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Coffee berry disease in Kenya. II. The role of Glomerella cingulata in the Colletotrichum population, colonizing the bark of Coffea arabica

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Abstract

On the maturing bark of cut branches of *Coffea arabica* previously sprayed with copper fungicides perithecia of *Glomerella cingulata* were easily found after two to ten days of incubation. Without fungicides the number of perithecia was decidedly lower. On prunings left on the ground under the coffee trees for 6 to 24 weeks the perithecia could also be found, but the numbers declined rapidly with time.

Perithecia of *G. cingulata* could forcibly discharge ascospores under laboratory conditions. Monospore cultures obtained by catching ascospores on agar, invariably belonged to three *Colletotrichum* types. It was rarely possible to isolate a *Colletotrichum* able to infect green coffee berries. Growth-rate, colour of the mycelium, number of conidia produced *in vitro* and infectivity on green coffee berries, however, differed substantially from *C. coffeanum*, the cause of coffee berry disease.

In Kenya no evidence has been obtained that ascospores from perithecia on bark could infect wounded or unwounded green coffee berries. Neither has any infection been obtained with ascospores from perithecia grown *in vitro*. Possible explanations for the difference with previous findings are offered. Based on the data presented in this paper, it is concluded that *G. cingulata* is not likely to play a role in the epidemiology of the coffee berry disease in Kenya.

Introduction

Macdonald (1926) and Small (1926) described the incidence of *Glomerella cingulata* (Stonem.) Spaud. & V. Schrenk in relation to certain saprophytic isolates of *Colletotrichum coffeanum* Noack obtained from *Coffea arabica* L. in East Africa. In West Africa, Meiffren (1957) and Boisson (1960) published data on the occurrence of *G. cingulata* also on Arabica coffee. No information on the incidence of *G. cingulata* under field conditions was available from East Africa until Nutman and Roberts reported at the First Coffee Specialist Conference at Nairobi, Kenya in 1966 that they had found perithecia of this ascomycete on cut branches of coffee. At that time it was still uncertain which role *G. cingulata* played in the epidemiology of the coffee berry disease (CBD), the most serious coffee problem in East Africa, caused by a parasitic *Colletotrichum* species, now specifically named *C. coffeanum* Noack (Hindorf, 1970).

Hocking *et al.* (1967) reported on findings in Tanzania with *G. cingulata*, while Nutman and Roberts (1969) provided information on the effects of some fungicides on *G. cingulata* in relation to the control of CBD. No further details were, however,

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available on the role of *G. cingulata* in the *Colletotrichum* population colonizing, coffee bark.

This paper reports on the following aspects of *G. cingulata*:

a. the occurrence on sprayed and unsprayed coffee trees,
b. the discharge and the distance of discharge of ascospores;
c. the various forms of *Colletotrichum*, which could be obtained after passing through the *G. cingulata* stage;
d. the pathogenicity of the *Colletotrichum* isolates;
e. the pathogenicity of the ascospores of *G. cingulata*.

Materials and methods

Unsprayed branches from a farm near Nairobi, at 1800 m, or those sprayed with copper from the National Agriculture Laboratories, Nairobi, at 1700 m of 'S.L. 34', 'French Mission', 'Harar' or 'Mocha' varieties of *C. arabica* were cut off; the distal portion of each branch was discarded, the cut being made one internode above the one showing obvious signs of bark maturation. The basal portions were also discarded, leaving an eight-internode long twig (Nutman and Roberts, 1961, 1969). These were divided serially in screw-topped jars. The first bottle contained the internodes $1+2$, the second $3+4$, the third $5+6$, and the fourth $7+8$. The internodes were washed either with water containing 10 ppm 'Teepol' and afterwards washed in three changes of water or with 0.1% $HgCl_2$ for three minutes and afterwards rinsed in three changes of water.

Prunings left on the ground under coffee trees for periods ranging from 6 to 24 weeks were also collected and treated in almost the same way, although it was very difficult to distinguish any previous signs of the progress of bark maturation on the then moribund tissue. Therefore the three distal internodes were usually discarded and the remaining part of the branch divided serially as described above.

