Conidial morphology and development of *Alternaria cassiae* from cowpea

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Van den Berg N, Aveling T A S & Van der Merwe C F 2002. Conidial morphology and development of *Alternaria cassiae* from cowpea. *African Plant Protection* 8(1&2): 69–74.

Alternaria cassiae (PPRI 6393) produced a mixed population of three distinct conidial types both in vitro and in vivo. Conidia with aseptate, long, filiform beaks were produced more frequently on various media except potato dextrose agar. Conidial body and beak sizes were variable when measured in culture and on cowpea leaves. Conidia produced on cowpea leaves in vivo were smaller in size than those produced in culture. Conidia of *A. cassiae* were large, obclavate and formed singly or in chains. Conidiophores emerged directly through the epidermis or stomata of inoculated cowpea leaves. Hyphae growing on the leaf surface also differentiated into conidiophores. Conidiophores were straight or curved and enlarged apically at the site of conidium production. Smooth, bud-like conidial initials were produced at the apex of the conidiophores. As conidia matured, they became elliptical to obvate and densely verrucose. Once the mature conidium had seceded, a small pore was visible at the apex of the conidiogenous cell.

Key words: conidial types, cowpea, culture media.

Alternaria cassiae was first described from *Cassia holosericea* Fresen. by Jurair & Khan (1960). Simmons (1982) and David (1991) subsequently provided detailed descriptions of the morphology of conidiophores and conidia of the species and showed that it produced three different conidial types on *Cassia* species. More recently, La Grange & Aveling (1998) reported *A. cassiae* to cause a destructive foliar disease of cowpea (*Vigna unguiculata* (L.) Walp.) in South Africa, and also noted different conidial types in field-collected material. Preliminary studies (unpubl. data) furthermore indicated that the body and beak sizes of conidia produced in vivo and in vitro differ.

Conidial morphology, including dimensions of the spore body and beak, are important criteria in the classification of *Alternaria* (Neergaard 1945; Simmons 1967; Rotem 1994). The existence of different conidial types in *A. cassiae* therefore could have diagnostic significance, since few other *Alternaria* spp. display this feature. However, confusion will obviously be created if a culture produces more, less or none of a specific conidial type when maintained on different media. No information is available on the conidial development of *A. cassiae* on cowpea or on artificial media. The objective of this study was to determine the effect of different substrates on the conidial types and morphology of *A. cassiae* and to describe the development of conidia of *A. cassiae* on infected cowpea leaves.

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Materials and methods

Plates were prepared of the following nutrient media in 90-mm-diameter Petri dishes: cornmeal agar (CMA) (Merck), Czapek Dox agar (CDA) (Oxoid), malt extract agar (MEA) (Merck), potato-dextrose agar (PDA) (Merck), V8-agar [V8-juice (Campbell), agar (Merck)] and water agar (WA) (Merck). An isolate of *A. cassiae* (PPRI 6393) from cowpea in South Africa, known to produce conidia differing in size, was cultured on V8-agar at 25 °C under intermittent (12 hour light/dark) near-UV (320–420 nm) illumination. A plug of medium, 7 mm in diameter, was removed from the periphery of an actively growing culture and placed inverted in the centre on each of five replicate plates of each of the above media. Plates were incubated at 25 °C under intermittent near-UV light.

For inoculation of plants, cowpea (cv. Rhino) was sown, two seeds per pot, in 15-cm-diameter plastic pots containing pasteurised soil. Pots with seedlings were maintained in a greenhouse at 25 ± 1 °C and were watered daily. Six leaves on each of five 6–8-week-old seedlings were inoculated by brushing a 1×10^5 ml^{-1} conidial suspension of *A. cassiae* PPRI 6393 onto each leaf with a sterile paintbrush until run-off occurred. After inoculation, seedlings were transferred to a mist

Table 1. Spore dimensions of *Alternaria cassiae* in vivo and in vitro.

Variable	Measurements in µm[a]							
	In vivo[b]	CDA	CMA	MEA	PDA	V8	WA	LSD
Spore length								
Mean	60.71 a	92.04 e	85.40 d	66.85 b	65.96 b	68.89 b	72.21 c	3.216
Range	50–85	80–110	62–103	53–87	53–81	50–85	62–87	
SD[c]	8.56	8.24	7.92	7.92	7.01	6.37	6.07	
Spore width								
Mean	17.42 b	23.32 d	20.23 c	17.13 b	17.28 b	17.52 b	14.64 a	1.273
Range	13–25	19–25	12–25	15–22	12–22	12–23	12–25	
SD	2.85	4.67	2.97	2.07	1.92	1.96	2.47	
Beak length (when present)								
Mean	119.50 c	77.38 a	112.60 cb	65.40 a	98.70 b	111.80c b	177.00 d	15.42
Range	10–238	56–87	18–198	10–260	18–173	10–229	24–330	
SD	56.64	15.19	31.35	62.48	35.16	49.08	72.16	
	Percentage[a] spores with:							
No beaks	8	15	11	6	11	17	7	
Short, septate beaks	30	31	21	38	66	30	31	
Long, filiform aseptate beaks	62	54	68	56	23	53	62	

