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Components of coconut fruit susceptibility to *Phytophthora katsurae* (Pythiaceae) in Côte d'Ivoire

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ABSTRACT

The present study was to evaluate the susceptibility of coconut fruit to *Phytophthora katsurae* (Ko and Chang) according to their age and the position of lesions. The infection rates of nuts under natural conditions and by inoculation techniques on wounds and deposition of inoculum on the pericarp were compared. Under natural conditions, immature adult nuts between 8 and 11 months old are the most susceptible stage to infection by *P. katsurae*. The rounder part is the most receptive site. The proximal part is most liable to attack in young 4-8 months old fruits, which are less numerous in the susceptible host stage. Ripe nuts of at least 12 months old are totally immune to infection. The distal part of all categories of nuts is not invaded by the lesions. There is a highly significant difference between the susceptibility of the proximal zone of young and adult wounded nuts at 1% significance level. A non responsiveness of the distal zone of the young nuts was observed on nuts not wounded. Based on artificial inoculations on fruit of different ages, the resistance mechanism in the fruit seems to be much more physically linked to nut anatomy than chemically.

Keywords: Immature nutfall, field infection, rough and gentle inoculation, success rate, physical penetration.

INTRODUCTION

Coconut palm (*Cocos nucifera*) is an economically important crop grown in Côte d'Ivoire (Zakra, 1989; Centre National de Recherche Agronomique (CNRA, 2003). It is attacked by heart rot and immature nutfall (Thevenin, 1990; Pohé, 1992 (a and b), 1996; Mariau, 1999; Yao et al., 2010).

As early as 1984, immature nutfall was observed in Côte d'Ivoire on fist-sized to virtually ripe nuts (Quillec and Renard, 1984). *Phytophthora* attacks on nuts are characterized by mottling in various patterns, light brown in the centre, yellowish towards the edges, with a more or less translucid border. The infection generally begins around the floral parts, sometimes in the equatorial section and extends towards the apex of the nut and in towards the shell. The infected husk becomes oily-looking, and then turns brown and black. Nutfall occurs when the nut stalk is affected. *Phytophthora katsurae* found on coconut is located inside the leaf axil from where it attacks the surrounding fruit before

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being disseminated into the whole tree. From the leaf axil, it could move to the bud through wounds causing damp bud rot. Other symptoms included the wilting of the spear and the death of bud rotten trees. The investigation conducted in 1983 by ex-palm industry company revealed a high level of nutfall (50-70 %) during the rainy season on the “West African Tall” coconut variety grown in Côte d’Ivoire. Control measures include chemical (Allou and De Franqueville, 1989; Pohé et al., 2003; Allou et al., 2010) and genetic (Allou et al., 2002) means.

Previous observations had shown that only large green nuts from 8 to 10 months old were the most receptive to *Phytophthora*, whereas younger nuts generally remained disease-free (Quillec et al., 1984). However, these observations did not involve precise data to determine the importance of different lesion positions and age of the most vulnerable nuts.

The knowledge of nut anatomy and all the fruit susceptibility characters is important for a better understanding of the disease epidemics.

The coconut is a drupe, comprising several parts from the outside to the inside (Ministère de la Coopération, 1980; Ministère des Affaires étrangères, 2002):

- **Pericarp:** it is the outer part whose appearance varies depending on nut age;
- **Mesocarp** (fibrous): the fibre is called coir. Right after pollination, the fibres begin to form the distal part, which is harder than the rest of the nut. The proximal part which is the last section to differentiate remains a zone of lower resistance for a long time. It is partly covered by the floral organs;
- **Endocarp:** it differentiates very soon after pollination and appears first in the distal part. It is totally formed by the time the nut is 10 months old;
- **Meat or albumen:** the meat begins to form by deposits in the distal part of the endocarp cavity. This begins when the nuts are 7 or 8 months old and is completed on ripeness (12 months).

The inner cavity of the endocarp is filled up by coconut milk (liquid endosperm) whose volume diminishes as the meat forms.

Under natural conditions, some differences in the behaviour of plant materials have been observed, thus suggesting the possibility of improving plants as a way to control *P. katsurae* (Allou et al., 2002). In order to determine the resistance or susceptibility of those plant materials, tests of inoculation have been carried out on nuts (De Franqueville and Allou, 1992). Because of the unsatisfactory results of such tests (De Franqueville and Allou, 1992), the small number of nuts involved and the lack of information on lesion position and the age of vulnerable nuts, this study has been carried out. It is aimed at determining some epidemiological factors including lesion position and the age of nuts under natural and artificial conditions in the development of rot symptoms.

