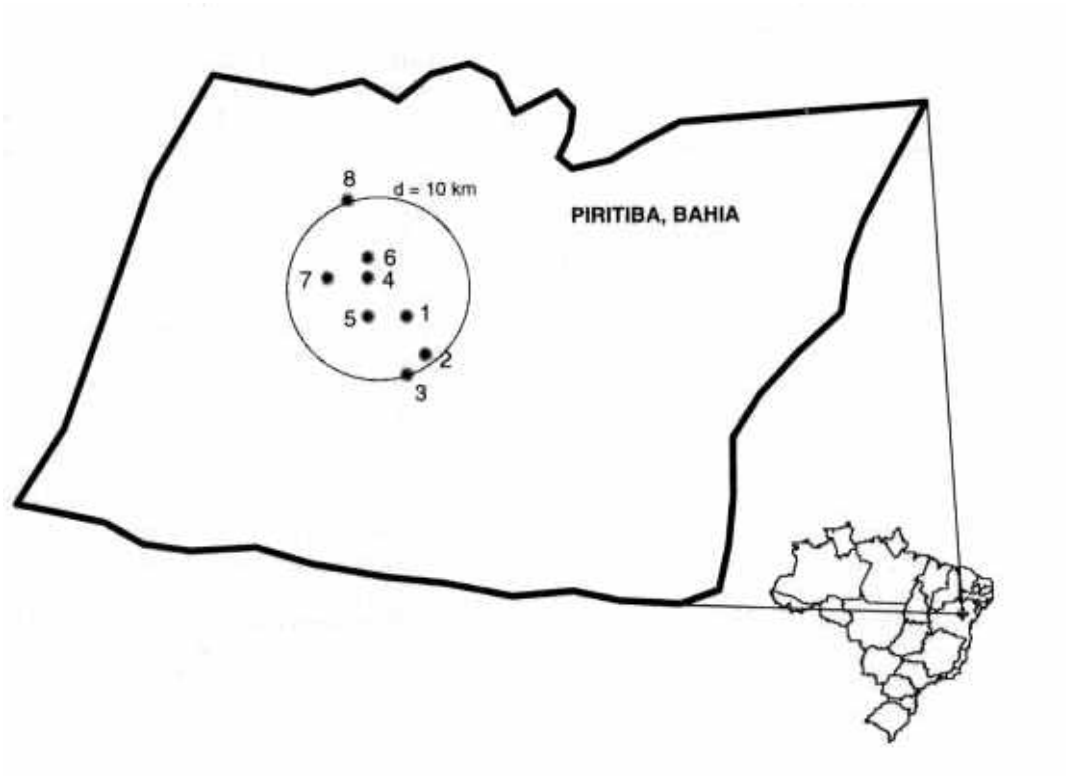

Figure 1. Location of cassava fields used in study of temporal variability of *Neozygites* sp. in Piritiba county, state of Bahia, Brazil. 1994.

The central lobe of each leaf used to estimate CGM density as described above was collected to obtain live specimens for determination of percent infection with *Neozygites* sp. The lobes were placed in paper bags and stored in a Styrofoam cooler with ice for transport to the laboratory. Two CGM females were randomly chosen from each central lobe and were mounted in Aman blue and Hoyer's medium (1:1). Live females were preferentially chosen. However, in some cases high mite mortality due to the long period between sampling and evaluation was observed; in these cases dead females without symptoms of disease were chosen. When the CGM density was very low, extra lobes were collected to assure a total of 100 CGM per field in each sample. First, CGM with capilliconidia adhering to the body were counted. Then, CGM were crushed on a microscope slide to expose body contents. These were examined for presence of *Neozygites* sp. hyphal bodies and resting spores.

Statistical analysis was carried out on 56 measurements taken in eight fields during the epizootic. The infection levels estimated by the proportion of mites with capilliconidia or hyphal bodies in apical leaves were compared with the levels estimated by the same proportions in median leaves using the z-test. We tested if the differences of each of these two binomial proportions was greater than zero. The CGM densities (ordinal scores) in apical versus median leaves were compared using the chi-square-test. Then, a binary logistic regression was used to measure the effect of the independent variables 'field' and 'CGM density' on the response variable 'infection'. The CGM density variable was used as the proportion of leaves with more than 50 mites per leaf. Finally, the two methods used to estimate the infection rate, 'capilliconidia' and 'hyphal bodies' were compared using z-test and correlation. The analyses were conducted using MINITAB 11.13 (1996).

