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# The incidence and distribution of *Phytophthora cinnamomi* Rands on macadamia in Kenya

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**Key words**
Macadamia, root rots, incidence, distribution

## 1 SUMMARY

**In Kenya, macadamia (*Macadamia integrifolia* Maiden and Betche and *Macadamia tetraphylla* L.A.S. Johnson) is grown by over 100,000 small-scale rural farmers. Eighty three percent of the Kenyan macadamia nuts are exported to Japan, USA and China. Root rot and stem canker are major macadamia nut production constraints. This study was carried out to establish the incidence and distribution of the causal organism (*Phytophthora cinnamomi*) in different macadamia growing areas of Kenya. Disease surveys were carried out between December 2005 and April 2006 in all the macadamia growing areas of Kenya. A questionnaire was administered to capture data on macadamia production practices. The location of each sampling site was marked using a Global Positioning Satellite (GPS) instrument, Gamin®. *Phytophthora cinnamomi* was recovered from soil and diseased plant parts by plating onto synthetic media (corn meal agar). The recovered isolates of *P. cinnamomi* were characterised on the basis of pathogenicity and growth. Determination of mating types was done by matching with isolates of known mating types acquired from Australia. Root rot was described as the major disease of macadamia (by 85% of the respondents in the survey areas). Reported yield losses due to macadamia root rot were as high as 36.6% in one of the districts. Disease incidence was higher in flat areas. The Phytophthora root rots affected all macadamia cultivars across the regions but the most commonly affected cultivar was *M. tetraphylla*. Ninety percent of the interviewed farmers reported that they did not manage the disease in any way. The results show that *P. cinnamomi* associated with root rots and stem canker of macadamia has a wide distribution in all macadamia growing areas of Kenya. There is need to develop, validate and disseminate the best bet technologies for management of the disease to save the macadamia nut industry in Kenya. Development of integrated pest management (IPM) options for macadamia root rot has been initiated at KARI-Thika and training of farmers and extension field officers is planned to be done through the farmer field school approach.**

## 2 INTRODUCTION

The two types of cultivated macadamia nuts are the smooth shelled (*Macadamia integrifolia* Maiden and Batche) and the rough shelled (*Macadamia tetraphylla* L. Johnson). Macadamia nuts are eaten raw, cooked in oil, or roasted. Both shell and husks are good sources of fuel (Jenkins & Ebeling, 1985). The oil is used in the pharmaceutical and cosmetic industries. Macadamia cake, a by-product of the oil extraction process, is used as a livestock feed.

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In Kenya macadamia is an important cash crop grown by over 100,000 small scale growers in different parts of the country (Onsongo, 2004). In 2007, an estimated 3,276 hectares was under macadamia cultivation (MoA, 2007). From these, about 18,161 metric tons of in-shell nuts valued at KES 822,002,292 (USD 11,742,890) were produced (MoA, 2007). The main export markets are in Japan, United States of America and China (Onsongo, 2004). Macadamia was ranked as a major cash crop by farmers in Meru where it contributed to 40 % of the income (Muthoka *et al.,* 2005).

Root rot and stem canker caused by *Phytophthora cinnamomi* Rands were reported as major diseases causing 60% yield losses (Muthoka *et al.,* 2005). The pathogen causes a rot of the fine feeder roots while the leaves are smaller than normal, light green to yellow rather than dark green, branches' die back and there is substantial reduction in incremental growth rate (Zentmeyer, 1960). Infected trees gradually die regardless of their age (Fig. 1).

