COMMON/SPEAR ROT CROWN DISEASE IN OIL PALM (*Elaeis guineensis* Jacq.): ANATOMY OF THE AFFECTED TISSUE

Keywords: *Elaeis guineensis*; crown disease; common spear rot; anatomy.

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Bent rachises in palms with symptoms of "crown disease" had fibres with thinner and less lignified cell walls than normal cells. Also, vascular bundles were less numerous, thinner, and had fewer fibres than healthy tissue. The first symptom observed before necrosis in leaflets was the collapse of the hypodermal cells. Fungal mycelium was found intercellularly in some preparations. The structural changes observed explain, in part, the softness of these tissues, which causes the bending of the rachises that characterizes the disease. Glyphosate applied to young palms caused anatomical changes similar to those observed in palms affected by crown disease.

INTRODUCTION

Symptoms of spear rot/crown disease are common during the juvenile growth of oil palm, particularly during the first two years after field transplanting. However, diseased palms may appear at the nursery stage, and some may still show symptoms after seven or more years. Symptoms may be recurrent in a particular palm and each attack may persist for a few weeks or several months. More frequently, affected palms recover and, after several months, show no signs of the disease (Turner 1981; Chinchilla, 1987; Breure and Sebagjo, 1991; Monge *et al.*, 1993).

Yield losses due to the disease depend on the severity and persistence of the attack. In commercial oil palm plantations the incidence rarely exceeds 5%, but more than 50% of the palms may be affected in some highly susceptible progenies (Turner, 1981; Chinchilla, 1987; Breure and Sebagjo, 1991; Monge *et al.*, 1993). In general, yield losses are in the range of 2%–3% (Turner, 1976; Turner,

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1981; Hartley, 1988). In Indonesia, crown disease reduced vegetative growth significantly, and the cumulative yield of oil during the first six years was reduced by 4.5% (Breure and Sebagjo, 1991). Vegetative growth and yield during the first 40 months after transplanting were compared in Costa Rica between a group of healthy palms and a group of palms with symptoms of the disease. The cumulative yield of the affected palms was reduced by 4 t/ha as compared with the healthy group (Chinchilla, unpublished).

From the description of symptoms in the literature, it is not easy to determine if crown disease is really a different condition from common spear rot. The first symptom of crown disease is described as the appearance of small, brown, watery lesions on leaflets located on the spear leaf. The lesions grow and several contiguous leaflets may be affected before the spear leaf expands. A characteristic of the disease is the presence of one or several bent rachises. The description of the initial symptoms of common spear rot is almost identical, except that in this condition no bent rachises are expected to develop (Turner and Bull, 1967; Turner, 1981; Hartley, 1988).

The microorganisms isolated from the infected tissue (necrotic lesions) in both types of disease are also similar (Turner, 1981; Monge *et al.*, 1993). Furthermore, when the disease is followed in individual palms over a period of several months or years, the symptoms of either type may precede the appearance of the other, and many plants may show a combination of symptoms (Chinchilla, 1991).

The genotype has a profound effect on the incidence of disease (Gai, 1969; Blaak, 1970; Breure and Sebagjo, 1991). The genotype will determine the initial level of disease, the highest incidence reached, the predominant symptom (tissue rotting with or without bending of rachises), the percentage of plants with recurrent infections and the rate of increase of the disease with time (Monge *et al.*, 1993). There is evidence of a recessive gene involved in susceptibility (De Berchoux and Gascon, 1963) and another that may inhibit its manifestation (Blaak, 1970).

Heusser (cited by Turner, 1981) thought that the bending of the rachis could be caused by an abnormal lignification of the elongating spear leaves. A lack of mechanical support would make the frond bend as it elongated and became heavier. As cited by Hartley (1988), Thompson wrote in 1934 that the lack of rigidity of the rachis was related to a reduced lignification of the parenchymatious tissue. Some authors think that certain fungi like *Fusarium* spp. may be involved in the development of the symptoms, affecting the physiology of the plant by producing, or inducing the plant to produce, some growth regulators (Turner and Bull, 1967). On the other hand, compounds like Glyphosate can induce symptoms similar to crown disease in young palms (Monge *et al.*, 1993).

