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Association of fungi with dead *Nzinga palmivora* Wilson (Homoptera: Cicadellidae) in coconut plots in Ghana

R. N. Quaicoe[1] and G. K. Yawson[1]

Abstract

Nzinga palmivora is one of the putative vectors of the Cape St. Paul Wilt Disease of coconut in Ghana. During a study to determine the seasonal population dynamics of the insect on coconut palms in the field, many of the insects were found dead and covered with fungal mycelia at one location. To investigate the role of the colonizing fungi in the death of the insects, two treatments of crude inocula, prepared from dead insects ground in sterile distilled water, were used to treat live insects in fyltis sleeve cages built around leaflets of coconut fronds. Insects treated with sterile distilled water (sdH_2O) alone, served as the control. The insects treated with the crude inocula showed higher mortality compared with the control ($p<0.001$). The crude inocula also had adverse effect on oviposition, hatching of eggs into nymphs and the emergence of adults from nymphs. Fungal isolations from dead insects revealed apparently a total of 24 colonizing species of fungi. The most frequently associated fungus with dead insects after surface sterilization was a species of *Penicillium* (tagged as *P.* sp. 1). This was followed by *Pestalotiopsis* sp. and *Cladosporium herbarum.* The surface unsterilized dead insects yielded certain of these fungi more frequently than sterilized ones, besides several other species of fungi. Among the fungal isolates, the only known entomopathogen was *Verticillium* sp., however, it was isolated from only 2% of the dead insects studied and this may not account for the insect mortality encountered in the field, due to its low frequency of occurrence. The implications of these findings and future direction of the study are discussed.

Key words: *Nzinga palmivora*, population dynamics, fungal species, entomopathogens, fyltis sleeve cage, fungal isolation.

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[1]CSIR-Oil Palm Research Institute, P. O. Box 74, Kade, Ghana.

Introduction

Lethal yellowing disease of coconut palm, locally called Cape St. Paul Wilt Disease (CSPWD), is the single most serious threat to the coconut industry in Ghana, of which about 4.2% of the population depend for livelihood. In the Western Region alone about 20% of the rural people depend on coconut for their sustenance (Adams *et al*., 1996; Dery and Arthur, 1996). CSPWD was first noticed in Ghana near Cape St. Paul, in the Volta Region, in 1932 (Westwood, 1953). After a spell of erratic spreading within that region, the disease 'jumped' to Cape Three Points in the Western Region, a distance of 450 km in 1964, leaving the intervening stretch of land free of the disease. Later the disease appeared at Ayensudu in the Central Region in 1983. From these points, the disease is fast advancing on all fronts (Ofori and Nkansah-Poku, 1995). The disease, which is phytoplasma-mediated, also occurs at many other places where it is designated by local names, as for example: Kaincope disease in Togo, Awka or Bronze leaf wilt in Nigeria, Kribi disease in Cameroon, Lethal Disease in Tanzania and Kenya and Lethal Yellowing in the Caribbean and Florida (Eden-Green, 1995).

Phytoplasma-mediated diseases are transmitted by insect vectors notably Homopterans (Nielson, 1979). In Florida and Caribbean the plant hopper *Myndus crudus* van Duzee (Homoptera: Cixiidae) has been confirmed as the vector of lethal yellowing (Howard *et al*., 1983; Harrison and Oropeza, 1995). In Tanzania, two Auchenorrynchous Homoptera, *Diastrombus mkurangai* (Homoptera: Derbidae) and *Meenoplus* spp. (Homoptera: Meenoplidae) have been implicated as putative vectors of the Lethal Disease (Mpunami, 1997). In Ghana, *Nzinga palmivora* (Homoptera: Cicadellidae) besides *Myndus adiopodoumeensis* Synave (Homoptera: Cixiidae) and many other insect species belonging to the Derbidae family, are listed as putative vectors of CSPWD (Dery and Arthur 1996; Dery *et al.*, 1995).

