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Evidence of cork barrier formation as a resistance mechanism to berry disease (Colletotrichum coffeanum) in arabica coffee

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Abstract

The histology of resistance to coffee berry disease (CBD) was studied in artificially inoculated berries and hypocotyls of 6-week old seedlings of a number of varieties of *Coffea arabica* L. In resistant varieties a phellogen was quickly formed some cell layers below the site of infection and progress of the fungal invasion was effectively blocked by a complete barrier of suberized cells. Such barriers were absent or incompletely developed in CBD susceptible varieties. A highly significant correlation ($r = 0.87$) was found between the frequencies of complete barrier formation in berries and in hypocotyls of young seedlings, while both were also highly correlated to observed mature plant resistance ($r \geq 0.93$). Resistance to CBD in arabica coffee may to an important extent be based on the formation of cork barriers. These cork barriers confine the pathogen to the small volume of tissue external to the barrier so that its growth is severely restricted. Such a resistance mechanism is likely to be stable (race-non-specific). The almost identical response to infection observed in berries and hypocotyls provides further evidence that plants with resistance to CBD can be reliably preselected by the hypocotyl inoculation test.

Additional keywords: Coffea arabica, coffee berry disease, coffee, cork barrier formation, hypocotyls.

Introduction

Coffee berry disease (CBD) is an anthracnose of the green and ripening berries and may cause very heavy crop losses in arabica coffee in Kenya if not controlled by intensive programmes of fungicide sprays (Griffiths et al., 1971). In the long term, CBD resistant cultivars are considered the only means of effective control. A breeding programme was started in 1971 and the development of a preselection test, by which resistance to CBD can be detected in very young coffee seedlings (Van der Vossen et al., 1976), greatly accelerated progress of introgressing CBD resistance from exotic varieties into local cultivars. Although resistance to CBD appears to be determined by only a few major genes (Van der Vossen and Walyaro, 1980), absence of differential pathogenicity of CBD iolates (Masaba and Van der Vossen, 1980; Van der Graaff, 1978) suggests that the resistance is race-non-specific.

Information on the mechanism of resistance to CBD operating in coffee berries is likely to have a direct bearing on the stability of this resistance. Nutman and

Roberts' (1960) contention that preformed chemical substances in the cuticle could play a role in CBD resistance was investigated by Steiner (1972) and Lampard and Carter (1973). They confirmed the presence of antifungal compounds in the cuticular wax layers of green berries of certain coffee varieties, which reduced in vitro germination of the pathogen, but they failed to establish a close relation between the concentration of such substances and the level of mature plant resistance.

Suggestions of a resistance mechanism to CBD based on the formation of a phellogen that produces cork layers and isolates the lesion from healthy tissue, were first made by R.T.A. Cook (1974, unpublished) and Gieskes (1976), following observa tions in hypocotyls of young coffee seedlings after inoculation with a conidiae suspension of the CBD pathogen. This has been further elaborated in the present study by histological work with hypocotyls and berries of a wide range of CBD resistant and susceptible varieties.

Materials and methods

Source of CBD inoculum. Green infected berries with active lesions provided inoculum for initial isolation. Inoculations were carried out using a conidial suspension (2×10^6 conidia/ml) freshly prepared from pure cultures of *Colletotrichum coffeanum* Noack *sensu* Hindorf on malt extract agar and with proved viability of the conidia in excess of 80% (Van der Vossen et al., 1976).

Varieties of arabica coffee included in the histological studies are listed in Tables 1 and 2. They cover the full range from high resistance to complete susceptibility.

Methods in inoculation. Hypocotyls, attached and detached berries were inoculated as described by Van der Vossen et al. (1976) except that for hypocotyls the conidial suspension was not sprayed but applied by fixing paper wicks, dipped into the conidial suspension, around the upper part of the hypocotyls just below the cotyledons.

Scoring of symptoms on hypocotyls. The lesions have been graded on a scale with a range 1-12 (Van der Vossen et al., 1976). Grade I indicates no visible lesions, then lesions appear as small brown superficial 'scabs' gradually enlargening to grade 4; grades 5-6 indicate the presence of small black lesions, which become deeper, larger in size, and/or greater in number and coalesce until grade 10 when the stem is completely girdled; grades 11 and 12 indicate that a large portion of the hypocotyl stem is black and shrivelled (seedling completely dead). Seedlings with infection grades 1-4 possess a high level of resistance; those with grades 5-6 have a medium level of resistance; and those with grades 7-12 are susceptible to CBD.