Progression of *Neozygites* sp. Within Cassava Fields. Disease progression within CGM populations was documented in two fields. One field (field 7 of the previous section) was planted in January 1993; the second (field 9) was planted in March 1994. A 50 x 50 m area in the approximate center of each field was selected and subdivided into a 2 x 2 m grid using nylon strings attached to wooden stakes. In each cell of the grid, one plant was randomly selected and four leaves (N, S, E and W) were examined with a 10x-hand lens for dead CGM with disease symptoms. Evaluations were carried out every third day until *Neozygites* sp. was found in all cells of the grid. In field 9, CGM density in each plot was also estimated to study the association between host density and pathogen dispersion within the field. Predominant wind direction was recorded daily, during May and June by morning observations using a windsock.

Results

Temporal Variability of *Neozygites* sp. Epizootics. Data on infection levels, estimated by averaging the percentage of CGM with capilliconidia adhered to their bodies with CGM containing hyphal bodies across apical and median leaves, are presented in Fig. 2. *Neozygites* sp. was initially detected in fields 1, 3, 4, 6 and 8 (March). During this month less than 1% of CGM had capilliconidia adhering to their bodies, and no CGM were found to contain *Neozygites* sp. hyphal bodies. During April and the first three weeks of May, there were no signs of *Neozygites* sp. in any of the fields. The onset of epizootics was observed between May 24 and June 16. There was a difference of 23 days between the onset of the epizootic in the earliest field and the onset in the latest one.

Infection levels and epizootic progression varied between fields. Infection of >50% of CGM population was observed in five fields with infection reaching 100% in fields 1 and 4. Lower levels of infection were detected in fields 5 and 8 where maximum infection was 37% and 40%, respectively. Epizootics progressed rapidly in some fields. Within nine days of onset, fields 3 and 6 achieved 58% and 43% infection, respectively. In contrast,

