

Coffee Anthracnose; Development of Morphological and Histological Symptoms

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Abstract. Morphological and histological symptoms of leaf anthracnose were studied on inoculated seedlings and detached leaves of three coffee cultivars; Hammadi, Iriani and Inici. Plesionecroses followed by holonecroses were obvious 7 days after inoculation under greenhouse and laboratory conditions. Epidermal cell collapse followed by accumulation of inclusions in epidermal and mesophyll cells was observed. The fungus produced mycelial mats over the host epidermis from which great numbers of setose acervuli arose. Frequency of acervuli differed on the different cultivars. Anici was the most susceptible among the tested. Numbers of acervuli, however, were higher on sound unscratched leaves than on abraded leaves on all tested cultivars.

Introduction

Morbid anatomy of plant tissues infected with anthracnose fungi has been studied in several host-pathogen systems. Major changes in mitochondria, chloroplasts and cell cytoplasm were found in tangerine tissues infected with *Colletrichum gloeosporioides* [1]. Brown [2], however, reported that intracellular hyphae of *C. gloeosporioides* did not cause immediate severe cell disruption; cells were not killed in advance of invasion. Hyphase of *C. acutatum* f. sp. *pineae*, on the other hand, spread leading to cell collapse in primary needles of *pinus radiata* [3]. An area of discoloration was observed around penetration sites on cotton lint invaded by *C. capsici* [4]. Papaya fruits inoculated with *C. gloeosporioides* showed deposits in several layers of parenchyma cells adjacent to infection sites. Cuticle and epidermal cell walls of the fruits were ruptured during fungal sporulations and acervuli formation [5].

The present study was conducted to investigate the development of morphological and histological anthracnose symptoms caused by *C. coffeanum* on coffee leaves.

Materials and Methods

1–Morphological symptoms

A – On seedlings

Nine month-old coffee seedlings of three cultivars namely; Hammadi, Iriani and Anici along with an isolate of *Colletotrichum coffeanum* Noak were used in this study. The fungus was originally isolated from infected leaves of coffee seedlings grown in a greenhouse at the College of Agric., King Saud Univ. The three coffee cultivars were brought from Arab Republic of Yemen. Spore suspension of the fungus was prepared in sterilized distilled water at a concentration of 1×10^4 spores/ml. Seedlings were inoculated by spraying with the fungal spore suspension and then covered with polyethylene bags for 24 hr after which bags were removed. Seedlings were then returned to the greenhouse ($25 \pm 2c$) and symptoms were regularly observed for development of morphological symptoms.

B – On detached leaves

Detached leaves (4th from the top) from the three coffee cultivars were inoculated with the fungal spore suspension (1×10^5 spores/ml). Just before inoculation, leaves (Six of each cultivar) were thoroughly washed with distilled water and half of the leaves were gently rubbed with carborundum to create tiny wounds on the leaf surfaces. Leaves were placed, singly, over glass rods in petri dishes containing filter paper (Whatman No.1) saturated with sterilized distilled water. Inoculum was applied, as spore suspension drops, on leaf surfaces using a micropipette. Inoculated leaves were incubated at room temperature and regularly observed for disease development.

2–Histological symptoms

Four to seven days after inoculation of the detached leaves, leaf peices of cultivar Anici were collected from infected sites and fixed in “FAA” for 24 hr. Fifteen micron transverse sections were prepared from leaf tissues using a paraffin [6]. Sections were double stained with safranine and light green and then mounted in canada balsam. Sections were examined under the light microscope to observe the invading fungus and its effect on host cells.

Results

1–Morphological symptoms

A – On seedlings

Disease symptoms on leaves of all three cultivars developed at first as minute plesionecrotic spots one week after inoculation. Fleck-like spots enlarged from a few millimeters to more than one centimeter in diameter. Upon symptoms manifestation, the developed spots appeared on both surfaces of the infected leaf as holonecrotic circular areas with a slightly sunken center which assumed a light grey colour but with reddish to brownish edges (Fig. 1 a, b). Spots became enlarged and often coalesced (Fig. 1 c). In the advanced stage of disease development, the enlarged spots assumed a dark colour with a somewhat rough center due to the development of acervuli just beneath the epidermis. The affected area enlarged to include most of the leaf blade and acervuli developed in concentric circles in the holonecrotic areas. Necrotic spots later assumed characteristic blotch symptoms (Fig. 1 d).

B – On detached leaves

Symptoms on detached leaves of all three cultivars developed four days after inoculation as necrotic areas which enlarged rapidly and finally shriveled. Numerous acervuli soon developed in concentric rings over necrotic areas. Acervuli on detached leaves started to develop 5 days after inoculation and became quite obvious after 10 days. The frequency of acervuli differed in the three tested coffee cultivars. Acervuli, in all tested cultivars, were more frequent on sound unscratched leaves than on scratched ones. Mean numbers of acervuli were 7, 9 and 22 per microscopic field ($10\times$ objective lens) on sound leaves of Hammadi, Iriani and Anici, respectively. The numbers, however, were 0, 2 and 7 on the corresponding scratched leaves.