Microscopic observations. Hypocotyls were sampled randomly from the 4th to 21st day after inoculation; portions with lesions were cut out and sectioned by hand using a sharp razor blade. Similarly, berries with or without visible lesions were sampled at regular intervals after inoculation and the pericarp sectioned with a Lab-Line/Hooker plant microtome at a thickness of 12μm (Hooker, 1967). When not

Table 1. Varietal differences in the reaction of hypocotyls to infection with *C.coffeanum.*

Varieties	Mean grade in hypocotyl inoc. test	Total number of hypocotyls sectioned	% lesions with complete barrier 21 days after inoculation		
			total %	% lesions with A type barrier	% lesions with B type barrier
1. Rume Sudan	4.0	117	100	87	13
2. Guatemala	4.9	63	100	55	45
3. Boma Plateau	5.3	104	94	72	22
4. Hibrido de Timor	5.2	82	85	45	40
5. Padang	6.1	78	100	25	75
6. S 288	4.4	32	100	36	64
7. Marsabit	6.4	49	96	79	18
8. K 7	5.8	121	77	34	43
9. B.A. 36	5.2	83	60	60	0
10. Mundo Novo	11.9	100	56	11	44
11. Geisha 9	11.6	110	0	0	0
12. Erecta	10.8	87	43	14	29
13. Caturra	11.8	103	9	7	2
14. H 66	10.1	110	0	0	0
15. N 39	11.9	97	21	0	21
16. SL 28	11.5	186	19	6	13

Tabel 1. De reactie van verschillende rassen na infectie van hypocotyle stengelleden van koffiekiemplanten met C.coffeanum.

sectioned immediately, both types of materials were preserved in a formaldehyde – acetic acid – ethanol (5 + 7 + 90, v/v/v) fixative solution for at least 48 h. Selected sections were subsequently stained in safranin-aniline blue using a slightly modified schedule of Johansen (1940). All sections were examined under a light microscope.

Results

Histology of lesions produced on coffee hypocotyls. A wide range of symptoms occurred on hypocotyls after artificial inoculation with the CBD pathogen. A histological study revealed two main types of host cell reactions where penetration by the fungus had occurred. In the first type of reaction, no histological changes were observed in the host cells undergoing invasion. These cells, however, took up the safranin stain indicating a change in the cell wall probably due to enzyme action of the fungus. The adjacent healthy cells showed no change. Affected cells (those behind the invading hyphae) had collapsed and were necrotic (Fig. 1a). An intermediate type of reaction, where 1-4 cells in the healthy cortex below the invading hyphae were dividing but did not seem to have an effect on the invading hyphae, was also observed (Fig. 1b). The above type of reaction occurred under all

Table 2. Varietal differences in the reaction of berries to infection with *C.coffeanum.*

Varieties	Corrected max. field score, % infection	Total number of berries inoculated	% berries infected	% with active lesions	% with visible scab lesions	% lesions with complete barrier (mainly type A)
1. Rume Sudan	2	457	13	12	1	66
2. Guatemala	4	426	28	21	6	52
3. Boma Plateau	12	384	14	11	4	47
4. Hibrido de Timor	20	196	22	15	8	43
5. Padang	15	813	12	11	1	38
6. S 288	27	308	15	11	4	34
7. Marsabit	30	437	60	55	6	33
8. K 7	30	374	50	37	13	17
9. B.A. 36	55	90	22	17	5	17
10. Mundo Novo	70	384	23	14	9	9
11. Geisha 9	76	353	23	19	4	0
12. Erecta	58	609	41	38	4	0
13. Caturra	93	78	30	27	3	0
14. H 66	78	478	60	56	4	0
15. N 39	73	213	68	67	1	0
16. SL 28	83	435	89	82	7	0

[1] Active lesions on resistant varieties were consistently smaller in size than those on susceptible varieties.
[2] Corrected maximum field score: see Van der Vossen et al., 1976.

Tabel 2. De reactie van verschillende rassen na infectie van koffiebessen met C.coffeanum.

the deep and black lesions, with infection grade 7 or higher, which correspond to the susceptible classes of the hypocotyl inoculation test.

In the second type of reaction , a lateral meristem (phellogen) was formed by radial and tangential divisions of the cortical parenchyma internal to the infected cells within 2-3 days after inoculation. The phellogen cells were rectangular and included one to several tangential rows. Cork cells (phellem) differentiated from the phellogen externally. These were uniform in size and radially elongated with tangential walls. The phellogen and phellem cell layers served as a barrier between the infected tissue and the healthy tissue. There were two ways in which this barrier was produced. In one way (type A barrier) the barrier tissue developed near the surface of the hypocotyl pushing the sealed-off tissue outward, and resulting in a distinct bulbing of the hypocotyl at the point of infection. The tissue outside the barrier remained attached as an irrigularly shaped mass, often called 'scab'. This either persisted or disappeared quite quickly with weathering (Fig. 2a, b, c). The cork barrier was also observed to be produced in a depression (type B barrier) at some distance below the hypocotyl surface, where it lined a cup-like cavity filled with a mass of dead cells intermingled with hyphae. It effectively isolated the infected tissue from the healthy tissue (Fig. 2d, e). Where a complete layer of cork tissue had formed, no trace of fungal hyphae was detected in the healthy tissue lying below either barrier. Complete cork barriers developed under all scabs, brownish superficial lesions of grades 2-4, and most of the small but deep black lesions of grades 5-6 of the hypocotyl inoculation test.