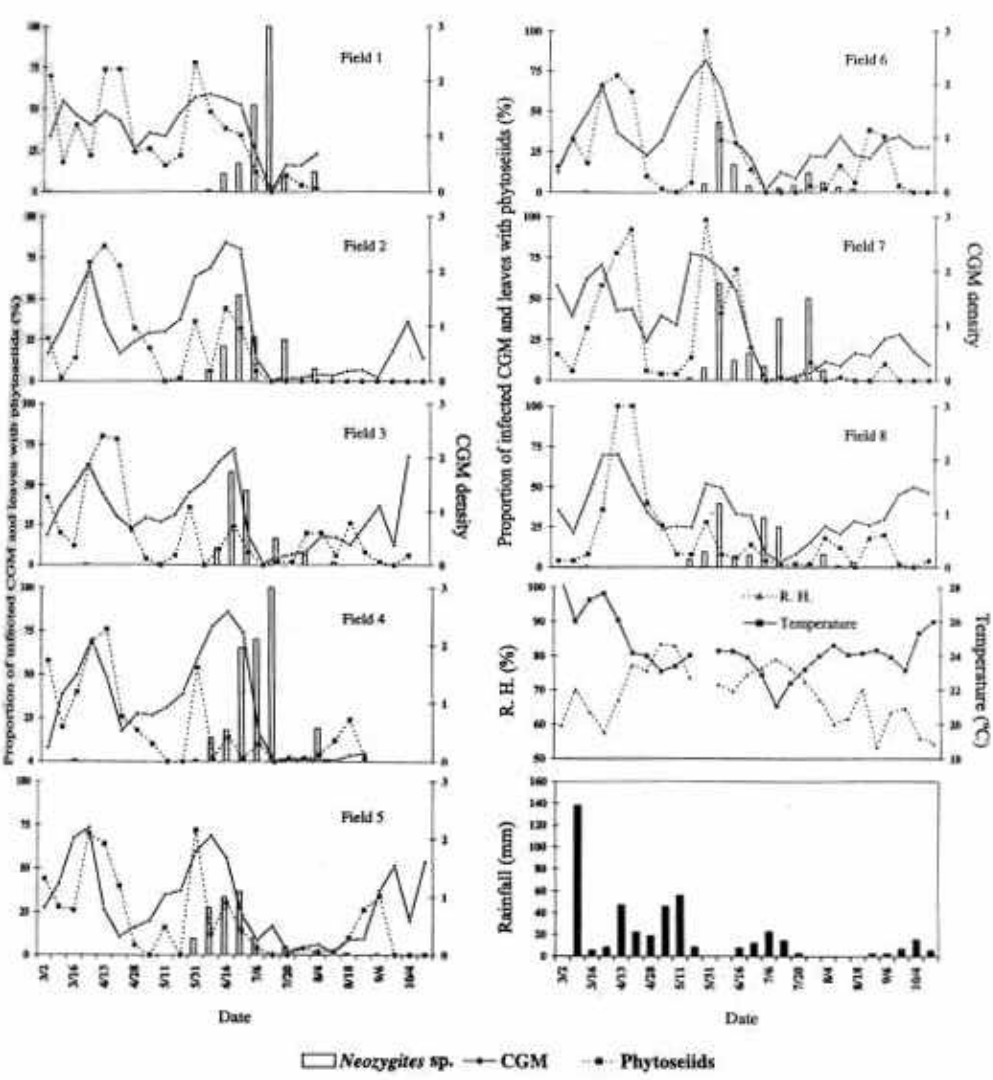

Figure 2. Population dynamics of *M. tanajoa* (CGM), phytoseiid mites and infection (%) of *M. tanajoa* by *Neozygites* sp. in eigth cassava fields in Piritiba county, state of Bahia, Brazil. 1994 (CGM density score: 0 = no mites, 1 = 1-24, 2 = 25-200 and 3 = > 200 mites per leaf).

maximum infection occurred in fields 1 and 4 at 36 and 45 days after the first observation of infected CGM, respectively. In field 4, a high level of infection (> 65.5%) was maintained for 21 days (from June 25th until July 15th). In field 5, *Neozygites* sp. did not reached

as high infection level as in other fields but infected CGM were observed for a longer period (106 days). Very low number of infected mites was sampled in the last 27 days of the epizootic.

Influence of Field Location, Climatic Factors and CGM Density on Occurrence of *Neozygites* sp. The fields where *Neozygites* sp. were first detected were fields 7 and 8. The appearance of the pathogen was last detected on field 3, the furthest field from 7 and 8. Infected CGM were detected first in northwestern fields, with an apparent progression to the southeast.

Between April 28th and May 11th, prior to the onset of epizootics, daily mean relative humidity (RH) was greater than 83% and temperatures were moderate ranging from 23.1°C to 23.4°C. During the epizootic phase (late May to late June), daily mean RH ranged from 70% to 79% and the daily mean temperatures were between 21.1°C and 24.3°C. All fields experienced epizootics, however, these epizootics began on various dates and lasted for varying lengths of time. The onset and span of epizootics appeared to be independent of the oscillations in temperature and humidity during this period (Fig. 2). After July 26th, a post-epizootic phase was observed in all fields with a drastic reduction in CGM density and infection by *Neozygites* sp. This coincided with a reduction in mean R.H. to < 70% and an increase in mean temperature to > 24°C.

In all fields, except for field 8, the pathogen first appeared when CGM density was moderate to high (CGM score ranging from 1.8 to 2.4). In field 8 the CGM population was relatively low (0.7) when *Neozygites* was initially observed.

Effects of Rainfall and Natural Enemies on CGM Dynamics. There were at least two peaks in CGM populations in each field. The first peaks occurred between March 8th and April 13th. The increase in CGM density up to these dates occurred after the onset of the period of heavy rains. Except for fields 7 and 8, significant increases in CGM density were observed between March 2nd and 8th, during which time cumulative precipitation was 138 mm. The decrease in CGM infestation in all fields after the first peak until April 28th, coincided with an increase in the number of phytoseiid mites and considerable rainfall. In all fields, >70% of leaves contained phytoseiids in at least one of the collections made during this period. Phytoseiid densities were greatest in field 8, where they were detected on 100% of the leaves collected on April 13th and 20th. Evaluations carried out in fields 1, 2 and 8 indicated that more than 95% of collected phytoseiid specimens were *Amblyseius idaeus* (Denmark & Muma).

Beginning on May 11th, a second increase in CGM density was observed in all fields, culminating with population peaks occurring between May 24th and June 25th. The reduction of CGM density after this second peak coincided with an increase in the levels of infection by *Neozygites* sp. This period was characterized by reduced rainfall and, excepts in fields 6 and 7, small numbers of phytoseiid mites were recorded. In these two fields, an increase and subsequent decrease of phytoseiids also occurred parallel to the increase and decline in CGM density.