MATERIALS AND METHODS

The study is partly based on observations from natural infections and on inoculation tests in the field.

Phytosanitary survey

During weekly rounds, in the infected 3 000 ha of Assinie fields of ex-palm industry, in Southeast Côte d’Ivoire; diseased nuts were collected and observed to locate the position of the lesions. It was often necessary to dehusk or break open the nuts to complete these observations. The nuts were classified into age categories: the young, adult and ripe nuts were respectively 4, 8 and 12 months old, according to the differentiation of the shell and level of dehydration of the husk. The shell is membranous, partly differentiated, with a dry husk respectively in very young (1-4 months old), young (5-7 months old), immature adult (8-11 months) and ripe (≥ 12 months) nuts.

Verification test

Nut inoculations were carried out *in situ*.

Inoculation

Nature of parasite inoculum: The inoculum was obtained from a *P. katsurae* culture isolated from a diseased nut on a water agar medium. The inoculum was a disc of a five days old culture taken using a cork borer (7 mm diameter). It contained parasite mycelium fragments and zygotes.

Planting material

The rank of a bunch indicated the age of its nuts, which means that it was easy to distinguish between adult and young nuts. Bunches ranking 4, 5, 6, 7 and 8 which are respectively 4, 5, 6, 7 and 8 months old bore young nuts; those of ranks 9 to 11 bore immature adult nuts. The nuts to be inoculated were chosen without any fruit below in order to avoid contamination due to rainwater runoff from lesion zones to healthy nuts.

Inoculation techniques

Two techniques were used:

- inoculation on a wound;
- inoculation by depositing the inoculum on the pericarp.

Inoculation on wounds

Inoculations were carried out on nuts still attached to the palm. A disc of tissue was removed from the zone to be inoculated using a cork borer, after washing it in alcohol and rinsing it in sterile water. The inoculum was placed in the hole made. The inoculation involved placing a culture disc containing mycelium fragments in the hole left after removal of the disc of husk. The disc of husk was then replaced and held in place with a strip of adhesive tape.

Inoculation by depositing the inoculum on the pericarp

This method involved depositing *P. katsurae* inoculum on the pericarp cleaned beforehand as in the previous case. The inoculum was deposited on water-soaked cotton wool and the external surface of the parasite culture was applied to the pericarp. The cotton wool and parasite culture were held in place with adhesive tape.

Lesion position in artificial infections

As described previously, adult or young nuts were inoculated either in the proximal, mid or distal section. Proximal inoculation was carried out just around the floral parts. Lateral inoculation was carried out on the equatorial section of the nut. Distal inoculation was carried out near the distal invagination of the nut, a zone characterized by the hardness of the tissues.

The inoculated nuts were monitored for a month: at the beginning, only nutfall was recorded. On the last day of observations, all inoculated nuts were collected and examined. Any rot with doubtful symptoms was cultured on water agar.

Statistical analysis

The infection rates recorded in each category of inoculated nuts either on wounds or on the pericarp were statistically analysed using the test of proportions (Jayaraman, 1999).The test of proportions determines whether the proportions that are designated p1 and p2 and observed in two independent and classified samples of large size n1 and n2, reflect significant differences in the incidence of a character in the two populations. The alternative hypothesis H1, where $p1 \neq p2$ was tested. The statistical criterion used is z. It is given by the formula:

$$z = \frac{p_1 - p_2}{\sqrt{\frac{p_1 q_1}{n_1} + \frac{p_2 q_2}{n_2}}}$$

Where q1 = 1- p1
q2 = 1- p2.

This statistic follows a standard normal distribution. The value of z was compared to the value given by the table of normal distribution at a chosen significance level (1% or 5%). If this value was less than the value given by the table at the level of significance, there was no significant difference between the proportions designated for the impact of such character. There were some significant differences in the case where z was greater than the value given by the table (Jayaraman, 1999). The proportions were represented by the different infection rates obtained during the two inoculation techniques applied on young and adult nuts.

RESULTS

Susceptibility of nuts depending on their ages under natural conditions

The rotten nuts observed may have come from bunches from ranks 5 to 11, that means from those with the smallest fruits to those with the largest (11 months). However, 12 months old ripe nuts observed during the survey remained disease-free. Their pericarp and husk were hard and difficult to be penetrated by the fungus.

Two nut age categories were adopted for analysis:

- young nuts from 4 to 8 months old, whose shells are still not differentiated, indicated by the letters YN.
- immature adult nuts from 9 to 11 months old, with a totally formed shell, indicated by the letters AN.