Individual lesions were comparatively but frequently coalesced to form a large corky patch. Occasionally the small lesions did not coalesce and under such lesions the barrier appeared as a waving ribbon under the infections (Fig. 2f).

Histology of lesions produced in coffee berries. In the present study, three types of disease symptoms were observed on the surfaces of attached berries inoculated with *C. coffeanum.* The first type of 'active' lesions correspnds well with the descriptions given by several earlier workers for the susceptible expression of host reaction (McDonald, 1926; Rayner, 1952; Bock, 1956; Nutman, 1970). Microscopic examination of this type of lesions indicated no mechanical barrier of any kind formed by the coffee berry as a reaction by the pathogen. The fungus spread freely through the host when conditions remained favourable (Fig. 3).

The second type of visible symptoms appeared as slightly sunken lesions, of limited size, light brown and with numerous minute black, erumpent fruiting bodies scattered over the surface. This type of lesions has been described as 'scab' sesions by the previous workers, most of whom stated that 'scab' lesions are active lesions

Fig. 1. Cross-sections of hypocotyls of CBD susceptible varieties after inoculation.
1a: An active lesion on the susceptible variety SL28, four days after inoculation; whilst invaded cells became necrotic and collapsed, the cells surrounding the lesion remained histologically unchanged. × 66.
1b: Camera-lucida drawing of an active lesion; the invaded cells are necrotic (n); two cells (d) were dividing under the lesion, but they did not seem to develop into a phellogen to limit the fungal spread; variety SL28, seven days after inoculation. × 66.

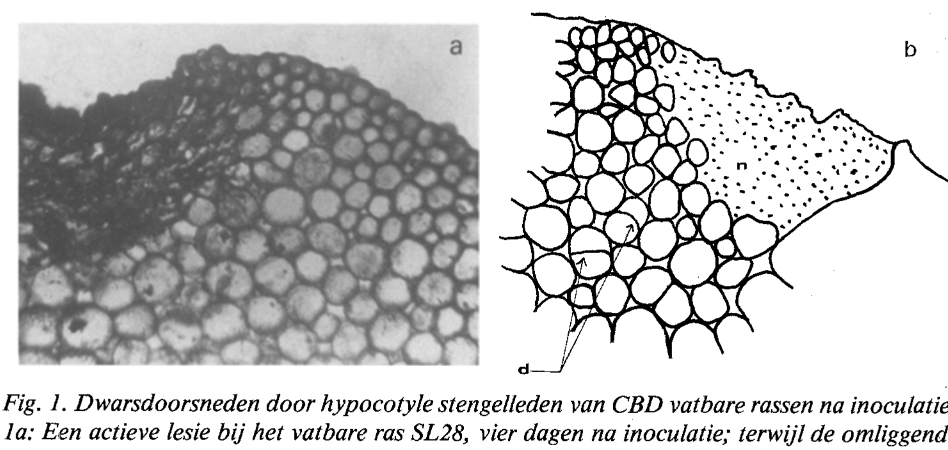


Fig. 1. Dwarsdoorsneden door hypocotyle stengelleden van CBD vatbare rassen na inoculatie.
1a: Een actieve lesie bij het vatbare ras SL28, vier dagen na inoculatie; terwijl de omliggende cellen waar de schimmel is binnengedrongen doodgingen, bleven de omliggende cellen onaangetast. × 66.
1b: Camera lucida tekening van een actieve lesie; de aangetaste cellen zijn necrotisch (n); twee cellen (d) vertoonden deling beneden de lesie, maar niets wijst er op dat dit zal ontwikkelen tot een fellogeen, dat de verdere verspreiding van de schimmel zal kunnen tegengaan. × 66.

Fig. 2. Reaction to CBD infection in hypocotyls of resistant varieties.
2a: Type A: two individual, small and superficial scabs occurred close to each other; both the fungus and the infected tissue were pushed away from the stem surface by effective cork barriers; a highly resistant variety Rume Sudan, seven days after inoculation. × 16.
2b: Type A barrier in a medium resistant variety K7, 21 days after inoculation. × 66.
2c: Type A: the necrotic and infected tissue is being sloughed off from the hypocotyl surface of another medium resistant variety Padang, 21 days after inoculation. × 66.
2d: Type B: cork barrier has been produced in a depression to isolate the infected tissue; medium resistant variety Marsabit, 21 days after inoculation. × 66.