The objective of this study was to characterize anatomically young affected tissue in oil palms showing the common spear rot/crown disease.

MATERIALS AND METHODS

Tissue used

Tissue was collected from three-year-old palms belonging to a Deli x AVROS cross, which is particularly susceptible to the disease. Crown disease symptoms (bent rachises) in this cross are normally accompanied by severe rotting of young tissues.

Leaflet and rachis samples from healthy and diseased palms were taken from leaves on positions –1, –2, –3, +2 and +5 on the phyllotaxis. Another group of samples was obtained from another Deli x AVROS cross that had been sprayed with 50 ml of Glyphosate (8000 mg a.i./litre) two weeks earlier.

The foliar tissue analysed included very young lesions, before extensive necrosis developed. Rachis preparations of diseased palms were obtained from the bent portion of the frond.

Sample preparation

Three methods were tried for the examination of tissue under a light microscope; these used a) paraffin, b) polyethylene glycol, and c) resin. The last method was the only one that allowed an appropriate handling of the samples and was selected for the rest of the study.

The tissues were fixed in F.A.A. or glutaraldehyde (2.5% in phosphate buffer, pH 7.2) for a minimum of 24 hours. After three washes (10 min/each) in a phosphate buffer, the tissues were dehydrated for one hour in seven successive alcohol solutions of increasing concentration (50%, 70%, 80%, 90%, 95%, 100% and 100%). Later, the tissues were placed for at least one hour in each of three mixtures of 100% alcohol and propylene oxide (reagent grade) in the proportions of 3:1, 2:2 and 1:3. Finally, the samples were kept for one hour in each of two fresh preparations of pure propylene oxide.

Mixtures of propylene oxide and resin (Spurr) were prepared in the proportions of 3:1, 2:2, and 1:3. The tissues were kept for one hour in the first two preparations and overnight in the last one. The following morning, the samples were placed in pure resin for three hours, and this was repeated three times using fresh resin each time. The samples were then placed in plastic capsules with resin and put in an oven (70°C) for 24 hr in order to polymerize the resin. The blocks obtained were cut in sections on 2 µm thick with a Sorval ultramicrotome. For better observation under the microscope, the samples were stained with toluidine blue. Photomicrographs were obtained of the cross sections on leaf lamina and the rachis. All samples were prepared and examined at the Unit of Electron Microscopy of the University of Costa Rica.

RESULTS

Leaf lamina

The first abnormality observed in diseased tissue was a brown deposit on both the abaxial and adaxial hypodermis (*Figure 1*). Later, this tissue appeared macerated (*Figure 2*) and the parenchyma disorganized (*Figure 3*). Cells nearer the abaxial surface appeared more affected. Septate mycelium was observed growing intercellularly in some preparations (*Figure 4*).

Rachis on healthy fronds

In cross section, the distal portion of the rachis showed a flat triangular view. The curved side (exterior) corresponded to the ventral portion. The epidermis had a layer of rectangular cells and the hypodermis was formed of two or three layers of cells which were larger than those of the epidermis. Vascular bundles were numerous, with their size depending on their position within the rachis. These bundles were surrounded by many fibres, as described by Tomlinson (1961), and were concentrated toward the periphery of the rachis (*Figure 5*).

Bundles closer to the epidermis, both on the adaxial surface and the sides, were smaller than those located near the abaxial surface. The larger size of bundles on the abaxial surface was probably the result of the fusion of adjacent bundles (*Figure 5*). The size of the vessel elements was similar on both epidermis; however, the number of bundles and vessel elements on the abaxial surface was smaller than on the adaxial portion of the rachis.

The fusion of vascular bundles could have been the result of the lignification of the cells of the parenchyma located among bundles. Fibres had a thick cell wall and were highly lignified (*Figure 6*). In a cross section of the base of the rachis, small fibre bundles, with no vascular tissue, were observed on the abaxial surface.