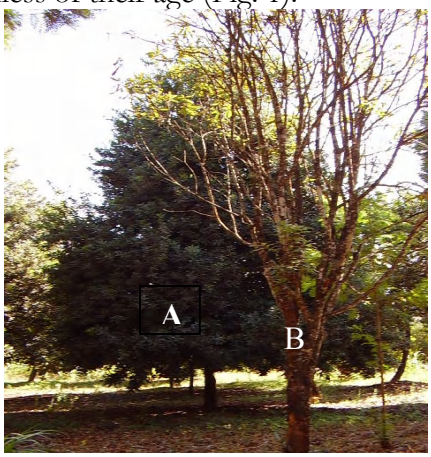


**Figure 1:** Macadamia trees A: Healthy tree; B: with disease symptoms.

*P. cinnamomi* has a global distribution and infects over 3000 species of trees and crops such as avocado, peach, pineapple, chestnut and macadamia (Adrienne, 2005). *P. cinnamomi* survives in dead plant material and can survive for long periods in this substrate. It may also survive in the soil as mycelium, sporangia, zoospore cysts, chlamydospores and oospores. *P. cinnamomi* can be spread by soil splashed by water movement and run off in drainage or irrigation ditches. The most likely means for distant movement is in contaminated soil or plant debris. Propagules can also be carried on machinery used for cultivation or harvesting and on planting materials. Infected seedlings are a major source of spread to previously clean areas, which is a major problem for field nurseries (EPPO, 2004).

Phytophthora species in soil or plant parts are isolated by baiting and then cultured on media containing fungal and bacterial inhibitors that favour the growth of *Phytophthora* species (Eden *et al.,* 2000). Various baits like seedling plants, or plant parts, including avocado fruits, blue lupine seedlings, apple fruits, seedlings of *Perseae indica,* and cotyledons of Eucalyptus, pineapple leaves, jacaranda seedlings and pine needles can be used to isolate Phytophthora from soil. Corn meal agar (CMA) has been used for isolations from small roots. The organism is readily identified by its colony morphology that has characteristic hyphal swellings to distinguish it from other *Phytopthtora* species (Erwin & Ribiero, 1996).

Cases of death of macadamia trees were reported in eastern and central Kenya in early 1980s. The cause of death was identified as root rot caused by *P. cinnamomi* (Sikinyi, 1983). However, the distribution of the pathogen was not established. The objective of this study therefore was to establish the distribution of *P. cinnamomi* in macadamia growing areas of Kenya and its infection on macadamia in different agro-ecological zones.

## 3 MATERIALS AND METHODS

**3.1 Survey sites:** A survey was conducted between December 2005 and April 2006 in macadamia farms within the five main macadamia growing provinces including Central, Eastern, Western, Rift Valley and Coast. Eleven districts representing the major

macadamia production areas were visited including Muranga, Maragua, Thika, Meru Central, Kirinyaga, Nyeri, Embu, Machakos, Taita Taveta, Baringo and Bungoma. For each district, except Baringo, Taita Taveta and Bungoma, which had fewer macadamia orchards 30 farms were selected on the basis of their accessibility and availability of not less than 30 bearing macadamia trees. A structured questionnaire to capture data on macadamia production practices was administered alongside face to face interviews with the selected farmers.

Field observations on the incidence of macadamia root rot were done by the team of trained enumerators. Where root rot was observed, severity was recorded as a score of 1 to 3 (1=less than 20% of the trees infected, 2= 20-50% of the trees infected and 3=more than 50 % of the trees infected). Root rot incidence was recorded as the percentage of farms where macadamia root rot was reported. In total 285 farmers were interviewed. The soil type and gradient (flat or steep slope) were also recorded. The location of the sampling site in each farm was marked by use of a Global Positioning Satellite (GPS) instrument, Gamin®. The descriptive survey data was analysed by use of Statistical Program for Social Scientists (SPSS) version 9.1 and the Microsoft Excel Program.

**3.2 Sampling, isolation and identification of *P. cinnamomi*:** In each farm, nine macadamia trees were randomly selected and observed for symptoms of root rot. Soil samples (1 kg) were taken from the rhizosphere of each tree at 0-10 cm depth. The samples were put in polythene bags, labelled and sealed. Plant parts (roots and bark) from symptomatic trees were collected, placed in polythene bags and stored at 4°C. Isolation of *P. cinnamomi* from soil was conducted using host bait. A soil sub-sample (250 g) was put in a plastic container and saturated with a similar amount (250 ml) of distilled water. This was done for all the samples from different survey areas.