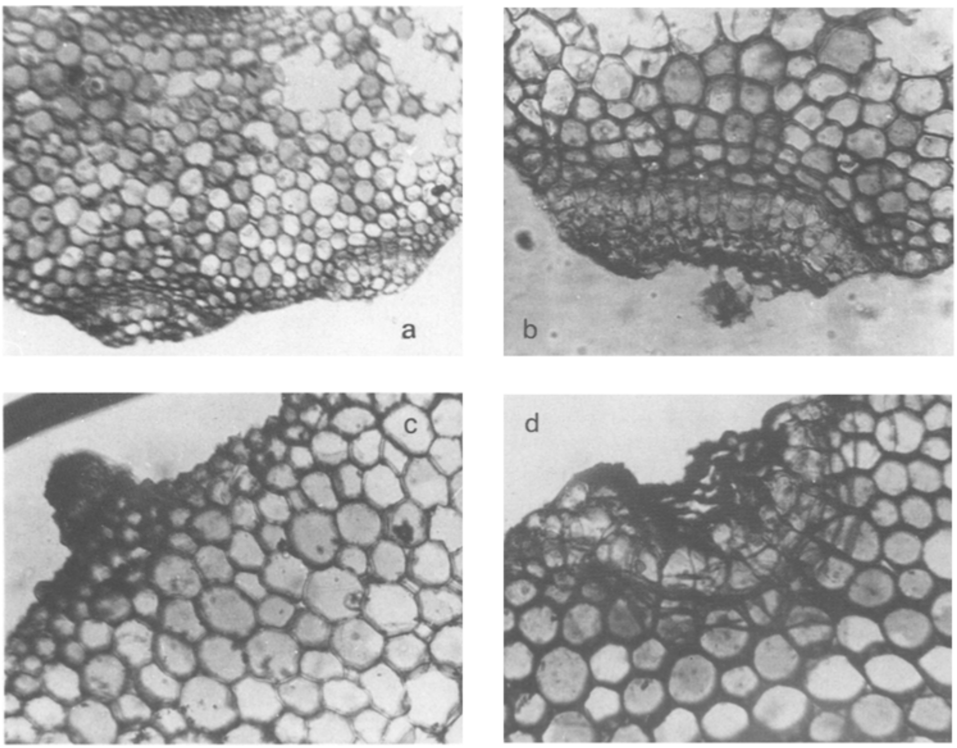
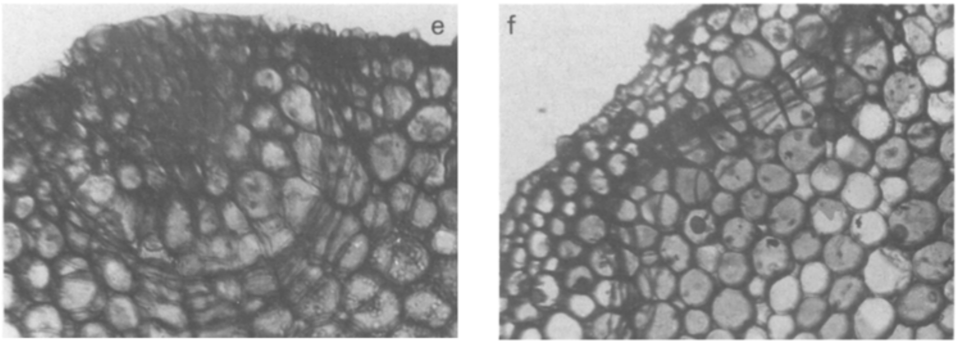


Fig. 2. De reactie in hypocotyle stengelleden van resistente rassen tegen infectie door CBD.
2a: A type: twee afzonderlijke, kleine en oppervlakkige 'scab' lesies kwamen dicht bij elkaar voor; de schimmel werd samen met het aangetaste weefsel afgezonderd en weggedrukt door wondkurk bij het zeer resistente ras Rume Sudan, zeven dagen na inoculatie. × 16.
2b: A type resistentie reactie bij het middelmatig resistente ras K7, 21 dagen na inoculatie. × 66.
2c: A type reactie: het afstervende geïnfecteerde weefsel wordt afgestoten van het oppervlak van het hypocotyle stengellid bij een ander middelmatig resistent ras Padang, 21 dagen na inoculatie. × 66.
2d: B type reactie: wondkurk is gevormd dieper weg in het waardplantweefsel van het middelmatig resistente ras Marsabit, 21 dagen na inoculatie. × 66.

2e: Another type B barrier developed on the variety Rume Sudan, 21 days after inoculation; some hyphal strands can be seen in cells lining the outer portion of the cork barrier. ×66.
2f: Type B cork barrier developed as a waving ribbon under several coalescing lesions on resistant variety Guatemala, 21 days after inoculation. ×66.