2–Histological symptoms

Light microscopic examinations of cross sections of inoculated leaves of cultivar Anici, prepared at different stages of symptom development, demonstrated that plesionecrosis was often associated with fungal growth and fruitification. Plesionecrosis was expressed as discoloration of infected host tissues. Epidermal tissue was the first to be affected particularly when the invading fungus produced stroma-like mycelial mats. At this stage, palisade tissue appeared healthy in most cases (Fig. 2a). As the acervuli developed, they appeared as an open bed of short conidiophores growing side by side, arising from a stromatic mass of hyphae and bearing unicellular conidia. The epidermal as well as palisade and spongy tissues were discoloured and some of the parenchyma cells became filled with dark brown inclusions (Fig. 2 b&c). At later stages of disease development, a complete collapse of mesophyll tissues took

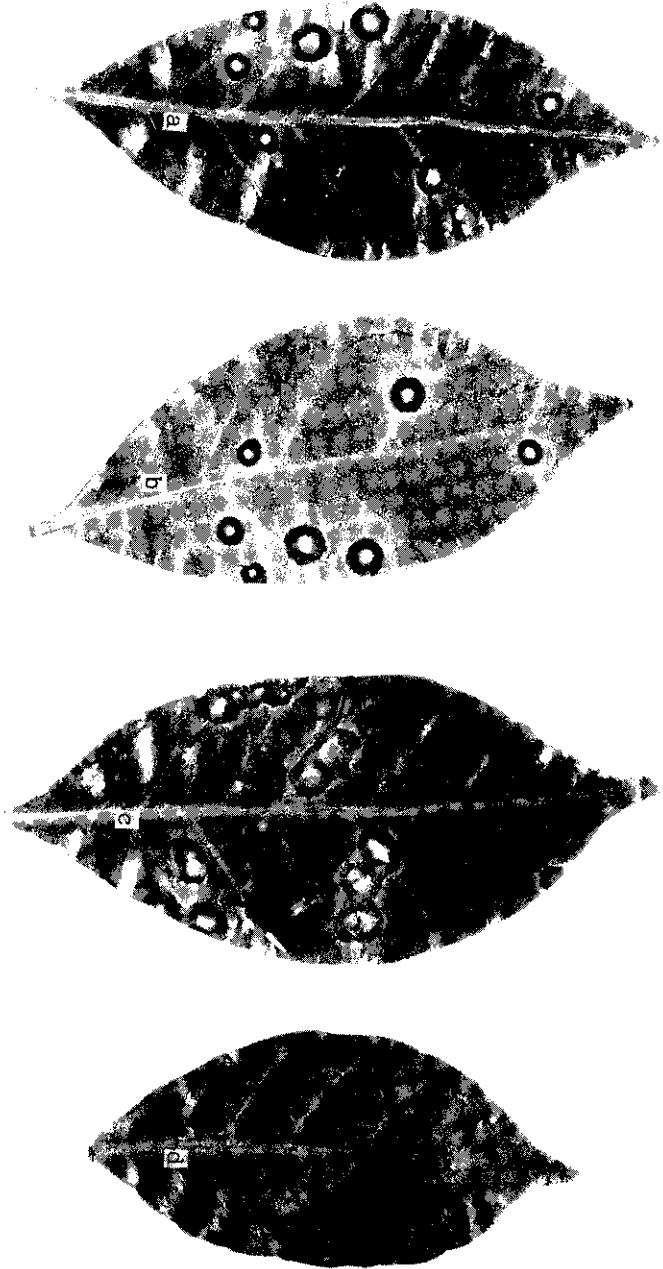


Fig. 1. Different stages of anthracose symptoms on leaves of Iriani coffee cultivar:

- a, b:** Circular spots with light grey center and reddish margin (a: upper surface, b: lower surface),
- c:** Coalesced spots.
- d:** Holonecrotic enlarged areas with dark colour.