Comparisons of Disease Prevalence Estimated Using Different Fungal Structures and Between Different Leaf Locations. Infected CGM were observed in greater proportions on apical leaves compared with median leaves when analyzed by the proportion of mites with capilliconidia or by the proportion of mites with hyphal bodies ($P < 0.001$). CGM densities in apical leaves were higher than the densities in median leaves ($P = 0.015$). CGM density was significantly and positively associated with the infection level measured by proportion of mites with attached capilliconidia in apical leaves and measured by proportion of mites with capilliconidia and hyphal bodies in median leaves ($P < 0.002$). CGM density and infection level, when measured by proportion of mites with hyphal bodies in apical leaves, are not related ($P = 0.602$). 'Field' also partially explains the variation in

the infection rate ($P < 0.013$). In all regressions the discordance between the response variable and predicted probabilities ranged from 32.6% to 35.3%.

The proportions of infected CGM estimated using the number of mites with capilliconidia were 0.08 ($P = 0.000$) and 0.02 ($P = 0.014$) higher than the proportion estimated by the presence of mites with hyphal bodies in apical and median leaves, respectively. These parameters are significantly and positively correlated ($P = 0.000$) with Pearson correlation coefficients equal to 0.655 and 0.832 for apical and median leaves, respectively.

Resting Spore Formation. Resting spores of *Neozygites* sp. were recovered from collected mites between June 9th and July 20th, the period of highest infection (Fig. 3). Very low levels of CGM containing resting spores occurred at the beginning of the epizootics.

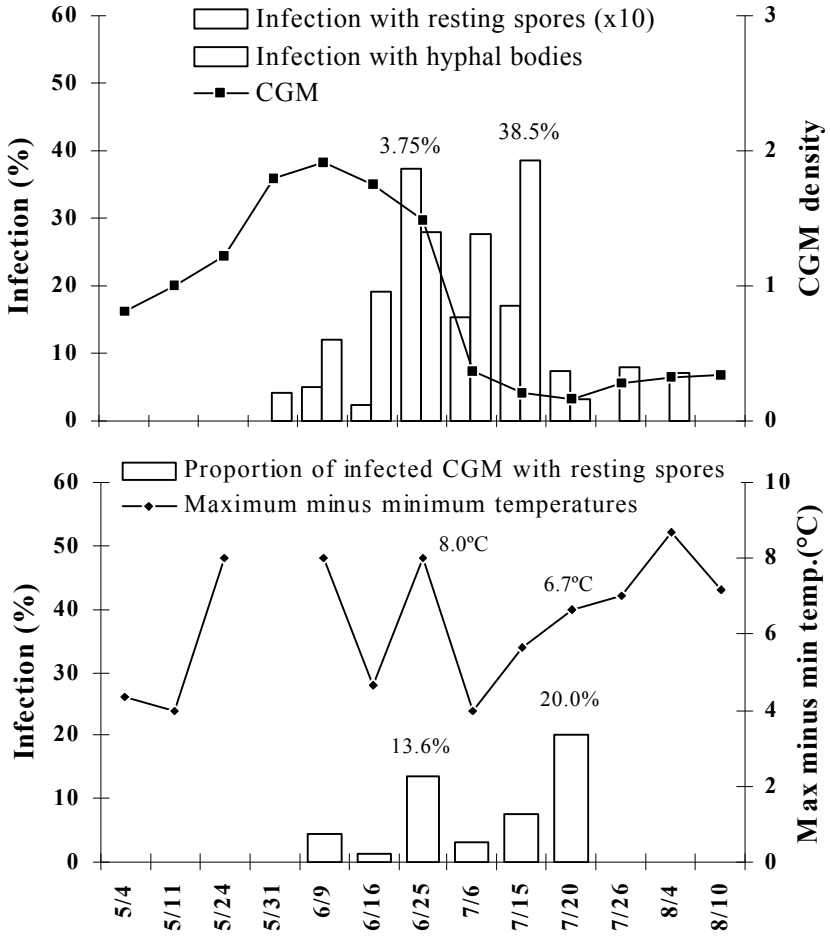

Figure 3. Percentage of infected *M. tanajoa* (CGM) with resting spores and hyphal bodies of *Neozygites* sp. in eight cassava fields and variation of temperature during 96 hours before each evaluation in Piritiba county, state of Bahia, Brazil. 1994 (CGM density score: 0 = no mites, 1 = 1-24, 2 = 25-200, and 3 = > 200 mites per leaf).