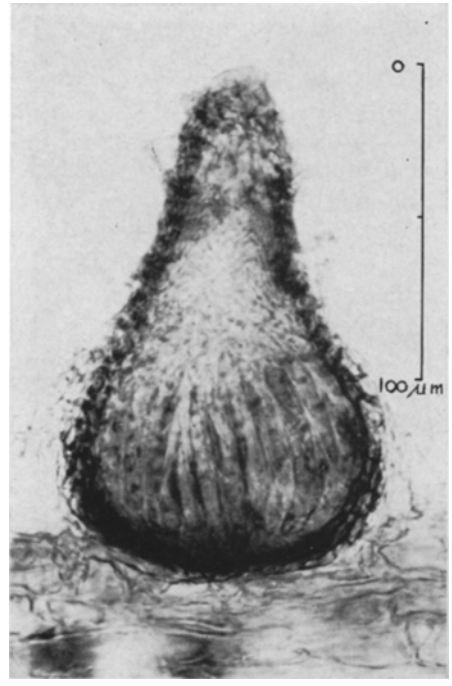
The identification of perithecia of *G. cingulata* was relatively easy as the translucent rostra of the ripe perithecia were typical of this fungus (Fig. 1). None of the other fungi colonizing the bark produced fruiting bodies similar to those of *G. cingulata*.

The discharge of ascospores from perithecia on the bark of cut branches was observed by putting small pieces of the bark material, on which perithecia could be found after two to ten days, facing upwards in the lids of inverted Petri dishes. Vertically discharged ascospores could be found on the agar inverted above the bark material. The horizontal discharge was observed by keeping the Petri dishes with the bark stuck to the lids stored sideways. The distance between bark material and agar could be changed by using different amounts of agar in the Petri dishes. During all experiments the temperatures varied between 22°–24°C.

When the ascospores were found on the agar above the bark material, mono-spore isolations were made under the low-power magnification of the microscope by cutting with a fine, sterile needle small blocks of agar containing one ascospore and transferring these blocks to malt agar plates. The isolates grown from these transfers were after ten to fifteen days classified according to Hindorf's system (1970): 1. *C. coffeanum* (Gibbs, 1969: *C. coffeanum* '*var. virulans*'), 2. *C. acutatum* Simmonds (Gibbs, 1969: *ccp*). 3. *C. gloeosporioides* Penzy (Gibbs, 1969: *ccm*), 4. *C. gloeosporioides*, 5. *C. gloeosporioides* (Gibbs, 1969: *cca*) and 6. *G. cingulata*. This classification and nomen-

Fig. 1. Section through a perithecium of *G. cingulata*, growing on the bark of *C. arabica*.

Fig. 1. Doorsnede van een perithecium van G. cingulata op de bast van C. arabica.



clature have been used throughout this paper. In case of cultural resemblance with *C. coffeanum*, infection tests on green coffee berries (cv. 'S.L. 34') were carried out to establish the pathogenicity of the isolate (Bock, 1956).

To establish the pathogenicity of ascospores, mature perithecia on bark, previously treated for three minutes with 0.1 % $HgCl_2$ and rinsed with sterile water were selected under the binocular microscope. The contents of the fruiting bodies, showing clearly the translucent rostra of the ripe *C. cingulata*-perithecia, were transferred with a fine needle to a bottle with water. After transferring the contents of 100–150 perithecia in this way, the suspension was centrifuged. The supernatant water was then decanted, so that a pellet of ascospores and asci remained in the centrifuge tube. This pellet was suspended in one or two ml water and the concentration assessed on a haemocytometer. Green berries were then inoculated with this suspension; the berries were divided into wounded (needle-prick after inoculation in the centre of the inoculation-droplet) and unwounded berries. Ripe perithecia of *G. cingulata*, grown *in vitro*, were treated in the same way. For all these tests the berries of the CBD-susceptible 'S.L.-34' variety were used, while also in all tests concurrently a batch of berries was inoculated with a fresh conidial suspension of *C. coffeanum* of the same density of inoculum as the ascospore suspension.

Tests were also carried out to assess the effect of sterile tap-water on the germination of ascospores. The Petri dishes were partly filled with sterile tap-water. Pieces of coffee bark with ripe perithecia were stuck to the lids of those dishes. Discharged ascospores were observed floating in the water.

Results

After four to seven days many ripe perithecia were found on the maturing bark of cut branches of coffee, previously sprayed with copper fungicides. Sometimes mature perithecia occurred even after two to three days incubation, only the numbers were much lower than after four to seven days. It was also possible to find new ripe perithecia after eight, nine or ten days. Then numbers of maturing fruiting bodies decreased rapidly. The number of perithecia on coffee branches, which had never been sprayed before with any fungicide was 60–90% lower than on sprayed branches. It was still possible, however, to find fruiting bodies of G. *cingulata* on unsprayed branches. A few perithecia were observed on prunings left for 6 to 24 weeks, but it became increasingly difficult to detect perithecia in the course of this period because of the growth of secondary fungi.