[a]After 12 days in vivo and 18 days in vitro; each value is the mean of five replicates with 20 conidia per replicate, except CDA and MEA where each replicate comprised 10 conidia; values in the same rows followed by the same letter do not differ significantly, according to Duncan's multiple range test ($P = 0.05$); CDA = Czapek Dox agar, CMA = cornmeal agar, MEA = malt extract agar, PDA = potato-dextrose agar, V8 = V8 agar, WA = water agar.
[b]Conidia measured in vivo on cowpea leaves.

chamber at 25 ± 1 °C.

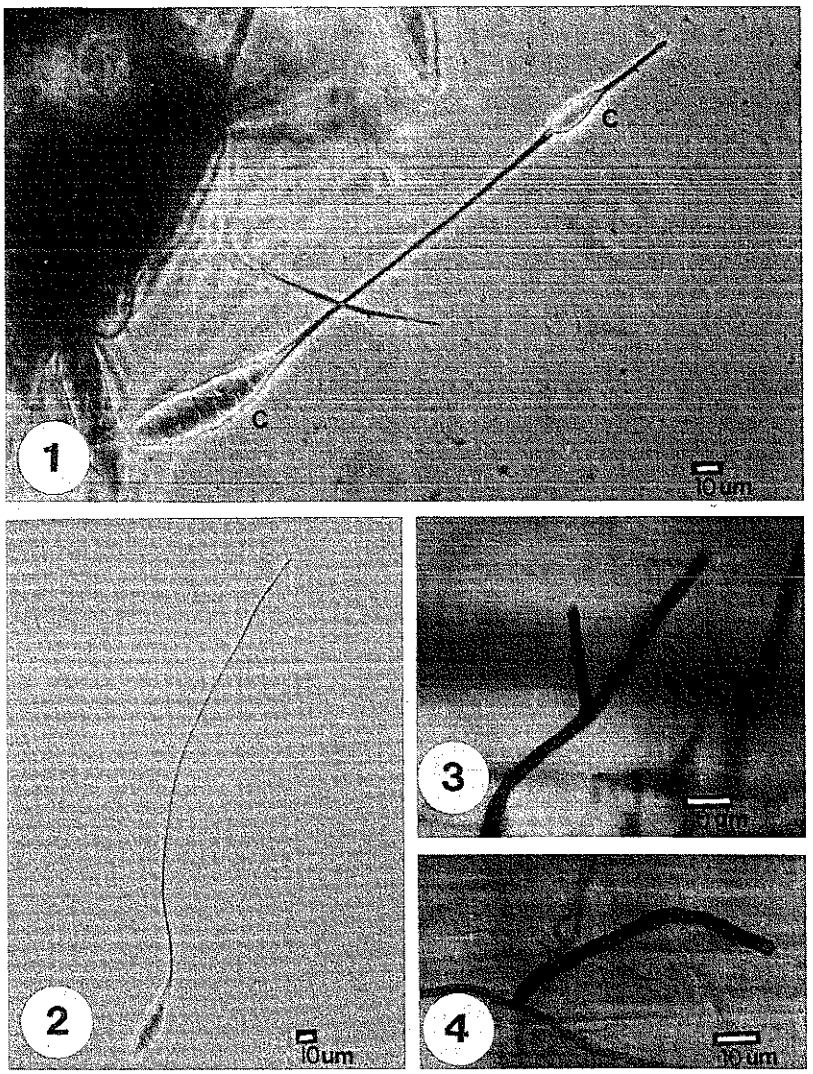
To study conidial development and morphology, fungal material was scraped from the various nutrient media after incubation for 18 days, stained with lactophenol blue, and viewed, measured and photographed using a Nikon Optiphot or Zeiss photomicroscope. Twenty conidia from each of five replicate plates of each medium were assessed, except for MEA and CDA, where only 10 conidia per replicate could be measured due to poor sporulation. For observation of conidia on cowpea leaves, the leaves were cut into 5 mm^2 sections at various intervals after inoculation. The epidermis of specimens was removed with a pincette, mounted on microscope slides, stained with lactophenol blue and observed under a photomicroscope. Twenty conidia on each of five leaf sections collected 12 days after inoculation were measured. Data were analysed by analysis of variance and means differences were separated according to Duncan's multiple range test.