A sudden increase in CGM containing resting spores occurred as the epizootics progressed. The maximum percentage of CGM containing resting spores was observed shortly before the peak of CGM infection. However, the greatest relative proportion of infected CGM containing resting spores was observed shortly after this date (June 2nd), when CGM density in the fields was very low.

The greatest percentages of CGM containing resting spores were observed during periods preceded by large temperature variations. On June 25th, 3.75% of total sampled CGM and 13.6% of infected CGM (from 800 mites sampled from 8 fields), contained resting spores. A difference of 8°C between the average of maximum and minimum temperatures in 96 hours before the respective sampling was recorded.

Progression of *Neozygites* sp. Within Cassava Fields. Infected CGM were observed for the first time in field 7 on May 21st. On this occasion, infected CGM were found in 56% of the total area studied (Fig. 4). Four days later, 96% of the area had infected CGM, fin-

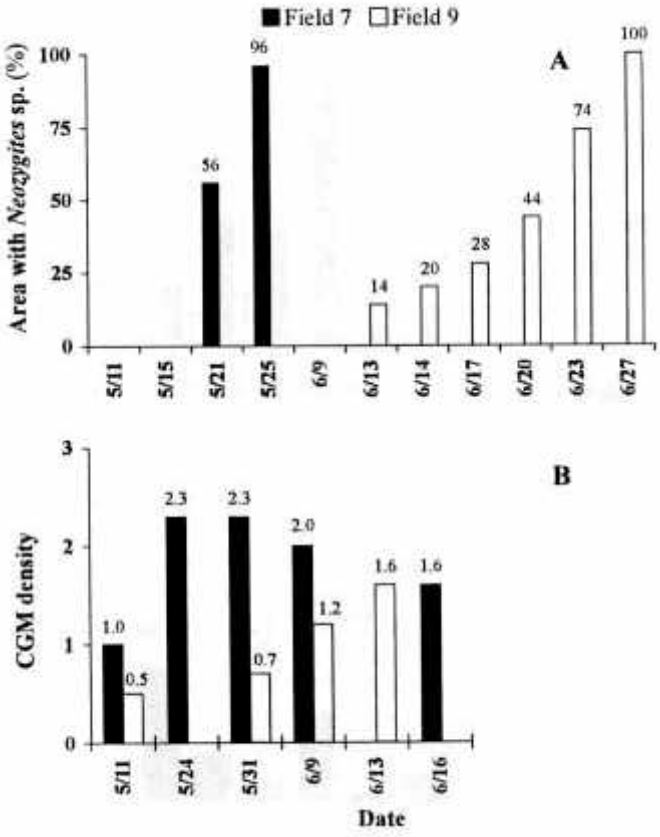

Figure 4. Dissemination of *Neozygites* sp. within two cassava fields in Piritiba county, state of Bahia, Brazil. 1994. A) proportion of area with the pathogen on each evaluation. B) population density of *M. tanajoa* (CGM) in fields 7 and 9 (density score: 0 = no mites, 1 = 1-24, 2 = 25-200, and 3 = > 200 mites per leaf).

ishing disease progression evaluations in this field. In field 9, the first infected CGM were observed 23 days later than in field 7. At that time, infected CGM were observed in 14% of the total area. The progression of the pathogen was slower in this field, increasing from 14% to 100% of the total area in 14 days. The first observations of *Neozygites* sp. occurred after increases in CGM population densities in both fields. Meanwhile, CGM reached elevated population levels more rapidly in field 9 than in field 7. The average scores for CGM density when *Neozygites* sp. was initially detected were 2.3 in field 7 and 1.6 in field 9.

The first mites infected by *Neozygites* sp. were observed scattered throughout each of the fields. The predominating wind direction during the period of *Neozygites* sp. emergence was from the northeast. However, the progression of the pathogen did not seem to be affected by predominant wind direction or position of field borders.

The pathogen was always detected in larger proportions in plots with the highest levels of CGM (Fig. 5). Despite this, *Neozygites* sp. was found in all plots regardless of CGM population density, on the same day (June 27th).