The infection rates recorded in each nut category are given in Table 1. For the diseased nuts as a whole, the infection rate of young nuts (YN) was much lower than for adult nuts (AN). There was a highly significant difference between the infection rates of young and immature adult nuts at 1% significant level.

Age of nuts and inoculation success

The inoculation carried out on both adult and young nuts gave the overall results shown in Table 2, which indicates the percentage of success obtained on young and adult nuts depending on the two inoculation techniques: wounding or inoculum deposit on pericarp.

There was no significant difference between the infection rates during pericarp inoculation, regardless of the age of the nuts at the significance level of 1%. No differences in susceptibility were detected between young and adult nuts. However, there was a highly significant difference between the infection rates observed during wounding on young and adult nuts that were inoculated at the significance level of 1%. There was also a highly significant difference between the rates of success of infection obtained by each category of nuts regardless of the inoculation techniques at the significance level of 1%. Inoculation with wounding was far more effective than inoculation without wounding.

Observations of the different types of *Phytophthora katsurae* attacks on nuts

Types of attacks under natural conditions

During the three years of observations, only proximal and lateral lesions were observed:

Proximal lesions

Phytophthora katsurae attacks could begin on the proximal part of the nut and spread towards the apex. Such attacks were responsible for the rapid fall of nuts that still had a healthy distal section at the time of observation. The attacks began either below floral parts or around them. The first case was very scarce and partly linked to the presence of insects. Indeed, insects such as *Temnoschoita* sp. and *Carpophilus* sp. were found under the sepals of diseased nuts, along with mites *Aceria* sp.

Lateral lesions

The lateral lesions were characterized by attacks in the equatorial zone, in the mid section of the nut. The lesions spread both to the apex and to the proximal section, but they spread much more quickly towards the peduncle than to the apex, hence lateral lesions could be confused with proximal lesions if the nut was not dehusked to determine the true direction of spread.

Regardless of where the attack began, nutfall occurred when the peduncle zone was reached. The spread of the disease to the proximal section through the internal tissues was very rapid, so the external size of a lesion provided no indication of the extent of internal rot.

These observations showed that the lateral infections were more important than the proximal ones. The distal part of the nut was not susceptible to the fungus.

A comparison between types of lesions in each fruit category revealed highly significant differences at the significant level of 1% (Table 3). On young nuts, proximal attacks reached 76% (68.9-83.1) as opposed to 24% (16.9-31.1) for lateral attacks (Table 3). On adult nuts, there were significantly more lateral attacks 60.2% (58.9-71.5) than proximal lesions 39.8% (38.49-40.11).
These observations showed that lateral infections were much more common than proximal lesions and the distal section of the nut was not receptive to the parasite.

Tables 4 and 5 show the overall results for wound inoculation and inoculum deposit on the pericarp respectively.

Analysis of the inoculation results obtained on wounds

There was a highly significant difference between the susceptibility of the proximal zone of young and adult nuts at the significance level of 5%.The success rate for inoculations on wounds was very high (Table 4). These results also showed that large numbers of distal lesions can be obtained artificially when inoculation takes place after wounding. There was no significant difference in responses to infection depending on the inoculation site at the significance level of 5%.

Analysis of the inoculation results obtained with inoculum deposited on the pericarp

The success rate for inoculation in this way was low and revealed no clear difference between the various lesion sites on adult nuts (Table 5). Proximal lesions seemed substantial for both nut categories, with 20% on young nuts and 18.33% on adult nuts. There was a highly significant difference between response to infection on the proximal and lateral sections of young nuts, at the significance level of 5%.

The inoculation results were not similar to those obtained by infections in the field (Table 3).The success rates for inoculation by inoculum deposit on the pericarp were low (Table 5). There were clear differences depending on the infection site: the distal zone of the nut was hardly receptive to the parasite. However, it is worth noting that artificial inoculation on young nuts led to formation of lesions, whereas they remained symptom-free, under natural infection.

Table 1: *Phytophthora katsurae* infection of different categories of nuts under natural conditions.

Category of nuts	Total number of diseased nuts	Percentage[1] and limit
Immature adult nuts (AN)	5 186	97.40 %[a] (97-97.8)
Young nuts (YN)	138	2.60 %[b] (2.2-3)
Total	5 324	100 %

1: Each percentage represents the infection rate of young and adult nuts. The calculated statistical criterion z = 69.06 was higher than 2.576 (the value read at the table of the law of normal distribution) at the significance level of 1%. Values in each column not followed by common letters are statistically different at the significance level of 1%.