The most feasible and generally accepted control strategy for lethal yellowing-type diseases is the use of resistant coconut varieties (Been, 1995). Attempts to control the disease in Ghana have therefore mainly concentrated on the identification of resistant lines among coconut germplasms. Currently, 39 coconut types are under field screening for tolerance to the CSPWD. From these field screenings two ecotypes - Sri Lanka Green Dwarf (SGD) and Vanuatu Tall (VTT) and the hybrid of Malayan Yellow Dwarf and Vanuatu Tall (MYD x VTT), have been identified as showing some resistance to the disease (Dery *et al.*, 1995). The two ecotypes that have shown high level of resistance, have been crossed to produce a novel hybrid (SGD x VTT), which is under further study for use to rehabilitate the coconut industry in the country (Dery and Arthur, 1996; Dery *et al.*, 1995). Being a vector transmitted disease, it is also desirable that the population of vector(s) be controlled to reduce the disease pressure. Results of a pilot study indicated that "insecticidal hot-fogging" in new disease outbreak sites, followed by cutting out of infected trees, can help to slow down the spread of disease (Nkansah-Poku and Dery, 1998). An integrated disease management strategy comprising resistant planting material and effective vector management would therefore hold a high promise in the management of the CSPWD menace. Due to the high cost and environmental hazards of chemical insecticides, the use of biological agents like entomopathogens, is gaining importance in modern agricultural pest management measures (NAS, 1981) – as for example, Latch and Falloon (1976) recommended the use of the entomopathogenic fungus, *Metarrhizium anisopliae* (Metch.) Sorokin for the control of the rhinoceros beetle (*Oryctes rhinoceros* L.). It is reported that more than 750 fungal species representing approximately 100 genera, infect insects. It is also reported that nearly all major fungal groups and virtually every type of insect is represented in this type of parasitism (NAS, 1981). Death of *N. palmivora*, suspected to be due to entomogenous fungus, was observed in the Akwidae coconut plot. Therefore it was felt to understand the identity of the colonzing fungi observed on the dead *Nzinga palmivora* observed in the field, with the view to

identifying for possible entomopathogens for use in management of vectors of in CSPWD through biocontrol.

Materials and methods

Seasonal population dynamics of *Nzinga palmivora*

The seasonal population variation of live and dead adult of *N. palmivora* was studied throughout 1997 in the coconut varietal resistance trial plots of the Oil Palm Research Institute - Coconut Research Programme located at Akwidae, Agona Junction, Princess Town and Dixcove. In each plot, 10 coconut palms were randomly selected and tagged. From each palm, 5 fresh fronds were sampled. Monthly counts of the live and dead adult *N. palmivora* on the leaflets of the fronds were recorded.

Study of fungi associated with dead *Nzinga palmivora*

Preparation of crude inoculum from dead insects: Fifty dead adult of *N. palmivora* with fungal growth were ground into fine paste using sterile mortar and pestle, and then followed by thoroughly mixing with 50 ml of sterile distilled water (sdH_2O) to obtain a crude inoculum (designated as inoculum A). Inoculum B was similarly prepared but suspended in 100 ml of sdH_2O. Each inoculum was filtered though a muslin cloth to remove large particles, and then poured into hand-held spraying gun.

Construction of insect cage and artificial infection of insects: Twelve sleeve cages each measuring 30 cm x 50 cm, made of fyltis material (fine nylon net of 600µ mesh size), were used to house the test insects. Each cage was built around 3 healthy leaflets, which provided support and nourishment for the insects (Fig. 1).