Selected at the proper time, the perithecia in the inverted Petri dishes, started to discharge ascospores after approximately sixteen hours at 22°–24°C. About 72 hours after the bark material had been put in the Petri dishes, discharge was no longer observed. Discharge of ascospores also occurred after surface sterilization, although the numbers of spores caught were 20–40% lower than in the case of bark material only washed with water. The ascospores caught on the agar above the bark material were clearly distinguisable (Fig. 2). When water agar was used the ascospores very often produced not only a germination tube and an appressorium. but also a conidiophore bearing a *Colletotrichum* conidium (Fig. 3). This was also observed when the ascospores germinated in sterile tap-water.

Observations were made on the distance covered by discharged ascospores both vertically and horizontally. A classification was made on how this discharge occurred: the number of spores per discharged projectile in relation to the distance covered (Fig. 4). The data presented in Fig. 4 apply to the vertical discharge range from 0.1–1.2 cm. The horizontal discharge pattern is very similar to that of vertical discharge and the data are therefore not presented in Fig. 4. Projectiles containing four to eight

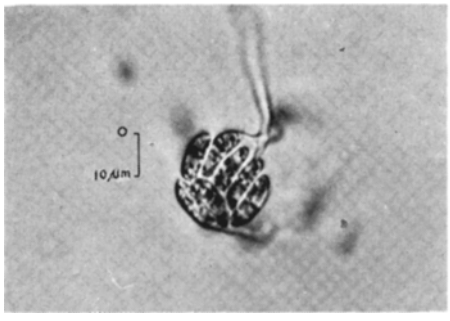


Fig. 2. A group of ascospores of *G. cingulata* caught on agar as one projectile. Germination tubes visible in the agar.

Fig. 2. Een groep van ascosporen van G. cingulata gevangen op agar als één projectiel. Kiembuizen zichtbaar in de agar.

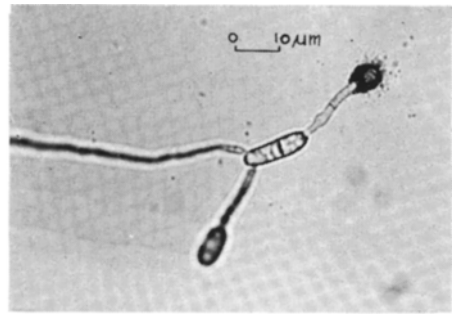
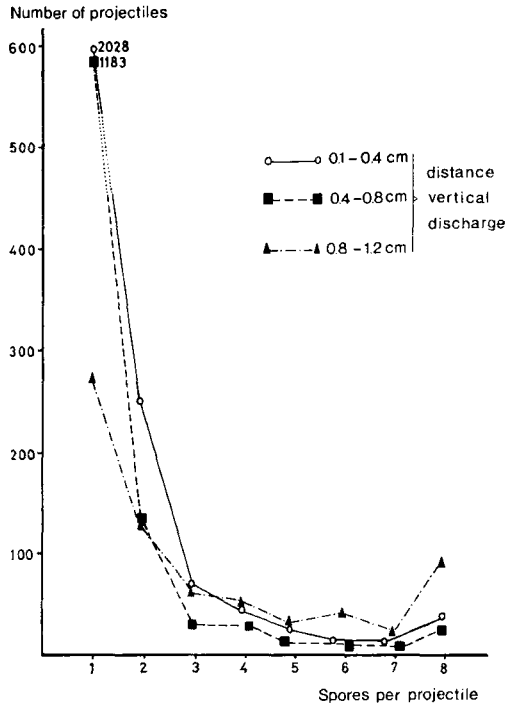


Fig. 3. Single ascospore of *G. cingulata* caught on agar. Germination tube, appressorium, and conidiophore bearing conidium are clearly distinguishable.

Fig. 3. Enkele ascosporen van G. cingulata gevangen op agar. Kiembuis, appressorium en conidiophoor met een conidium zijn duidelijk zichtbaar.

Fig. 4. Diagram of vertically discharged ascospores of *G. cingulata* in discharge distance and number of spores per discharged projectile.