Discussion

Based on the dynamics of *Neozygites* sp. in nine cassava fields we identified a few mechanisms driving the spatial and temporal spread of this pathogen between and within CGM-infested fields. Although we described many aspects of epizootics of *Neozygites* sp., our main focus was to understand the variability of the onset of the disease among fields.

The rise in humidity, reduction in temperature and the considerable rainfall after April 13th did not immediately result in the appearance of epizootics. The high levels of infection only occurring after June and the variability on the initial prevalence of *Neozygites* sp. among fields are probably related to the variation in CGM density, available initial inoculum and climatic factors. Variation in CGM levels among different fields until June could be the main reason for the delay in appearance of *Neozygites*. Experiments with mathematical models and field observations

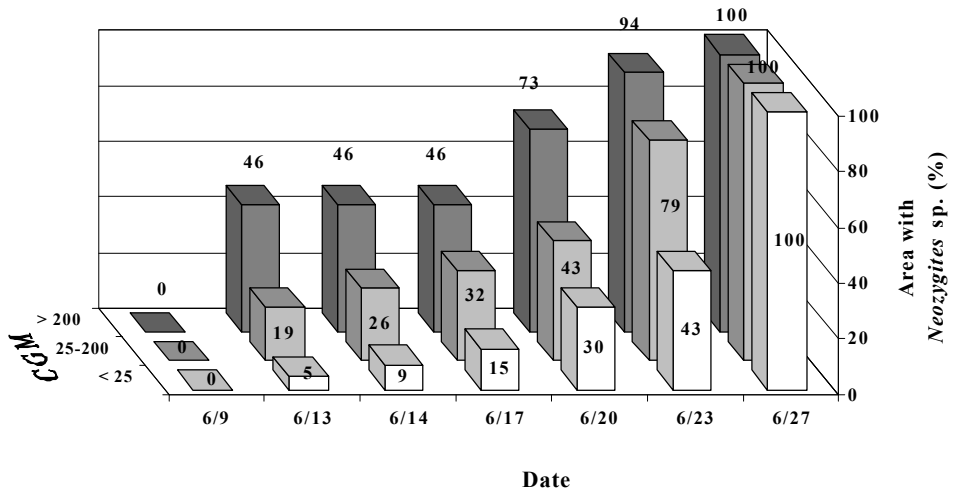

Figure 5. Cumulated frequency of *Neozygites* sp. recorded on areas with different *M. tanajoa* (CGM) densities in field 9, Piritiba county, state of Bahia, Brazil. 1994.

have shown consistent results in reporting the existence of a minimal host population for *Nosema pyraustra* Palliot, *Entomophaga maimaiga* Humber, Shimazu & Soper, *Neozygites parvispora* (MacLeod & Carl) Remaudière & Keller and other entomophthoralean species, below which epizootics do not occur or the pathogen does not persist (Remaudière *et al*. 1981, Onstad *et al.* 1990, Onstad & Carruthers 1990, Hajek *et al.* 1993, Vacante *et al.* 1994). Another possible reason for the delay in appearance of *Neozygites* sp. pertains to the reduction of available inoculum in the environment, which is responsible for the initial foci of disease. There could have been a reduction in available inoculum due to the intense drought in the region during 1992-1993. Primary infections, originating from the germination of resting spores, are important for production of an adequate level of secondary inoculum for development of epizootics of *E. maimaiga* in the gypsy moth (*Lymantria dispar* (L.)) (Hajek *et al.* 1993).

In general, epizootics of *Neozygites* sp. developed progressively among the evaluated fields during periods of mild temperatures and highest relative humidities. Despite a small temperature increase and a reduction in humidity during this period, epizootics persisted. This drop in R.H. could be one of the reasons why infection peaks in fields 6, 7 and 8, which occurred during the periods of lower mean R.H. (71%) during the epizootic, were considerably lower than corresponding infection peaks in fields 1 and 4. Many reports on epizootics of Entomophthorales in cropping systems consider R.H. a critical factor in development and maintenance of epizootics (Vacante *et al.* 1994).