Fig. 1. Picture of fyltis sleeve cage used to hold test Insects

Using sterile test tubes, 600 adult insects were collected in batches of fifty insects per tube, in the early morning at Akwidae, to avoid shock and stress to the insects. The insects from each tube were carefully transferred into a cage and the opening of the cage secured by clipping together with a paper stapler. Using a hand-held spraying gun, inoculum A was sprayed onto four of the cages, and similarly inoculum B was applied onto another four cages. The four remaining cages were only sprayed with sdH_2O, serving as controls. Care was taken to drain off any excess liquid in the sleeves to prevent drowning of the insects. As two of the control cages were lost due to bush fire, only 2 cages were left under control and utilized for the study. The number of surviving adult insects, number of dead insects, appearance of external fungal growth on dead insects, rate of oviposition, rate of hatching of eggs into nymphs and rate of adult emergence from nymphs, were monitored on daily basis for 60 days.

Isolation and identification of fungi associated with dead *Nzinga palmivora*

One hundred dead adult insects were collected from the Akwidae plot, and divided into two batches. One batch was surface-sterilized with 0.1% sodium hypochlorite solution, by immersion in the solution for three

minutes and followed by rinsing by two changes of sdH_2O, and then drying on dry sterile filter paper. The other batch of insects was used directly in the original state without surface sterilization. The insects were then placed onto petri plates (9 cm – diameter) containing potato-dextrose-agar (PDA) medium and incubated at 26°C till appearance of visible fungal growth (3 – 5 days). Using sterile needles, the fungal growths emerging from the insects were subcultured onto fresh PDA plates. Pure cultures of individual fungi as grouped were obtained after two subsequent subculturings onto PDA plates. Emerging fungal colonies grouped were identified based on their cultural and morphological characteristics, according to standard taxonomic keys (Bessey, 1964 and Barnett, 1965). Frequency of occurrence of fungi was calculated from the formula: (number of dead insects from which fungus was isolated / total number of dead insects examined i.e. 50) x 100%. The frequency patterns of the fungi were then tabulated.

Results

Population dynamics of *Nzinga palmivora* and its mortality

The seasonal population trends of adult *N. palmivora* at the resistance trial plots studied are illustrated in Fig. 2. Generally a high insect population and high mortality rates occurred in the dry seasons (January to April and September to November) while the lowest population was recorded during rainy season. With the exception of the Akwidae plot, the populations of both live and dead insects peaked around the same time in all the plots. At the Akwidae plot however, population of live insects was highest in January, but the mortality was highest in April. Between mid-March to mid-July there was an average of 5.99 live insects per frond, but the number of dead insects rose to 8.70 within this period.

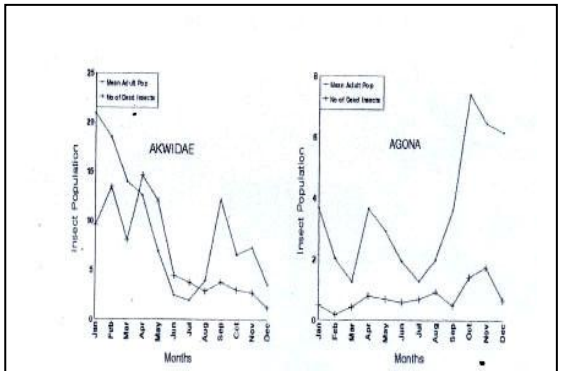

Fig. 2. Seasonal population trends of adult *Nzinga palmivora*

Effect of crude fungal inoculum - Artificial induction of infection in live insects

The survival rates of insects treated with crude inoculum from dead insects is shown in Fig. 3. Fifty percent reduction in adult insect population occurred in 20 days after inoculation under treatment A and similarly in 30 days under treatment B. Thirty five days after inoculation, when the survival count was terminated, the survival rate had dropped to 25% and 35% for treatments A and B, respectively as compared to 60% in the control.

The cumulative frequency distribution of test insects with external fungal mycelia is shown in Fig. 4. Fungal growth appeared on dead insects 5 days after inoculation under both treatment A and B, but in 10 days after inoculation in the control. The cumulative mean number of insects with fungal growth at the end of the 60 days of monitoring was 10.75, 11.25, and 8.00 for treatment A, treatment B and control respectively.