Green avocado fruits of variety Fuerte were carefully harvested to avoid injury and surface sterilized by wiping with cotton wool soaked in 70 % ethanol, then rinsed in sterile distilled water. One avocado fruit was pushed into each sample of wet soil and incubated at 25-27 ° C for seven days in the dark as previously described by Zentmyer *et al.* (1960). After seven days, the fruits were removed from the soil, rinsed under tap water and observed for development of a hard brown rot at the soil line. The fruits were surface sterilized by dipping in 5 % sodium hypochlorite (Jik®) for two minutes then rinsed in sterile distilled water and dried using Whatman No. 2 blotter paper. A 5 mm piece was cut from the edge of the rot (between healthy and necrotic area), plated on corn meal agar (CMA) (Himedia Laboratories Ltd, India) in 90 mm disposable Petri dishes (Greiner Bio-one GmbH, Austria) and incubated at 25±2 ° C in the dark. The isolates were characterized on the basis of colony morphology, habit characters under high power microscope, growth on media at the same temperature and pathogenicty to green apple fruits.

**Pathogenicity tests**:The use of green apple fruits was based on the principle that *P.cinnamomi* strains pathogenic to macadamia cause a hard brown rot on green apples in 2-3days after inoculation (Zentmyer *et al.*, 1960). Pathogenicity tests where green apple fruits are used take a shorter time.Green apple fruits were obtained from the local market. These were surface sterilized by wiping with cotton wool dipped in 70% alcohol. Triangular slits were made on each apple by use of a sterile scapel blade inside a laminar flow cabinet to avoid contamination. The same scapel was used to cut 3mm$^2$ agar plugs from the edges of actively growing *P.cinnamomi* cultures. The agar plugs with mycelia were placed on the slits on the apples and sealed with Vaseline petroleum jelly. The inoculated apples were incubated at 25±2$^0$C. Observations were done starting 3 days after inoculation. Development of a hard brown rot was indicative of virulence of the *P.cinnamomi* isolate (Fig.2).

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**Figure 2**: Brown root on apple fruits caused by *Phytophthora cinnamomi* infection (Photo: Mbaka J.).

Roots and bark from an individual tree were washed under running tap water for five minutes to dislodge soil particles and cut into 5-10 mm long segments. These were then surface sterilized by dipping in 5 % sodium hypochlorite (Jik®) for two minutes, rinsed in sterile distilled water three times and dried with a blotting paper (Whatman No.2) before plating on potato dextrose agar (PDA) (Oxoid Ltd. Basingstoke, Hampshire, England). After 48 hours, mycelial growth in the agar was examined microscopically for presence of *Phytophthora* species hyphae. Axenic cultures were established on corn meal agar and characterized in the same way as those from the soil samples.

## 4 RESULTS AND DISCUSSION

**4.1 Baseline survey:** The survey areas mainly fell in the Upper Midland (UM) agro ecological zones 3 and 4. These are the main coffee (UM4) and the marginal coffee (UM3) zones according to Jaetzold and Schmidt (1983). This was expected as macadamia in Kenya was introduced from Hawaii in the 1960s through the coffee factories and was mostly cultivated in coffee growing areas.

Macadamia was mainly grown as an intercrop with coffee and food crops such as bananas, maize and beans. The mean farm sizes, number of macadamia trees grown in each farm, incidence of root rot severity and percentage losses due to root rot are shown in Table1. Root rot was recorded in all the macadamia growing areas of Kenya. The highest incidence, severity and percentage losses caused by root rot were recorded in Meru Central district. This could be due to the fact that in that region, macadamia was mainly intercropped with avocado which is also a host of *P. cinnamomi* as reported in an ealier survey by Muthoka *et al.*, (2005). The lowest percentage losses caused by root rot were recorded in Maragwa and Muranga districts despite the relatively high disease incidence of 30 and 36 % respectively. This could be due to the fact that most of the dead trees had been replaced with improved cultivars from the Kenya Agricultural Research institute ( Gitonga *et a*l., 2009). The main cultivar in these two districts was MRG-20, which had less infection (Table 2).