The effects of rainfall on population dynamics of tetranychids has been discussed by some authors. Using mathematical simulation models, Gutierrez *et al*. (1988) and Yaninek *et al*. (1989a) reported that heavy rains induces mortality and reduces CGM populations in Africa. However, as observed in this study and another study conducted in Brazil by Delalibera *et al*. (1999), during 1989-1990, the decrease in CGM populations cannot be attributed solely to the direct effect of rainfalls. During 1994, despite the relatively high rates of rainfall at the beginning of May, CGM populations increased over this time period. Independent of the low levels of rainfall during the remaining period of observation, CGM populations were significantly reduced in fields 6, 7 and 8 at the end of May, and in fields 1 and 5 after the second week of June, coinciding with higher occurrence of *Neozygites* sp.

The greater prevalence of *Neozygites* sp. on apical leaves is most likely due to greater CGM density on these leaves. In three out of four regression analysis, CGM population density when used as the independent variable, explains the observed variation in infection rate. The positive relationship between CGM density and infection rate indicates that higher levels of infections should be observed with higher CGM densities. Although other factors not measured in this study might influence the infection rate, greater densities of mites on apical leaves could facilitate disease spread.

The determination of infection level by detection of hyphal bodies within mites is time consuming and infection levels are probably underestimated due to the difficulty in visualizing the initial phase of the infection inside the host. We consider that the percentage of mites with capilliconidia adhering to their bodies can be useful for determining the infection level in field studies of *Neozygites*, when frequent measurements are required. This method is faster than dissecting for hyphal bodies and can be carried out using any compound microscope. Due to the association between these two measurements, we suggest that solely counting capilliconidia gives an adequate representation of the percent infection. The presence of *Neozygites* sp. capilliconidia attached to the body of CGM indicates a potential for infection, considering that only one conidium is sufficient to cause disease and death in *M. tanajoa* (Oduor 1997).

Resting spores of *Neozygites* sp. have

rarely been observed (Delalibera, unpublished) in samplings of CGM in northeastern Brazil. In tropical regions, mainly in the semi-arid regions of northeastern Brazil, pathogens need an efficient mechanism of resistance to survive prolonged dry seasons that coincide with high temperatures. It is thought that resting spores are the main means of the pathogen survival during these periods. The factors that induce the formation of this type of spore are still unknown. We observed in this study that resting spores were detected during the winter mostly after periods preceded by large temperature variations. Based on laboratory studies of the *Neozygites* sp. life cycle (Oduor 1995), we think that the effect of climatic factors on the pathogen development, which may induce the formation of resting spores, should be more relevant during the first 96 hours of infection. In the next stage of this study we will investigate which factors are associated with *Neozygites* sp. resting spore formation. Hyphal bodies of *Entomophaga grylli* pathotype 2 (Fresenius) Batko and *E. maimaiga* only begin maturing to the final double walled resting stage after the death of their hosts, *Melanoplus differentialis* (Thomas) and *L. dispar*, respectively (Tillotson & Margolies, 1990, Hajek & Humber 1997). Live females of CGM were preferentially sampled and no mature resting spores were found. If resting spores of *Neozygites* sp. are formed predominantly after mite death the proportion of resting spores in the field was probably underestimated.

There were major differences between fields 7 and 9 which could be attributed to the delay in initial occurrence and to the slower spread of *Neozygites* sp. within field 9. The cassava plants in field 9 were young and small. The plants in field 7 were larger and had more leaves, greater numbers of CGM per leaf, and, consequently, a much greater density of CGM per area. The greater the CGM density, the higher probability of healthy mites contacting the initial foci of infection. Within field 9, we also observed that the progression of the pathogen was faster in plants with higher CGM density.

Brazilian isolates of *Neozygites* sp. are currently being released in Africa where CGM is an introduced pest. The importance of these isolates on the regulation of CGM populations in Brazil was reported recently by Delalibera *et al*. 1999 and Elliot *et al*. (in press). The impact of *Neozygites* sp. was assessed both in various agroecological regions and in the same region throughout a multi-season study. These studies demonstrated that *Neozygites* sp. is a major cause of mortality in CGM populations in the place of this pest's origin and has desirable characteristics for classical biological control of CGM in Africa. As discussed here, the possibility of using this pathogen in some regions where it is native is also considered.

The temporal variability in the initial prevalence of *Neozygites* sp. indicates the technical viability of pathogen inoculation from one field to another in the same region, with the objective of early induction of epizootics. In order to increase the efficiency of fungal releases in the field future studies should focus on the confirmation of the existence of a geographical progression of the disease and on the determination of the minimal host density below which epizootics do not occur.

Acknowledgments

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