Table 2: Distribution of infection rates depending on nut categories and inoculation techniques.

Inoculation technique	Nut category						TOTAL		
	Young nuts			Adult nuts					
	Nuts inoculated	Success	Percentage [1] and limits	Nuts inoculated	Success	Percentage and limits	Nuts inoculated	success	Percentage[1] and limits
Surface	180	15	8.33 % a (4.3-12.6)	180	20	11.11 % a (6.51-15.71)	360	35	9.72 % (6.66-12.78)
Wound	180	139	77.22% b (71.12-83.82)	180	159	88.33 % c (83.6-93.03)	360	298	82.77% (78.87-86.67)
Total	360	154	42.77 % (37.6-47.8)	360	179	49.72 % (44.6-54.8)	720	333	46.25 % (42.65-49.85)

1: Each percentage represents the infection rate of young and adult nuts depending on inoculation techniques.

Surface inoculation on young and adult nuts $z = 0.89 < 2.576$ (read at the table) at the significance level of 1%: no significant difference.

Wounding inoculation on young and adult nuts $z = 2.82 > 2.576$ (read at the table) at the significance level of 1%: highly significant difference.

Surface and wounding inoculations on young nuts $z = 18.40 > 2.576$ (read at the table) at the significance level of 1%: highly significant difference.

Surface and wounding inoculations on adult nuts $z = 23.06 > 2.576$ (read at the table) at the significance level of 1%: highly significant difference.

Values in each column not followed by common letters are statistically different at the significant level of 1%.

Table 3: Lesion distribution according to nut age: Results of three years of observation (overall observation).

Age of nuts	Lesion position						Total of diseased nuts
	Proximal		Lateral		Distal		
	Number of lesions	Percentage and limits	Number of lesions	Percentage and limits	Number of lesions	Percentage and limits	
Young nuts	105	76 % a 68.9-83.1	33	24 % b 16.9-31.1	0	0	138
Adult nuts	2064	39.80 % b 38.49-40.11	3122	60.2 % a 58.9-71.5	0	0	5186
Ripe nuts	0	0	0	0	0	0	0
All ages	2169	40.74 % 39.44-42.04	3155	59.26 % 57.96-60.56	0	0	5324

1: Each percentage represents the distribution rate of lesions on young, adult and ripe nuts depending on lesion positions.

On proximal and lateral sections of young nuts, $z = 6.12 > 2.576$ (read at the table) at the significance level of 1%: highly significant difference.

On proximal and lateral sections of adult nuts, $z = 14.78 > 2.576$ (read at the significance level of 1%): highly significant difference.

Values in each column not followed by common letters are statistically different at the significance level of 1%.

Table 4: Results of the inoculations in the proximal, mid and distal sections of young and adult wounded nuts.

Age of nuts	Proximal section			Mid section			Distal section		
	Number of inoculated nuts	Number of diseased nuts	Percentage of diseased nuts	Number of inoculated nuts	Number of diseased nuts	Percentage of diseased nuts	Number of inoculated nuts	Number of diseased nuts	Percentage of diseased nuts
Young nuts	60	46	76.66 % a 65.96-87.36	60	49	81.66 % a 71.66-91.46	60	44	73.33 % a 52.13-84.53
Adult nuts	60	59	98.33 % b 95.10-101.5	60	53	88.33 % a 80.21-96.45	60	47	78.33% a 67.91-88.75

1: Each percentage represents the success rate for wound inoculations on young and adult nuts depending on lesion positions.
On proximal section of young and adult nuts, z = 3.80 > 1.96 (read at the table) at the significance level of 5%: highly significant difference.
On lateral section of young and adult nuts, z = 1.02 < 1.96 (read at the significance level of 5%: no significant difference.
On distal section of young and adult nuts, z = 0.64 < 1.96 (read at the significance level of 5%: no significant difference.
Proximal and lateral sections of young nuts, z = 0.67 < 1.96 (read at the significance level of 5%: no significant difference.
Proximal and distal sections of young nuts, z = 0.42 < 1.96 (read at the significance level of 5%: no significant difference.
Lateral and distal sections of young nuts, z = 1.098 < 1.96 (read at the significance level of 5%: no significant difference.
Proximal and lateral sections of adult nuts, z = 2.24 > 1.96 (read at the significance level of 5%: significant difference.
Proximal and distal sections of adult nuts, z = 3.59 > 1.96 (read at the significance level of 5%: highly significant difference.
Lateral and distal sections of adult nuts, z = 1.48 < 1.96 (read at the significance level of 5%: no significant difference.
Values in each column not followed by common letters are statiscally different at the significance level of 5%..