The main macadamia cultivars grown and percentage root rot infection recorded on them is shown in Table 2. The main cultivars were *M.tetraphylla*, hybrid types (between *M. integrifolia* and *M. tetraphylla*), MRG-20, KMB-3, *M. integrifolia*, KRG-15 and EMB-1.. Other types included; Hawaii 508, MRG-27, KRG-4 and MRU-25.

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**Table 1:** Farm size, number of macadamia trees and root rot infection in 10 districts of Kenya.

| District | Total farm size (Ha) | No. macadamia trees per Ha | Root rot incidence | Root rot severity | % loss caused by root rot |
|---|---|---|---|---|---|
| Bungoma | 7.52 | 15 | 12.0± 2.6c | 2±0.2a | 27.1±1.3b |
| Embu | 1.34 | 35 | 33.3±0.4b | 2±0.2a | 12.2±0.3c |
| Kirinyaga | 1.86 | 38 | 29.3±0.9 | 1.4±1.0b | 11.3±0.4c |
| Machakos | 1.63 | 43 | 40.0±0.3a | 2.8±1.8a | 12.0±0.3c |
| Maragwa | 1.66 | 31 | 30.0±0.7b | 2.1±0.4a | 6.8±1.0d |
| Meru Central | 3.20 | 45 | 46.6±1.6a | 2.5±1.2a | 36.6±2.3a |
| Muranga | 1.50 | 28 | 36.0±0.5b | 2±0.2a | 9.4±0.6d |
| Nyeri | 1.65 | 47 | 29.6±1.5c | 1.4±1.0b | 13.8±0.1c |
| Taita-Taveta | 3.73 | 57 | 23.5±0.8c | 1.3±1.2c | 12.9±0.2c |
| Thika | 1.29 | 28 | 37.0±0.6b | 1.8±0.3ab | 12.2±0.2c |
| Mean | 2.53 | 36.7 | 31.7 | 1.9 | 15.3 |

Means followed by the same letter in the same column are not significantly different (P=0.0496, SNK), n=256farms

**Table 2:** Macadamia cultivars grown in Kenya and incidence of root rot on them.

| Macadamia cultivar | Percentage of the farms (n=256) | Percentage of the trees with root visible rot symptoms |
|---|---|---|
| *M.tetraphylla* | 30.2 | 15.1 |
| Hybrids | 18.0 | 4.0 |
| MRG-20 | 16.5 | 3.0 |
| KMB-3 | 14.5 | 6.7 |
| *M.integrifolia* | 13.2 | 2.1 |
| KRG-15 | 12.1 | 7.5 |
| EMB-1 | 11.3 | 6.9 |
| Others | 7.3 | 2.1 |

The high percentage (30.2) of *M. tetraphylla* in the farms despite its poor quality nuts could be attributed to high germination of fallen nuts. More than 85% of the respondents in the study areas reported macadamia tree death as a major constraint to production. All the macadamia cultivars were affected but most of the affected trees observed were of *M. tetraphylla*. (Table2). The high number of root rot infected *M. tetraphylla* trees observed could be due to the fact that *M. tetraphylla* is more tolerant to *P. cinnamomi* and the tree can survive for long periods with the infection without succumbing to the disease (Zentmyer, 1979). Other reported diseases were stem canker and flower blight.

The main method of weed control was by mechanical means using *pangas* (machetes) or *jembes* (hoes). Use of *jembes* and *pangas* for weed management could cause injury to the roots and create entry points for *P. cinnamomi* from other hosts such as avocado (Zentmyer, 1979). About 80% of the respondents reported that they did not apply disease management practices while 5% of them reported control using ash or by burning organic wastes under the tree to allow smoke to go up the macadamia tree to repel

pests (smoking). Smoking in macadamia orchards is a recommended management strategy for macadamia stink bug and nut borers but has no effect on root rot. There is no record on effective use of ash for control of any macadamia pests.

Most of the farmers did not apply fertilizers to their macadamia orchards. It was observed that in farms where organic manure was applied to macadamia or the coffee intercrop, there was no incidence of root rot. Observation of low incidence of root rot in farms where cow and chicken manure was used in the coffee intercrop was in agreement with Aryantha *et al.* (2000) who found that high levels of ammonia in chicken manure is toxic to *P. cinnamomi*.