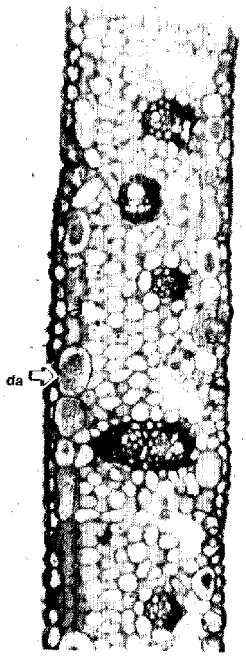

Figure 1. Abnormal deposition of substances (da) in the hypodermis of affected leaves in position –1 in the phyllotaxis; 200x.

Diseased rachis

The xylem was mostly occluded by tyloses. Fibres in vascular bundles had very thin cell walls (*Figures 7* and *8*). The bundles had a small diameter in cross section owing to the presence of a relatively small number of fibres. Fusion of vascular bundles was less common in the affected tissue than in normal rachises. On the abaxial surface, even fused bundles had more fibres with thin cell walls than those on the adaxial surface. Lignification was not uniform in fibres of bundles from the abaxial surface of the rachis. On the adaxial surface, bundles had fibres with thicker cell walls that showed an almost normal degree of lignification. Fibres tended to be thicker on those bundles located close to the epidermis (*Figure 8*). Tissue at the flaccid central portion of the rachis had some dark-coloured deposits in the parenchyma. Many of these observations coincided with those of Heusser in 1927 (cited by Turner, 1981) and Thompson in 1934 (cited by Hartley, 1988).

Crown disease symptoms (rachis bending) were consistently associated with some necrosis on leaflets. However, it was still possible to obtain young tissue (spears) with very mild necrosis. When such necrosis was present on the rachis the vascular bundles closer to the lesions had fibres with very thin cell walls and with little lignification.

Rachis of plants sprayed with Glyphosate

Fibres in vascular bundles were less lignified than in normal tissue. Most lignification observed was in that part of the bundle closest to the external surface of the rachis. Lignification was poor in the rest of the tissue. The cell walls of the fibres were thin, particularly in the internal tissue. Many fibres did not stain blue as expected and had sinuate walls. The phloem was occluded by a dark brown substance.

DISCUSSION

The anatomical characteristics observed in young rachises obtained from oil palms affected by crown disease made it easier to understand the lack of rigidity of these structures, and the development of the typical symptoms of the disease. The rachis bends or even breaks as it elongates and its weight increases. The disease causes changes in the normal lignification pattern of the rachis during its elongation. The loss of stiffness seems to be caused mainly by reduced lignin deposition on the cell walls of the fibres of the vascular bundles.

Plants sprayed with Glyphosate produced some young rachises with anatomical characteristics similar to those found in diseased plants: the rachises showed an abnormal lignification of the cell walls of the fibres in the vascular bundles. Palms sprayed with Glyphosate showed one or several fronds which were bent in a manner similar to those of the infected palms. Glyphosate inhibits the shikimic pathway affecting phenol and, eventually, lignin synthesis (Hahlbrock and Grisebach, 1979; Rubin *et al.*, 1984).

The significance of the fungal hyphae found within the infected tissue cannot be interpreted with the information available. The leaf lesions analysed were quite young, but a secondary organism could have gained access to the tissue. Some pathogens produce, or induce the host to produce, certain growth regulators such as auxins and ethylene causing imbalances that may affect lignin synthesis, among other things (Sequeira, 1973; Misaghi, 1982).

The close association between bent rachises and leaflet necrosis in palms affected by crown disease is a clear indication of the role that some microorganisms may be playing in the development of the disease syndrome. Two organisms are consistently isolated from diseased tissue: *Fusarium* spp. and *Erwinia* spp. (Kovachich, 1957; Duff, 1963; Turner, 1981; Watanavanich, 1982; Babu, 1989; Monge *et al.*, 1993). However, the inoculation of these microorganisms has not yet reproduced the typical symptom of crown disease (bent rachises).