Fig. 4. Grafiek van verticaal uitgeschoten ascosporen van G. cingulata in relatie tot afstand van uitschieten en het aantal sporen per projectiel.



spores were occasionally found at distances of 1.5 cm at most for the vertical discharge and 2.0 cm for the horizontal discharge. These observations are also not given in Fig. 4 because of the very low numbers. Mono-spore isolates from perithecia previously sprayed with copper fungicides yielded in about 90% of the transfers isolate 3, in about 5% isolate 5 and 2–3% isolate 6, the saprophytes classified by Hindorf (1970) as *C. gloeosporioides* (3 and 5) and *G. cingulata* (6). In four cases -during the rain periods of April-May 1967, March 1968, October 1968 and March-April 1969- an isolate mildly parasitic on green berries was isolated which was classified as *C. gloeosporioides*, isolate 4 (Hindorf, 1970). This type was always obtained from material of internodes 1 + 2 and 3 + 4, the younger internodes where the bark maturation had just started. The growth-rate of the greenish mycelium exceeded that of *C. coffeanum*, while relatively few conidia were produced *in vitro*. The pathogenicity of the conidia of isolate 4 on green berries at approximately 10^3 con./ml was about 1/4 of the pathogenicity of *C. coffeanum* at the same density of inoculum. As there were much less perithecia on non-sprayed bark the findings on the distribution of the four types are less reliable. Inoculations with ascospore suspensions, derived from perithecia on bark or perithecia grown *in vitro*, at concentrations ranging from 10^4–10^6 spores/ml. on green coffee berries did not result in obvious lesions on the berries, even after wounding. The *C. coffeanum* inoculations carried out concurrently at the same concentration range gave infections varying from 50–90%.

Discussion

Kaiser and Lukezic (1966) described the discharge of ascospores of *G. cingulata* on bananas and Hocking *et al.* reported the violent discharge of ascospores of the same fungus on coffee in Tanzania. Nutman and Roberts (1969) in Kenya, however, could not catch any airborne ascospores when they left coffee branches carrying large numbers of ripe perithecia suspended vertically above a spore trap in a polythene-covered chamber equipped with a humidifier. Therefore they concluded that *G. cingulata* on coffee lacked the ability to liberate ascospores directly into the air.

The experiments described in this paper and carried out from March 1967–July 1969 show that the ascospores of *G. cingulata* are discharged forcibly into the air under the conditions provided by the inverted Petri dish technique in the laboratory. Discharge also occurs after surface sterilization of the bark containing perithecia. This eliminates any possibility of contamination by *Colletotrichum* conidia sticking to the outside of perithecial tissues. The ascospores caught on the agar above the bark material had the typical form of ascospores of *G. cingulata*. When an ascospore germinated on water agar or in water, it very often produced a conidiophore bearing a typical *Colletotrichum* conidium. The mono-ascospore isolates always gave rise to *Colletotrichum* cultures.

It is difficult to offer an explanation for the discrepancy between the conclusion of Nutman and Roberts (1969) that ascospores cannot be discharged violently and the findings presented in this paper. It may well be that the difference in the findings are only caused by differences in laboratory techniques. Kaiser and Lukezic (1966) found that moisture was the most important environmental factor affecting ascospore discharge. Discharge only occurred when the perithecia were wet or had been kept at 100% R.H. for fifteen hours. This agrees with the findings described in this paper when discharge started after about sixteen hours in the inverted Petri dishes.

The detailed observations by Nutman and Roberts (1969) on the marked effect of spray treatments on perithecial production of *G. cingulata* could be confirmed. The unsprayed branches in the experiments described in this paper showed, however, a decidedly lower production of perithecia than the control treatments of Nutman and Roberts. This is probably due to the fact that the control material used by Nutman and Roberts may have had a spray history, while the unsprayed material used here had never been sprayed with fungicides in the past. This observation supports the suggestion made by Nutman and Roberts (1969) that fungicidal sprays can influence the relative abundance of certain components of the microflora of the maturing bark and in particular the *Colletotrichum* spp. with a *G. cingulata*-stage. Only types 3, 4, 5, and 6 could be grown from mono-ascospore isolates of *G. cingulata* and this agrees with the findings of Hindorf (1970). Isolate 3 being the most common *Colletotrichum* component in the maturing bark at this altitude, it is possible to explain its high level of incidence (Vermeulen, 1970), while the ability of this type to produce perithecia after a relatively short time (Hindorf, 1970) should also be taken into account. *C. coffeanum* and *C. acutatum*, were never obtained after passage through *G. cingulata*. The possibility that *C. coffeanum* would be a danger to the Kenya coffee industry through the transport of ascospores by air seems therefore to be negligible. The mildly parasitic nature of isolate 4, its low level of occurrence and the apparent low productivity of conidia *in vitro* do not seem to indicate that this particular strain will be a

threat to a country like Kenya with an already high level of CBD. This type might be of interest in countries with little or no CBD. Although isolate 4 occurred during the same time of the year and in the same internodes as *C. coffeanum*, this isolate could not be obtained directly from bark (Vermeulen, 1970). This would suggest an even lower level of incidence of isolate 4 than *C. coffeanum*. Research should be continued.