2e: Nog een type B wondkurk ontwikkeld bij het ras Rume Sudan, 21 dagen na inoculatie; de hyfen van de schimmel kunnen worden waargenomen tot vlak bij de buitenste cellen van het wondkruk. × 66.
2f: B type wondkurk ontwikkeld als een golvende lijn onder verschillende samensmeltende lesies bij het resistente ras Guatemala, 21 dagen na inoculatie. × 66.

which have been halted at some stage in their development by adverse weather conditions particularly low humidity. Microscopic examination showed that in these lesions the fungus, after penetration, was restricted to 1-5 cell layers below the epidermis. No barrier was observed to develop under the lesions, though the fungal hyphae appeared to be arrested. Affected cells collapsed and were necrotic and subepidermal stomata ruptured the cuticle at several points giving rise to the minute black fruiting bodies. The fungus as unable, however, to sporulate freely on the surface of the lesion even under favourable weather conditions. Occasionally, very slight cell division (1-4 cells) occurred under these lesions but this did not result in the formation of a cambium. These lesions were observed on berries of all coffee varieties, irrespective of the level of resistance (see also percentage scab in Table 2).

The third type of symptoms can be described as generally superficial but occasionally slightly sunken lesions, light brown with a few black fruiting bodies scattered on the surface. The margins of these lesions characteristically assumed a water soaked appearance. Under these lesions cork barriers, similar in all respects to those formed on hypocotyls, developed either pushing out the infected tissue or separating the infected tissue from the healthy tissue (Fig. 4a, b, c).

Because most of the attached berries belonging to resistant coffee varieties appeared free of any visible symptoms, an attempt was made to section a few symptomless berries from various coffee varieties. It was observed that quite a number of berries had been penetrated to a limited extent. Underneath these tiny microscopic lesions, cork barriers had also developed pushing up the infected tissue (Fig. 4d).

Fig. 3. Cross-sections through the pericarp of attached berries of the susceptible variety N39, 4 days after inoculation; an active lesion with no histological changes in the surrounding cells. × 31.

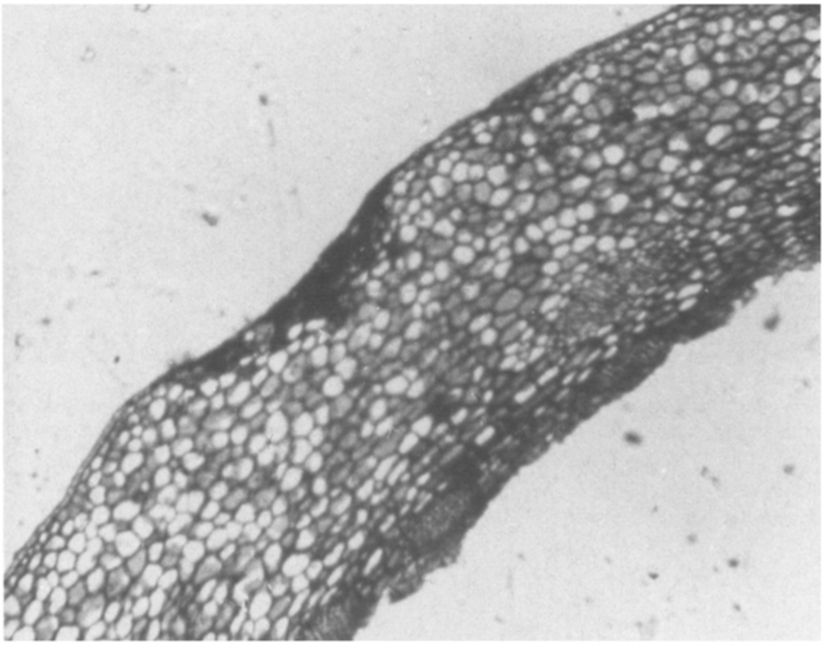


Fig. 3. Dwarsdoorsnede door het vruchtvlees van bessen van het vatbare ras N39, vier dagen na inoculatie in het veld; een lesie waarbij zich in het omliggende weefsel geen veranderingen hebben voorgedaan. × 31.

In detached berries no cork barrier development could be detected after inoculation in any of the varieties and detached berries were therefore excluded from the present study.

Barrier formation in relation to CBD resistance in coffee plants. A summary of the results showing varietal differences in the development of complete cork barriers on hypocotyls inoculated with CBD and the hypocotyl mean grades of infection is presented in Table 1. A similar summary of results from inoculated attached berries and their known field resistance is given in Table 2. Correlation coefficients between percentage of complete cork barriers in the hypocotyls and in berries, mean grades of infection in the hypocotyl inoculation test and field scores for CBD infection for 16 coffee variesties are presented in Table 3. All correlation coefficients were very high and significant ($r \geq 0.80$). The relation between barrier formation in hypocotyls and berries is also presented diagrammatically in Figure 5. The numbers in this figure correspond with the varieties in Tables 1 and 2 for easy reference.