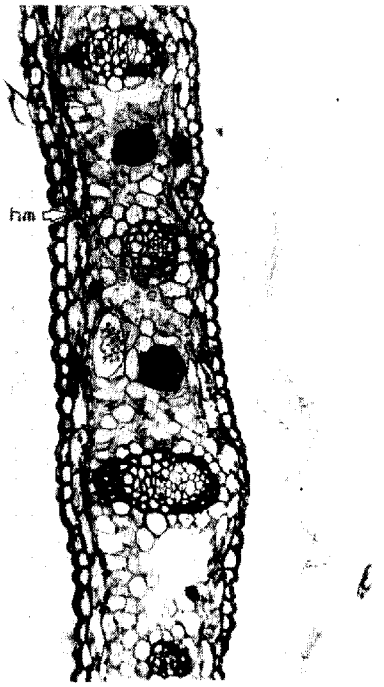

Figure 2. Macerated hypodermis (hm) in affected leaflets (crown disease) in position –1 in the phyllotaxis, 200x.

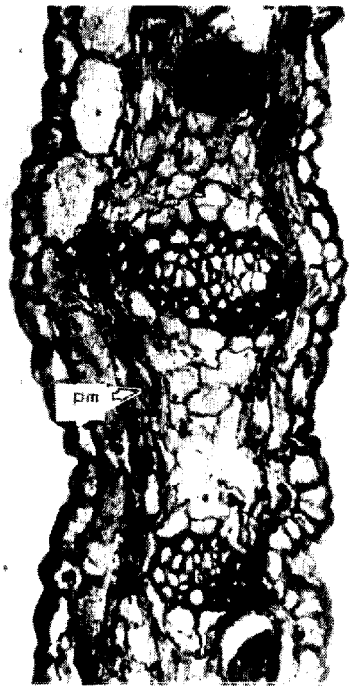

Figure 3. Macerated parenchyma (pm) of leaflets in position –2 in diseased palms; 200x.

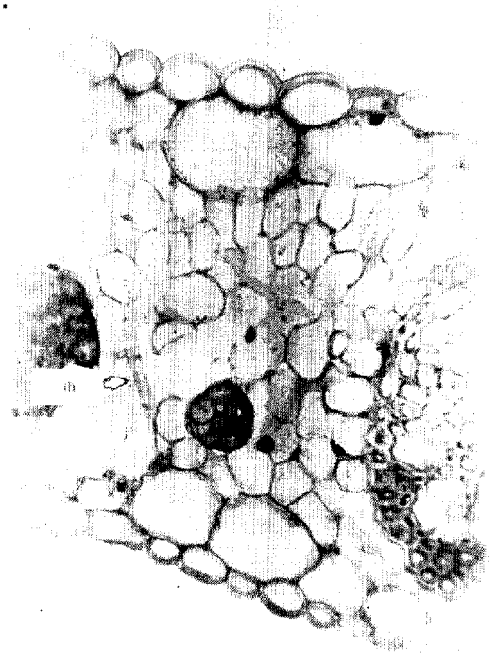

Figure 4. Mycelium (m) in the parenchyma of leaflets in position –3 in diseased palms; 200x.

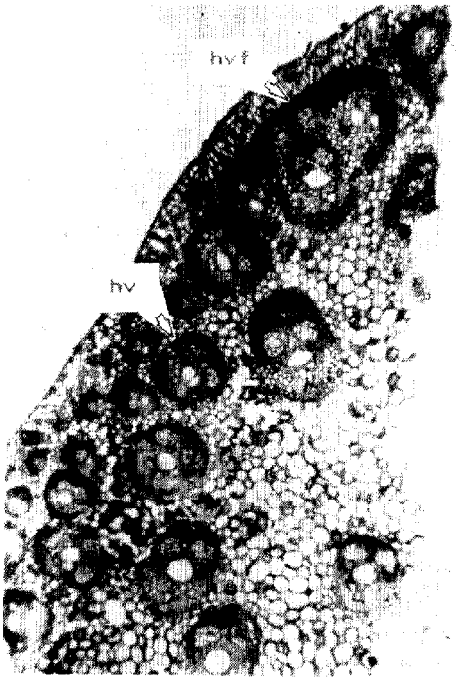

Figure 5. Vascular bundles (hv) and bundles fused (hvf) in the rachis of healthy fronds in position +5 in the phyllotaxis; 40x.