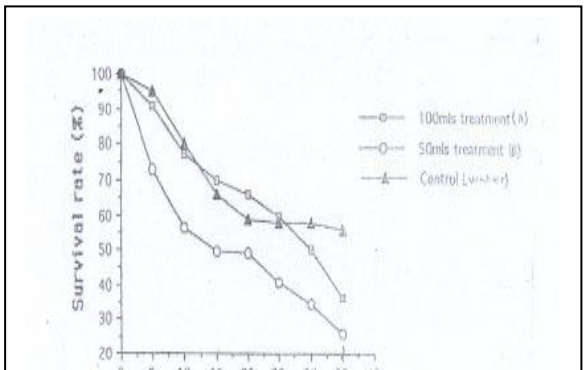

Fig. 3. Survival of adult *Nzinga palmivora* after inoculation

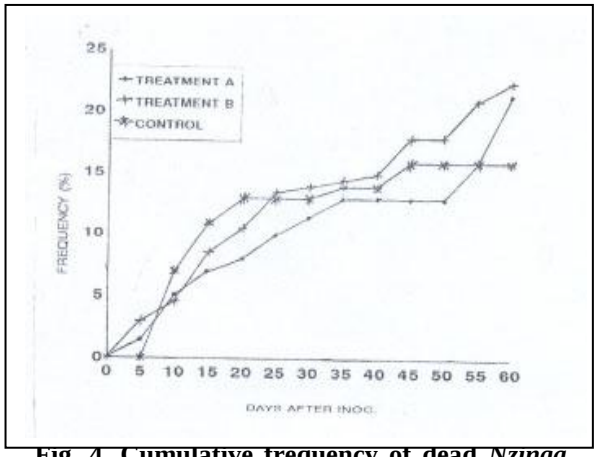

Fig. 4. Cumulative frequency of dead *Nzinga palmivora* showing external fungal growth

recorded 30 days after the treatment (Fig. 5a). At this peak period the egg production in the control was almost double that of each of treatments A and B. Over the 60 days period of observation, the mean total of egg batches produced were 20.25 for treatment A, 22.00 for treatment B and 37.50 for the control. In all the treatments, the highest production of nymphs was recorded 40 days after the treatment (Fig. 5b). At this peak period nymph production in the control was double that of either treatment A or B. The mean nymphal production over the 60 days of observation was 420.5 for treatment A, 323.5 for treatment B and 946.0 for the control. The mean of adults emerging from the nymphal stage was 75.5 for treatment A, 97.25 for treatment B and 115.5 for the control (Fig. 5c).

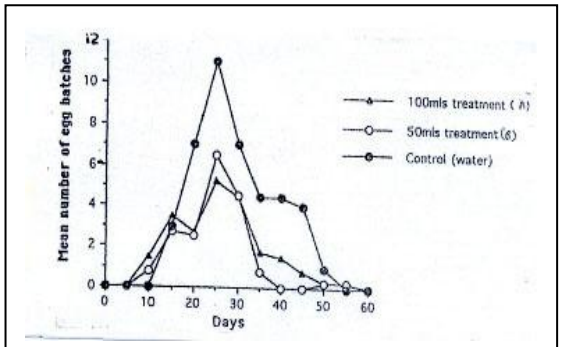

Fig. 5a. Graphical illustration of oviposition by *Nzinga palmivora* after inoculation