Fig. 4. Resistance reaction to CBD infection in berries of resistant varieties.
4a: Type A: part of a wide cork barrier occurring under a visible, superficial scab lesion on variety K7, 14 days after inoculation. × 66.
4b: Type A barrier on resistant variety Guatemala, nine days after inoculation (compare with fig. 2b). × 66.
4c: TypeB barrier on variety Hibrido de Timor, six days after inoculation (compare with fig. 2d). × 66.
4d: Type A barrier produced under a microscopic lesion on the variety Rume Sudan, six days after inoculation. × 66.

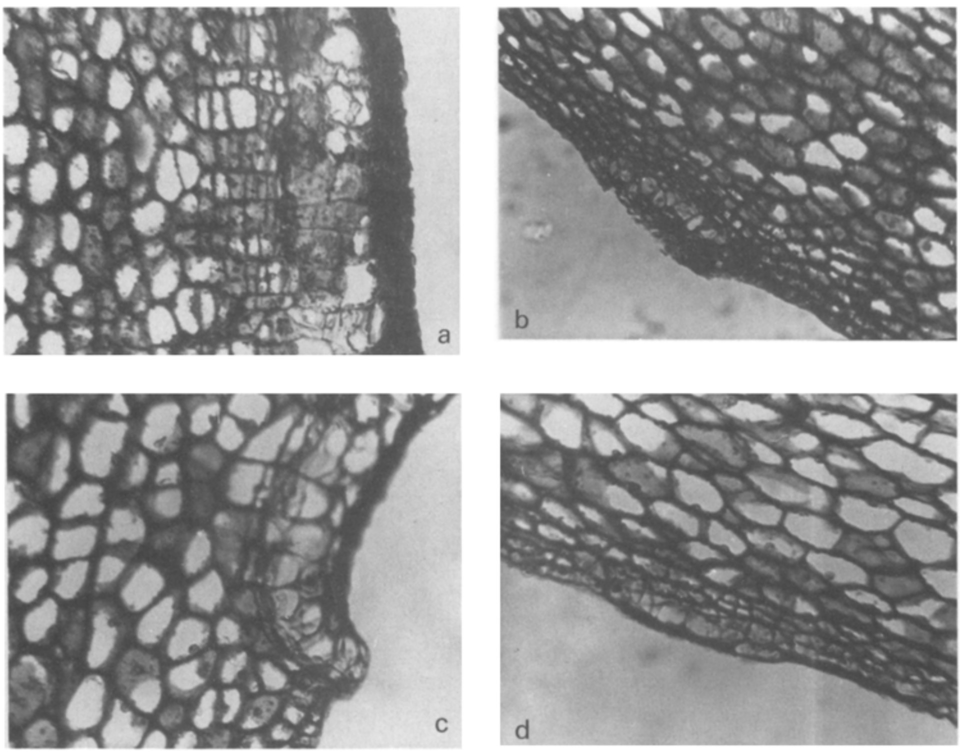


Fig. 4. Reacties van resistentie tegen binnendringen van de CBD pathogeen in bessen van resistente rassen.
4a: A type: een deel van de brede kurklaag onder een vrij grote maar oppervlakkige 'scab' lesie bij het ras K7, 14 dagen na inoculatie. × 66.
4b: A type reactie bij het resistente ras Guatemala, negen dagen na inoculatie (vergelijk met fig. 2b). × 66.
4c: B type reactie bij het ras Hibrido de Timor, 6 dagen na inoculatie (vergelijk met fig. 2d). × 66.
4d: A type wondkurk gevormd onder een miniscuul kleine lesie bij het ras Rume Sudan, zes dagen na inoculatie. × 66.

Table 3. Coefficients of correlation between barrier formation in hypocotyls and berries as well as the resistance to CBD in the preselection test and in the field of 16 varieties of *C.arabica.*

1	2	3	4	5
1. % lesions with complete barrier (hypocotyls)	0.81 (0.84)	0.87 (0.86)	−0.88 (−0.85)	−0.94 (−0.90)
2. % lesions with A type barrier (hypocotyls)		0.84 (0.83)	−0.85 (−0.86)	−0.80 (−0.81)
3. % lesions with complete barrier (berries)			−0.85 (−0.87)	−0.93 (−0.92)
4. Mean grades of infection (hypocotyls)				0.90 (0.89)
5. Corrected maximum field score for CBD infection				

N = 16.
In brackets: r after angular transformation of data for 1, 2, 3 and 5.
$r \geq 0.74$. Significant at $P \leq 0.001$

Tabel 3. Correlaties tussen de mate van vorming van wondkurk in hypocotyle stengelleden en in bessen en resistentie tegen CBD in de voorselectietoets en die in het veld bij 16 rassen van C.arabica.