There was positive correlation (+1.359, P =0.005) between landscape and root rot incidence with more root rot occurring in orchards located in flat beds. The high incidence of root rot in flat beds could be attributed to movement of fungal propagules (oospores, chlamydospores or oospore cysts) in the soil particles (Benson, 1987; Weste and Vithanage, 1979) from sloppy to flat areas. In addition, drainage in flat areas could be poor hence leading to increased soil moisture that creates an environment conducive for development of root rot. Weste and Vithnage (1979) found that mycelium of *P. cinnamomi* can survive in moist soil for over six years.

**4.2 Characterization of *P. cinnamomi* isolates:** The isolates were identified under high power microscope based on presence of coralloid type mycelia with hyphal swellings (Fig. 3). Isolates showed differences in growth on CMA, PDA and tap water agar at the same temperature. In total, 21 different *P. cinnamomi* isolates were recovered from the soil samples. However, only seven out of these were virulent to green apple fruits as observed in the pathogenicity tests. This implies that not all *P. cinnamomi* strains are pathogenic to macadamia. However these strains were pathogenic to avocado as they were recovered with the use of avocado fruit baits. This is in agreement with Zentmyer (1979) who found that *P. cinnamomi* isolates from avocado were only pathogenic to wounded macadamia to cause the trunk cankers but not otherwise. Declining avocado orchards should not be replaced with macadamia. *Phytopthtora cinnamomi* was widely distributed in all the macadamia growing areas of Kenya (Figure 4).

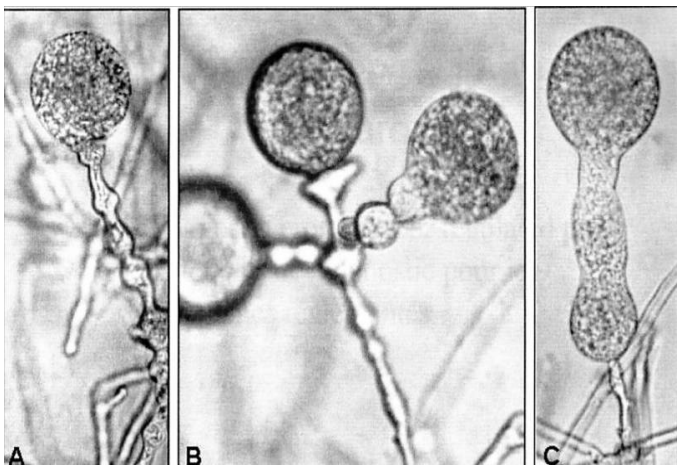


**Figure 3:** *Phytophthora cinnamomi* (X 400): A: Coralloid –type mycelium with a hyphal swelling. B: Cluster of hyphal swellings. C: Hyphal swelling with a bizarre shape.