Table 5: Results of inoculations by inoculum deposited on the pericarp in the proximal, lateral and distal zones, on young and adult nuts.

Age of nuts	Proximal section			Mid section			Distal section		
	Number of inoculated nuts	Number of diseased nuts	Percentage of diseased nuts	Number of inoculated nuts	Number of diseased nuts	Percentage of diseased nuts	Number of inoculated nuts	Number of diseased nuts	Percentage of diseased nuts
Young nuts	60	12	20 % a 9.9-30.12	60	3	5 % b 0.5-10.5	60	0	0
Adult nuts	60	11	18.33 % a 2.63-28.03	60	7	11.6 % a b? 3.5-19.7	60	2	3.3 % 1.3-7.8

1: Each percentage represents the success rate for inoculations on the pericarp of young and adult nuts depending on lesion positions.
On proximal and lateral sections of young nuts, z = 2.55 > 1.96 (read at the table) at the significance level of 5%: highly significant difference.
On proximal and lateral sections of adult nuts, z = 1.038 < 1.96 (read at the significance level of 5%: no significant difference.
On proximal section of young and adult nuts, z = 0.23 < 1.96 (read at the significance level of 5%: no significant difference.
Lateral section of young and adult nuts, z = 1.32 < 1.96 (read at the significance level of 5%: no significant difference..
Values in each column not followed by common letters are statistically different at the significant level of 5%.

DISCUSSION

Immature adult nuts are the most susceptible stage to infection by *P. katsurae*. These observations are similar to those reported previously (Quillec et al., 1984; Pohé, 1992 (a and b), 1996). The lateral infections were more important than the proximal ones. Pohé (1996) reported that the hardness of the husk slows down the penetration of tissues by the fungus. The distal part, presenting a very tough barrier, is not easily reached by the fungus.

The equatorial zone of the nut is the most receptive site under artificial inoculation. The observations of the success rates with wound inoculations are similar to those obtained in the Philippines (Concibido-Manohar and Abad, 1992). According to other authors, resistance to the premature nutfall disease is related to fruit age and depends on the phenolic compounds content in infected tissues which is higher than on healthy ones (Brahmana et al., 1992). The activity of some enzymes such as pectate lyase and laccase was analysed and correlated to pathogen aggressiveness on the plant (Yao et al., 2009).

The almost total success of artificial inoculations on wounds and the lack of success for inoculum deposit on the pericarp led to the conclusion that the nut resistance process occurs in the pericarp. Thus, with inoculation on wounds, this resistance mechanism in the fruit may be circumvented. The resistance mechanism seems to be much more physically linked to fruit anatomy, than chemically. It would appear to be a mechanical resistance to parasite penetration.

Based on the knowledge of the anatomy of the fruit, we can propose hypotheses as to the receptivity of the different zones of the nut to *P. katsurae*:

Proximal zone

The floral parts provide physical protection of the pericarp against the pathogen, but at the same time they are organs which may retain the inoculum, in particular through water droplet retention around their edges, or they may harbour insects or mites, which are inoculum vectors (Pohe, 1992 b). *Phytophthora katsurae* cannot penetrate through hard pericarp and husk that are found in 12 months old ripe nuts.

Equatorial zone

This zone, which definitely undergoes the greatest changes during nut development, is the site where free water is retained on the surface to varying degrees depending on the characteristics of the insect's wounds which are present.

Distal zone

This zone reaches maturity very quickly and remains smooth throughout its development, acquiring total resistance to the parasite very rapidly.

Conclusion

The age of the coconut fruit and the position of lesions are some important components of the susceptibility of the nuts to *P. katsurae*. Depending on natural or artificial infection, the infection rate of the nuts varies. Wounding seems to expose the nuts of all ages to more infections.

Other factors are involved in nut susceptibility and probably determine the susceptibility of different planting material and are connected with:

- evasion phenomena which take into account nut position and contact between nuts in the same bunch;
- physiology: it is linked to drying out of the pericarp during nut ripening;
- nut morphology: large and convex size of nuts leads to different degrees of water droplets retention.

Nuts are easily penetrated by *P. katsurae* and this may be a route for pathogen movement into the heart of coconut tree (Uchida et al., 1992). Inoculation of non wounded barks had showed that the pathogen does not penetrate the dry bark and is only able to kill the plant if the white inner bark is exposed. It is why the application of an appropriate chemical using syringe injection in the wounded bark seems effective. The cost of chemical applications is profitable in productive fields when copra is sold at a high price.

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