Discussion

The type of host tissue that can be readily infected by the CBD pathogen is very similar in berries and hypocotyl stems. It consists of the relatively thin-walled parenchymatic cells which make up the pericarp of berries at the soft green stage (6-14 weeks after flowering) or the cortex of the upper half of the hypocotyls of very young seedlings (5-7 weeks old). This may explain why the observed response to CBD infection by resistant coffee varieties was almost identical in berries and hypoctyls. Older berries, at the hard green stage (Mulinge, 1970) or the older (lower) part of the hypocotyls are normally not infected even when the coffee variety is very susceptible to CBD.

Penetration through the epidermal cells and initial progress of the hyphae in the underlying tissue appeared to be equally fast in berries an hypocotyls of susceptible and resistant varieties. The presence of hyphae close to the phelleem formed in resistant varieties suggests that these cork barriers are the main mechanism in coffee by which further invasion of the CBD parasite is blocked and not a secondary wound reaction after the fungus had been killed by some fungitoxic substance, as appears for instance to be the case with resistance to anthracnose in the watermelon (Anderson and Walker, 1962). Numerous examples of cork barrier formation in host tissue in response to infection by a pathogen have been cited in literature (Akai,

Fig. 5. Relation between complete barrier formation (angular transformation of percentages) in berries and that in hypocotyls of the 16 arabica coffee varieties studied (see also Tables 1 and 2).

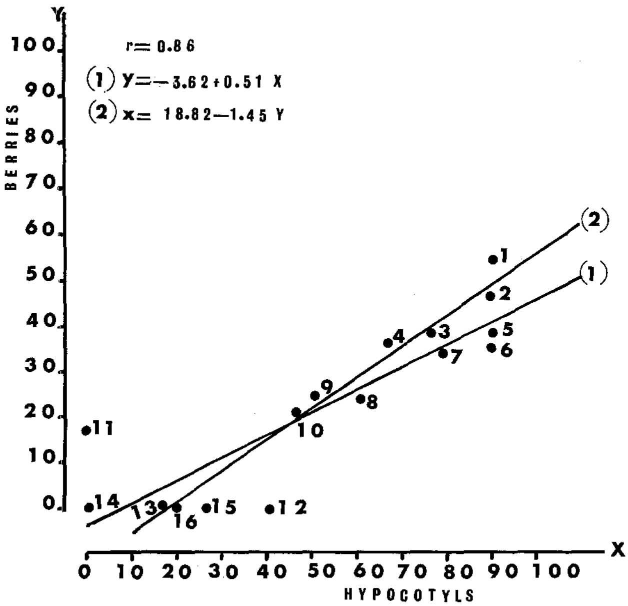


Fig. 5. De relatie tussen de mate van complete kurkvorming als barrière tegen CBD infectie (percentage omgezet in hoektransformatie) bij bessen en hypocotyle stengelleden van de 16 bestudeerde rassen van arabica koffie (zie ook Tabellen 1 en 2).

1959; Wood, 1967). In particular the resistance reaction to root rot in tobacco (Conant, 1927) and to scab in citrus (Cunningham, 1928) compare very well with that found in coffee after CBD infection.

This resistance mechanism to CBD depends apparently on actively metabolizing plant tissue. Detached berries quickly lost their ability to respond to CBD infection and no cork barriers could be detected in such berries after artificial inoculation in the laboratory even of the most resistant varieties such as Rume Sudan. Detached berries of resistant varieties were often actively infected after inoculation. Van der Vossen et al. (1976) found also little or no correlation between the results of the detached berry inoculation test and mature plant resistance.

The host-pathogen interaction is also much influenced by environmental factors, especially humidity and temperature. High humidity is obviously required for conidial germination and successful infection (Waller, 1971). Low temperatures reduce the ability of the host to respond adequately to invasion by the CBD pathogen, which is relatively more tolerant to low temperatures. When, for instance, temperatures in the preselection test on hypocotyl stems fell below the optimum range of 18-20 °C during the two-week incubation period, active infection lesions appeared on inherently CBD-resistant varieties. At high temperatures (> 25 °C) even the most susceptible varieties showed low infection, presumably because temperatures were unfavourable for the pathogen and the host had been able to respond timely by the formation of barriers.

Environmental conditions during artificial inoculation of attached berries in the field were naturally much more variable and a high humidity could be maintained only during the first four days after inoculation by an opaque-plastic bag surrounding the berries. Leaving the bags for longer periods resulted in poor infection and sometimes scorching of the berries due to overheating. As a result, symptom development was less consistent than in the hypocotyl test; active lesions were found even on berries of the most resistant varieties, although only at a low frequency and very small in size (Table 2). Most of the lesions occurring on resistant varieties were sealed off by a complete cork barrier (Fig. 4a). Visible scab lesions (those without cork barriers) were found at a low frequency on berries of resistant as well as susceptible varieties (Table 2). Such scab lesions appear to represent initially successful infections which are arrested because of unfavourable invironmental conditions for the pathogen, higher temperatures and low humidity.