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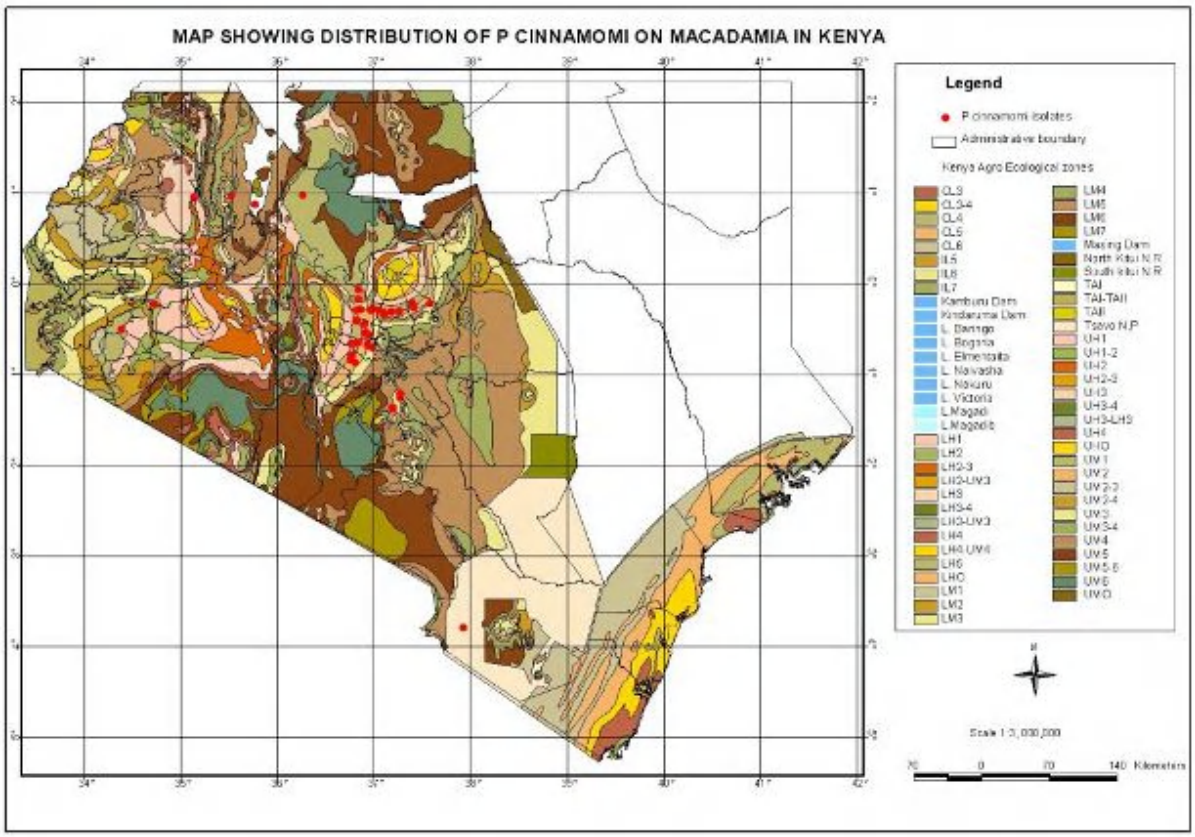


**Figure 4:** Distribution of *P.cinnamomi* on macadamia in different agro-ecological zones of Kenya.

## 5 CONCLUSION AND RECOMMENDATIONS

The survey established that macadamia root rot and stem canker occur in all the macadamia growing areas of Kenya. Farmers' perception of the disease is poor as decline can be caused by other biotic factors such as low soil fertility and water stress. There is no management strategy in place at the moment and the full macadamia yield potential is not exploited.

There is need to develop and disseminate an effective root rot management strategy for the macadamia farmers. The macadamia nursery operators (private and public) need training on nursery management to avoid infections in the nurseries. Sterilization of potting media at the nursery level can be done in different ways such as:

**1.** Solarization: use of solar energy can be effective in hot areas. The potting media is watered to saturation, covered with a black polythene sheet for two weeks. Temperatures rise to over 100 °C and kill soil borne pathogens, pests and even some weed seeds.This is an inexpensive envirometally safe method of soil sterilization.

**2.** Fumigation:Metham Sodium (Metham sodium anhydrous) is a soil fumigant that is available from agro-chemical stockists in the country in packages as small as 50 ml. This can be used by macadamia nursery operators to sterilize the potting media. This kills the soil borne pathogens, pests and weed seeds. Care should be taken when applying this chemical and also disposal of the packaging material

**3.** Biological method: Rootgard is a cocktail of microorganisms useful to the plant specially formulated with nutrients and enzymes. The microorganisms include *Trichoderma* spp, *Bacillus* spp, *Pseudomonas* spp, *Aspergillus* spp, *Chaetomium* spp, *Escherichia* spp and *Azotobacter* spp. The product is available in the country and can be used as a root dip, soil drench or foliar spray to control root rots in the field or the nursery. It

should however be noted that Rootgard cannot be used in combination with Metham Sodium as this will render the micro organisms in Rootgard inactive.

**4.** Nursery operators should only select healthy rootstocks when grafting macadamia to avoid early root rot infections.

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