The results described above for the yields of the *Colletotrichum* isolates (approximately 98% saprophytic), obtained through *G. cingulata*, agree with those of direct inoculations of green berries with suspensions of ascospores from perithecia either produced on branches or *in vitro*. Even after wounding no infections could be induced. The ascospore germination was not affected by the tap-water used, as the spores germinated easily in sterile tap-water. These results do not agree with the previous findings of Hocking, Johanns and Vermeulen (1967) who reported successful infections on berries, using ascospores from perithecia of *G. cingulata* on green coffee berries. Several possible explanations for the difference in findings may be offered:

1. The inoculation experiments reported by Hocking *et al.* from Tanzania were carried out with ascospores from perithecia on green berries and not from perithecia on branches or perithecia grown *in vitro*.
2. *C. coffeanum*, introduced only recently in the main coffee area of Tanzania, may not yet have lost its capacity to produce perithecia in that country[1].
3. *C. gloeosporioides*, isolate 4 (Hindorf, 1970) is more prevalent in Tanzania and has been mistaken for *C. coffeanum*, because of its capacity to cause infection on green berries.
4. The level of saprophytic components of the *Colletotrichum*-population is so high in Kenya, that the rare perfect stage of *C. coffeanum*, because of the low incidence of the parasite, has been overlooked.

The observations made on the discharge of ascospores in relation to discharge distance agree with the findings of Ingold (1965, p. 52–74). The discharge distance is amply sufficient to make the spores airborne. As it was hardly likely that *G. cingulata* plays a role in the epidemiology of *C. coffeanum* in Kenya, no further investigations were carried out to assess the liberation of ascospores under field conditions.

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Samenvatting

Koffiebesziekte in Kenia. II. *De rol van Glomerella cingulata in de Colletotrichum populatie, die de bast van Coffea arabica koloniseert.*

Op afgesneden takken van met fungiciden bespoten *Coffea arabica* konden de peritheciën van *Glomerella cingulata* (Fig. 1) in grote hoeveelheden worden gevonden op de

[1] J. C. Hudson has found recently again perithecia of *G. cingulata* on CBD-affected berries in Tanzania (pers. comm.).

rijpende bast na twee tot tien dagen incubatie. Op takken, die nog nooit met fungiciden waren bespoten, lagen de aantallen peritheciën aanzienlijk lager. Ook werd *G. cingulata* gevonden op snoeihout onder de koffiebomen, maar de aantallen peritheciën verminderden snel na het tijdstip van snoeien.

Het bleek mogelijk te zijn om onder laboratoriumomstandigheden peritheciën van *G. cingulata* ascosporen in de lucht te laten schieten (Fig. 2). Ook nadat de takken uitwendig waren ontsmet met sublimaat werden ascoporen uitgestoten. De opgevangen sporen vormden vaak op water agar en in water niet alleen een appressorium en een kiembuis, maar soms ook een conidiophoor met een *Colletotrichum* conidium (Fig. 3). De maximale afstand afgelegd door uitgeschoten ascosporen bedroeg verticaal ongeveer 1.5 cm, horizontaal ongeveer 2.0 cm (Fig. 4).

Een monosporenisolatie van een uitgeschoten en op agar opgevangen ascospore gaf altijd één van vier typen die tot de *Colletotrichum* populatie behoren. Eén van deze vier bleek een *C. gloeosporioides* te zijn, die in staat was groene koffiebessen te infecteren. Groeisnelheid, kleur van het mycelium, het aantal gevormde conidiën *in vitro* en de pathogeniteit op groene koffiebessen bleken echter aanzienlijk te verschillen van *C. coffeanum*, de veroorzaker van de koffiebesziekte. Er konden geen bewijzen worden gevonden, dat ascosporen afkomstig van peritheciën op de koffiebast in staat waren gewonde of niet-gewonde groene koffiebessen te infecteren. Ook ascosporen afkomstig van peritheciën van *G. cingulata in vitro* konden geen groene koffiebessen aantasten. Verschillende mogelijkheden werden gesuggereerd om het verschil te verklaren tussen deze waarnemingen en eerder gepubliceerde gegevens, waaruit bleek dat ascosporesuspensies verkregen van peritheciën op groene bessen wel in staat waren geweest infectie op koffiebessen te veroorzaken.

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