Whereas considerable penetration of resistant host tissue occurred on hypocotyls, the degree of penetration by the fungus on berries was usually limited so that in general only microscopic lesions were being observed (mostly with type A cork barriers). This may be partly due to uniformly optimum conditions for infection in the hypocotyl inoculation test. It was also difficult to locate microscopic lesions on the wide berry surface for sectioning since the inoculated sites were not as clearly marked as in hypocotyls. Hence the percentage of lesions with barrier formation was in berries (Table 2) lower than in hypocotyls. The differences in location within the coffee tissue of A and B barriers seem to result in two characteristic types of scab lesion, either the tiny brown superficial scabs or the slightly sunken scabs. Both types of barrier were observed to be produced by all CBD resistant coffee varieties and to effectively arrest fungal spread.

The minor damage caused by the inital infection and accompanying resistance reaction of host tissue (the pericarp of the berry) has little consequence, since the economic product is the coffee bean which is left untouched. Only when the invasion by the CBD pathogen is unchecked, as in susceptible coffee varieties, will the developing bean be destroyed together with the other berry tissue.

The level of resistance to CBD of a variety is characterized by the frequency of the formation of complete cork barriers in berries as well as in hypocotyls (Tables 1 and 2). This is borne out by the very high and significant correlation coefficients between percentage complete barriers in hypocotyls and mean grade of infection in the preselection test ($r = 0.88$) and resistance of the mature plant in the field ($r = 0.94$) and also between percentage complete barrier formation in berries and resistance in the field ($r = 0.93$) for the 16 coffee varieties studied (Table 3).

The observation that the resistance to CBD operating in the juvenile (hypocotyls) and mature (berries) stages of coffee plants correlates well with the formation of effective cork barriers ($r = 0.87$ for percentage complete barrier formation in hypocotyls and berries, see also Fig. 5) is a further indication of the reliability of the hypocotyl inoculation test applied in the breeding programme at Ruiru to preselect for CBD resistance (Van der Vossen et al., 1976).

Although the presence of higher concentrations of fungistatic substances in resistant varieties, as suggested by Steiner (1972) and Lampard and Carter (1973), could also play a role in slowing down infection, the histological evidence produced by the present study indicates that the main resistance mechanism in response to CBD infection is based on the formation of cork barriers which effectively seal off the invading pathogen from healthy host tissue. Such a resistance mechanism is usually non-specific and together with the apparent absence of differential pathogenicity of CBD isolates (Masaba and Van der Vossen, 1980) there is good reason to believe that the CBD resistance is of a stable nature.

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Samenvatting

Aanwijzingen dat bij arabica koffie de resistentie tegen koffiebessenziekte (Colletotrichum coffeanum) berust op de vorming van wondkurk

Het mechanisme van de resistentie tegen de koffiebessenziekte werd nader onderzocht bij een aantal rassen van *Coffea arabica* aan de hand van anatomische studies van groene bessen en van hypocotyle stengelleden van 6-weken oude kiemplanten, die vooraf waren geïnoculeerd. Bij resistente rassen bleek steeds enkele cellagen beneden het punt van infectie een wondkurk te ontstaan, dat verdere groei van de schimmel in het waardplantweefsel onmogelijk maakte. Dit kurk werd niet of zeer onvolledig gevormd bij vatbare planten. Er bleek een zeer hoge correlatie ($r = 0.87$) te bestaan tussen de mate van voorkomen van wondkurk na infectie bij bessen en bij hypocotyle stengelleden. De mate van vorming van wondkurk was bovendien zeer goed gecorreleerd met de mate van de in het veld waargenomen resistentie tegen de koffiebessenziekte ($r \geq 0.93$). Dit doet vermoeden dat resistentie tegen de koffiebessenziekte in hoge mate wordt bepaald door snelle vorming van wondkurk na infectie. Hierdoor wordt voorkomen dat nog meer gezond waardplantweefsel wordt aangetast. Ziekteresistentie gebaseerd op een dergelijk mechanisme zal zeer waarschijnlijk van stabiele aard zijn (niet fysio specifiek). Het nagenoeg identieke reactiepatroon bij bessen en hypocotyle stengelleden biedt een verdere ondersteuning aan de betrouwbaarheid van de voorselectietoets, zoals die wordt toegepast in het koffieveredelingsprogramma ter verkrijging van nieuwe rassen die resistent zijn tegen de koffiebessenziekte.

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