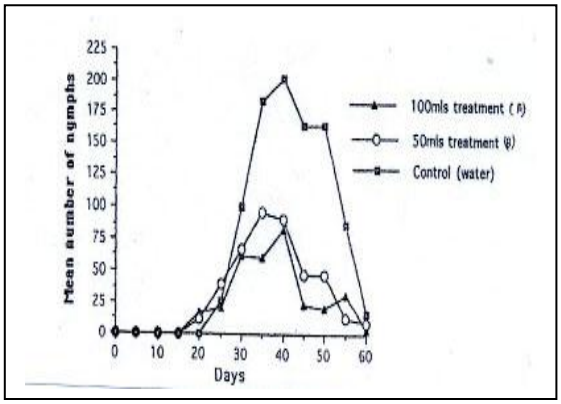

Fig. 5b. Nymphal production of *Nzinga palmivora* after inoculation

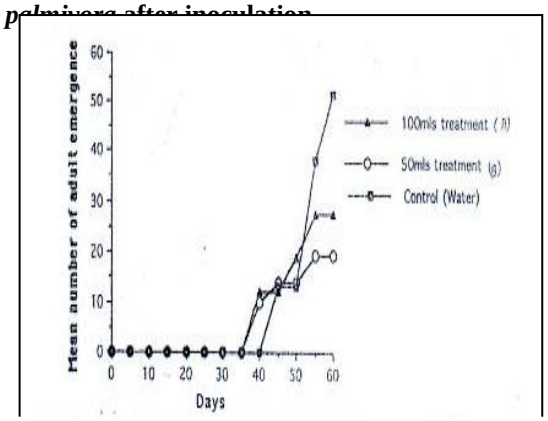

Fig. 5c. Emergence of adult *Nzinga palmivora* over a 60 day period after inoculation

The record of the fungi isolated from dead *N. palmivora* is presented in Table 1. A total of 24 fungi including unindentified species were isolated from the insects. The most frequently associated fungus with dead insects after surface sterilization was *Penicillium* sp. 1, followed by *Pestalotiopsis* sp. and *Cladosporium herbarum*. The surface unsterilized dead insects yielded certain of these fungi more frequently than sterilized ones, besides recording several other species of fungi. With the exception of

Cylindrocephalum sp., *Spicaria* sp. and *Penicillium* sp. 2, all the other 16 fungal species that were isolated from the surface-sterilised insects were isolated from the un-sterilized insects also. The fungi viz., *Aspergillus niger, Aspergiullus terreus, Penicillium* sp. 3, *Fusarium flocciferum, Fusarium* sp. 3, *Rhizopus* sp., *Neurospora* sp., *Hormiscium* sp., *Phoma* sp., and *Trichoderm viride,* which occurred on the un-sterilised insects were not recorded from the surface-sterilized insects.

Discussion

The seasonal fluctuation in population of live and dead insects followed a similar trend from January to December. The population patterns were similar at Agona Junction, Princess Town and Dixcove. The peak population periods in all cases were January – April and September – November, with May – August having the lowest populations (Fig. 2). In these 3 plots, the number of live adult insects always exceeded the number of dead insects. The insect population situation at Akwidae was slightly different. Whereas the periods of population fluctuations were similar to those in the other 3 plots, the actual numbers varied. Between mid-March to mid-July, there were more dead insects than live ones (Fig. 2). It is possible that the high mortality rate observed at Akwidae, which is a departure from the general trend in the 3 other locations, would be due to some external factor(s), such as interactions of entomopathogens, predation or adverse climatic factors. It is interesting to note that Agona Junction and Dixcove are active disease foci (with disease level being between 10% and 50%). Princess Town is a new disease focus with disease level less than 1%, whereas Akwidae is a disease re-surgence area after about a 9-year period of dormancy (Annonymous, 1996). The current disease level at Akwidae is about 4% (Annonymous, 1996). There seems to be some semblance of population control of *Nzinga palmivora* in the Akwidae plot, as indicated in this study and it is wondered if this phenomenon has any role in the relatively slow progress of the disease in this plot.

Most of the dead insects encounted in the field showed visible external fungal growth. Artificial infection of healthy adult insects with crude inoculum from mouldy insects was done to ascertain if death of insects was due to any of the fungi associated with the dead insects. Comparison of the survival graphs of Fig. 3 seems to suggest an adverse effect of the crude inoculum on the adult insects. Insects in treatment A showed the least survival level. This is understandable since the highest level of any harmful agent in the inoculum would occur in this treatment. Monitoring of surviving insects had to be terminated 35 days after treatment, because of the emergence of new adults, which could not easily be differentiated from the original test insects.