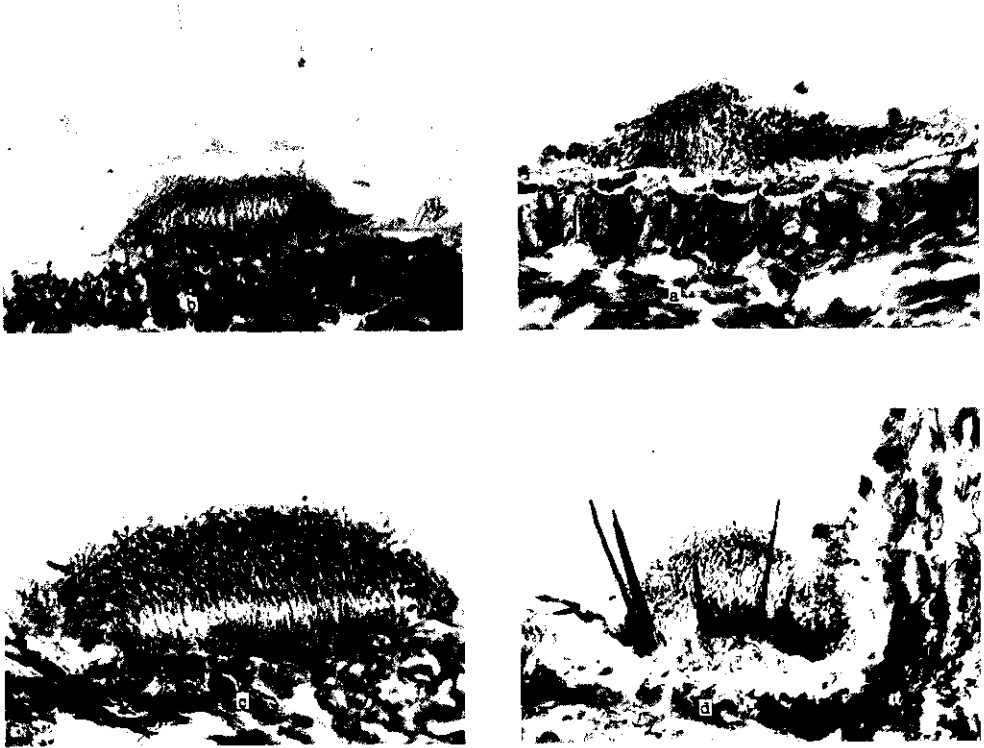


Fig. 2. Cross sections of inoculated detached leaves of “Anici” coffee cultivar at 10 days after inoculation with *C. coffeanum*

- a. Stromatic mycelial mat with conidia and conidiophores.
- b. Well developed acervulus over collapsed epidermal layer and discoloured palisade tissue.
- c. Well developed acervulus expressing hyaline conidiophores and unicellular conidia, ruptured host epiderms and partially disintegrated cortical tissues.
- d. Acervulus including conidia on conidiophores along dark seta, host tissues is completely collapsed and disintegrated (Holonecrosis).



Fig. 3. Cross section of inoculated detached leaves of "Anici" cultivar showing the development of acervuli on, both, upper and lower surfaces of the leaf

place and cells, in infected areas, shrivelled and disintegrated. Well developed acervuli became quite obvious in which long, stiff pointed, and dark setae were interspersed with the conidiophores (Fig. 2d). Although acervuli were more distinct on the upper surface of infected leaves they did, also, occur on the lower surface (Fig. 3).

Discussion

Morphological symptoms on leaves were more pronounced and severe on Anici when compared with those on the other tested coffee cultivars. The frequency of

acervuli on infected leaves was also highest on Anici. This might suggest that Anici could be considered the most susceptible among tested cultivars. This is in agreement with the findings of Kamara *et al.* (unpublished data). Breeding for anthracnose resistance or use of more tolerant coffee cultivars are of prime importance for disease control.

Symptoms developed faster on detached than on undetached leaves. This might be due to the fact that detached leaves become physiologically exhausted, when compared with undetached ones, and subsequently allow for comprehensive fungal growth and sporulation. Old and exhausted leaves of coffee seedlings would then be expected to become infected more easily and severely than young leaves.

Abrasion of leaves with carborundum just before inoculation diminished the severity of symptoms as expressed by decreased numbers of acervuli on inoculated leaf surfaces. This might suggest the release of inhibitory compound(s) from leaf tissue upon scratching. Our results support those of Firman [7] who found that wounded berries of some coffee cultivars were not as susceptible as would be expected if the cuticle was the only barrier to infection.

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مرض أنثراكنوز البن : تكشف أعراض المرض المورفولوجية والتشريحية

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ملخص البحث . أجريت دراسات مورفولوجية وتشريحية على تكشف أعراض مرض الأنثراكنوز على بادرات البن أو على الأوراق المنزوعة من ثلاثة أصناف بن هي : حمادي ، إيرباني ، وأنيسي . لوحظ ظهور أعراض النخر (الموت الموضعي) الجزئي والذي تبعه ظهور أعراض نخر كلي بعد سبعة أيام من إجراء العدوى تحت ظروف المعمل أو الصوبة الزجاجية . وعند دراسة قطاعات عرضية في أوراق مصابة ظهر تدهور في خلايا نسيج البشرة ثم تلاه تجمعاً لمواد داكنة اللون في خلايا البشرة وكذلك خلايا النسيج العمادي والنسيج الإسفنجي وذلك حول منطقة الإصابة . وبعد ذلك تكشف على نسيج البشرة وسائد هيفية تكون عليها العديد من الكويبات الجرثومية . ولقد اختلف عدد الكويبات الجرثومية باختلاف الصنف المختبر .

ولقد وجد أن صنف أنيسي هو أكثر الأصناف المختبرة قابلية للإصابة . كما لوحظ أيضاً أن عدد الكويبات الجرثومية كان أعلى عند عدوى أوراق النبات غير المخدوشة إذا ما قورنت بالأوراق المخدوشة بمسحوق الكاربورانوم وذلك في جميع الأصناف المختبرة .