Figure 6. Bundle of fibres with thick cell walls in the rachis of healthy fronds in position –2; f = fibres, l = lumen, p = wall; 400 x.

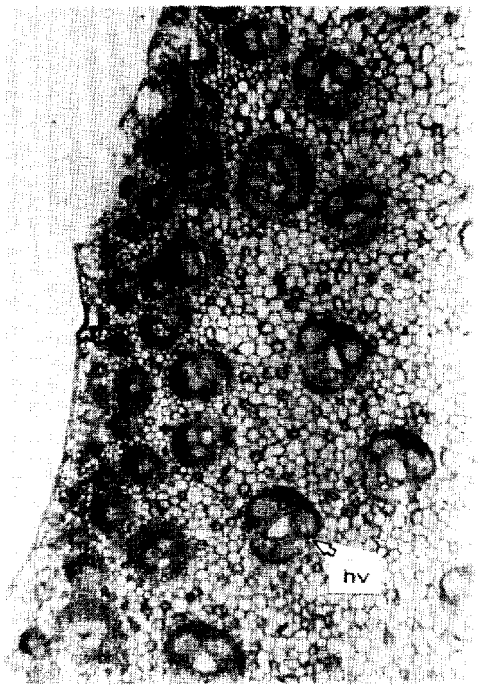

Figure 7. Fibres with thin cell walls, and vascular bundles (hv) abnormally lignified in the rachis of infected fronds in position +5 on the phyllotaxis; 40x.

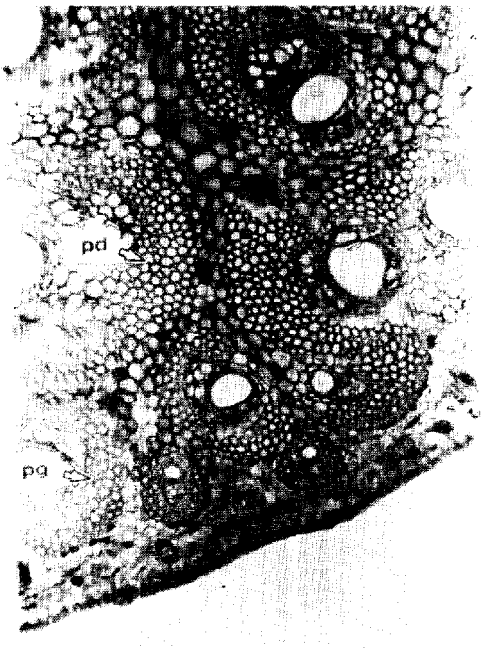

Figure 8. Fibres with thin cell walls (pd) and thicker walls (pg) in the region near the epidermis in the rachis of diseased fronds in position –2 on the phyllotaxis, f = fibres; 100x.

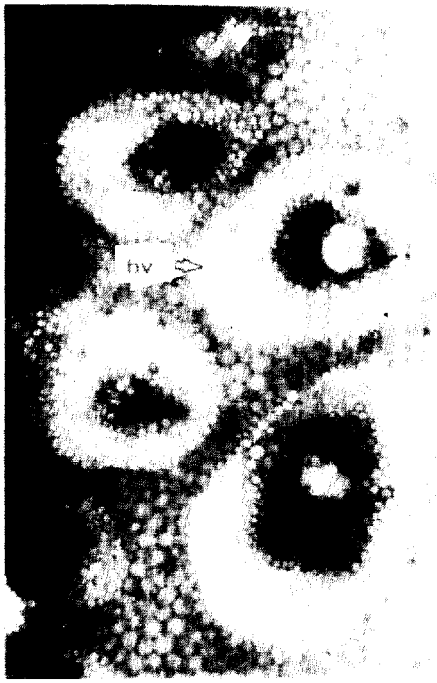

Figure 9. Vascular bundles (hv) with little lignification in the rachis (position –2) of a Glyphosate-treated palm; 100x.

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