Table 1. List of fungi isolated from dead adult of *Nzinga palmivora* and their frequency

Sp. No.	Fungus	Frequency of fungi recorded – with and without surface sterilization of *Nzinga palmivora* (%)	
		Surface sterilized	Surface un sterilized

01	*Aspergillus niger*	0	8
02	*Aspergillus terreus*	0	6
03	*Penicillium* sp. 1	20	8
04	*Penicillium* sp. 2	2	0
05	*Penicillium* sp. 3	0	8
06	*Spicaria* sp.	2	0
07	*Fusarium flocciferm*	0	2
08	*Fusarium oxysporum*	2	6
09	*Fusarium* sp. 3	0	6
10	*Cunninghamella* sp.	4	8
11	*Syncephalatstrum* sp.	3	6
12	*Rhizopus* sp.	0	4
13	*Pestalottiopsis* sp.	16	32
14	*Neurospora* sp.	0	4
15	*Helminthosporium* sp.	2	2
16	*Curvularia* sp.	6	6
17	*Verticillium* sp.	2	2
18	*Hormiscium* sp.	0	4
19	*Cladosporium herbarum*	14	20
20	*Botryodiplodia* sp.	2	4
21	*Phoma* sp.	0	2
22	*Trichoderma viride*	0	6
23	*Cylindrocephalum* sp.	2	0
24	Unidentified (*Mycelia sterillia?*)	8	2

Visible fungal growths appeared on dead insects in both treatments A and B just 5 days after treatment, whereas dead insects in the control showed no external fungal growth until the tenth day after treatment (Fig. 4). The mean of infected insects of the treatments (10.75 for treatment A and 11.25 for treatment B); was significantly higher compared to 8.0 for the control ($P<0.001$). Could there be entomopathogen(s) in the inoculum used in treating the test insects? This is a possibility and needs consideration. This unidentified fungal agent in the inoculum also appeared to adversely affect the developmental stages of the insects. It is evident from Fig. 5 that – egg and nymphal production, as well as emergence of adults from the nymphal stage were normal in the control but not in treatments A and B.

More fungal species were isolated from the un-sterilised insects as is to be expected, since surface-sterilization would have eliminated most of the superficial fungi found on the un-sterilized dead insects. Of all the fungi isolated, only *Verticillium* sp. is a known entomopathogen (NAS, 1981). However, its low frequency of occurrence (2% in the insects studied) does not seem to implicate it as the cause of the insect mortality observed in this study. The most frequently colonizing fungus that seems to have successfully colonized internal insect tissues appeared to be *Penicillium* sp. 1, with a frequency of 20% of surface-sterilised insects and 8% of un-sterilised insects.

In a previous study, *Penicillium* sp. 1 was isolated from 44% of the insects screened for entomopathogens (Annonymous, 1997). The close morphological resemblance between the genera *Penicillium*, *Paecilomyces*, and *Metarrhizium*, and the fact that entomopathogenic *Paecilomyces* and *Metarrhizium* species have been recorded

(NAS, 1981; Crawford, 1985), makes *Peniciullium* sp. 1 a possible candidate for further study. It is on record that in the Philippines, the population of the rhinoceros beetle, a destructive pest of coconut palm, is effectively managed through an integrated beetle control strategy that includes biocontrol with a *Metarrhizium anisopliae* (Crawford, 1985). It is believed that the potential of fungi in control of insect pests is under exploited, and that apart from the few known entomopathogens that are currently being used for biological control, other promising candidates could be explored (NAS, 1981). In this context *Penicillium* and other promising fungi hold promise for precise identifications, more screening of entomopathogenic capabilities and multiplication for field utilization. The biological control of insect pests involoved in transmission of phytoplasmal diseases such as CSPWD draws special importance.

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