Conidial formation and detachment behaviour were observed on cowpea leaf sections from 24 to 288 hours post inoculation (hpi). Leaf material was fixed in 2.5 % (v/v) gluteraldehyde in 0.075 M phosphate buffer (pH 7.4–7.6), rinsed in the same buffer and then post-fixed in 0.25 % (m/v) aqueous osmium tetroxide for 2–4 hours. After three successive washing steps in distilled water, the material was dehydrated in an ascending ethanol series, dried in a Bio-rad critical point dryer and mounted on stubs. Specimens were coated with gold in a Polaron sputter coater and examined with a Jeol JSM 840 scanning electron microscope at 5 kV.

Results

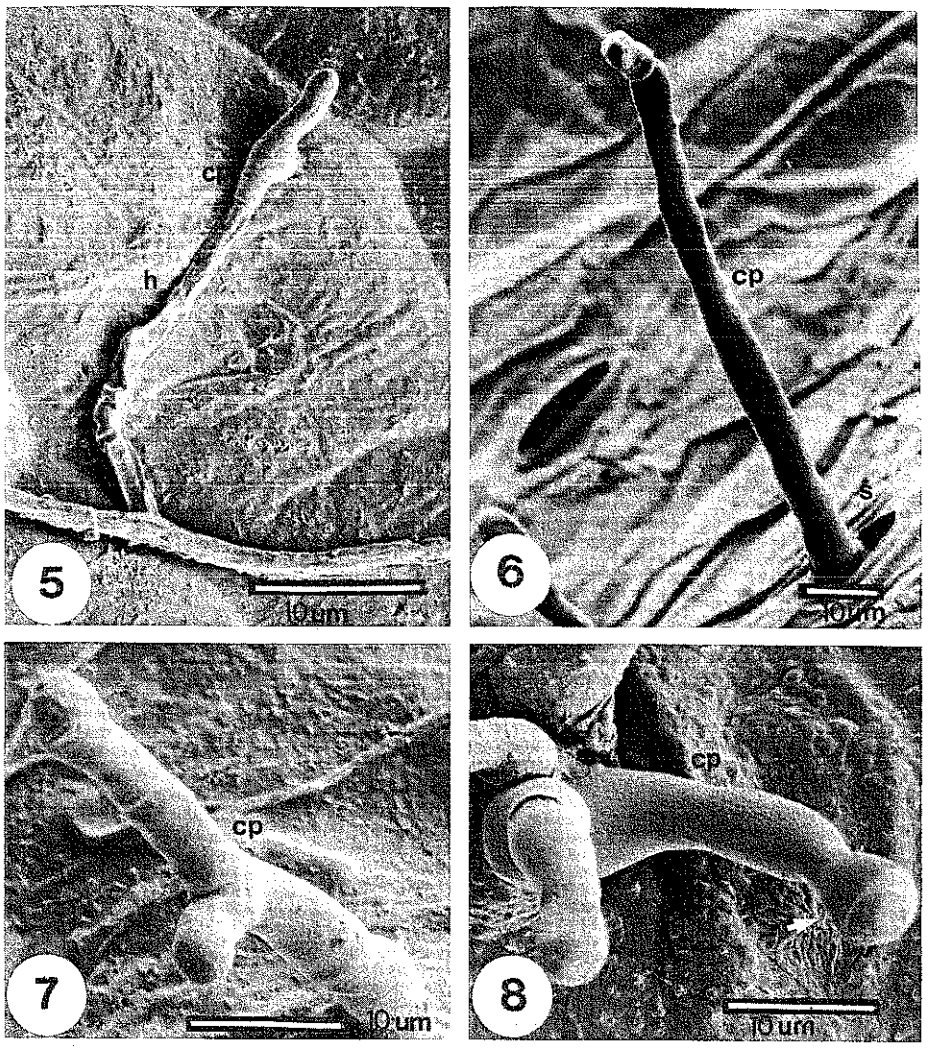
Conidia of *A. cassiae* were large, obclavate and each conidium was divided by transverse and longitudinal septa into multiple compartments (Fig. 1). *A. cassiae* produced a mixed population of conidia on all the different culture media and on the surface of inoculated cowpea leaves. Conidia were borne singly or in chains of 2–4.

Three distinct conidial types were produced: 1) conidia with long, aseptate, filiform beaks (Fig. 2), 2) others that have generated secondary conidiophores capable of producing additional conidia



Figs 1–4. **1**: Large conidium (c) of *Alternaria cassiae* with transverse and longitudinal septa and a long, filiform beak, which is converted into a secondary conidiophore giving rise to an additional conidium (c); **2**: conidium of *A. cassiae,* produced on water agar, with a long, aseptate, filiform beak; **3**: branched conidiophore of *A. cassiae* produced in vivo; **4**: unbranched conidiophore of *A. cassiae* produced in vivo.

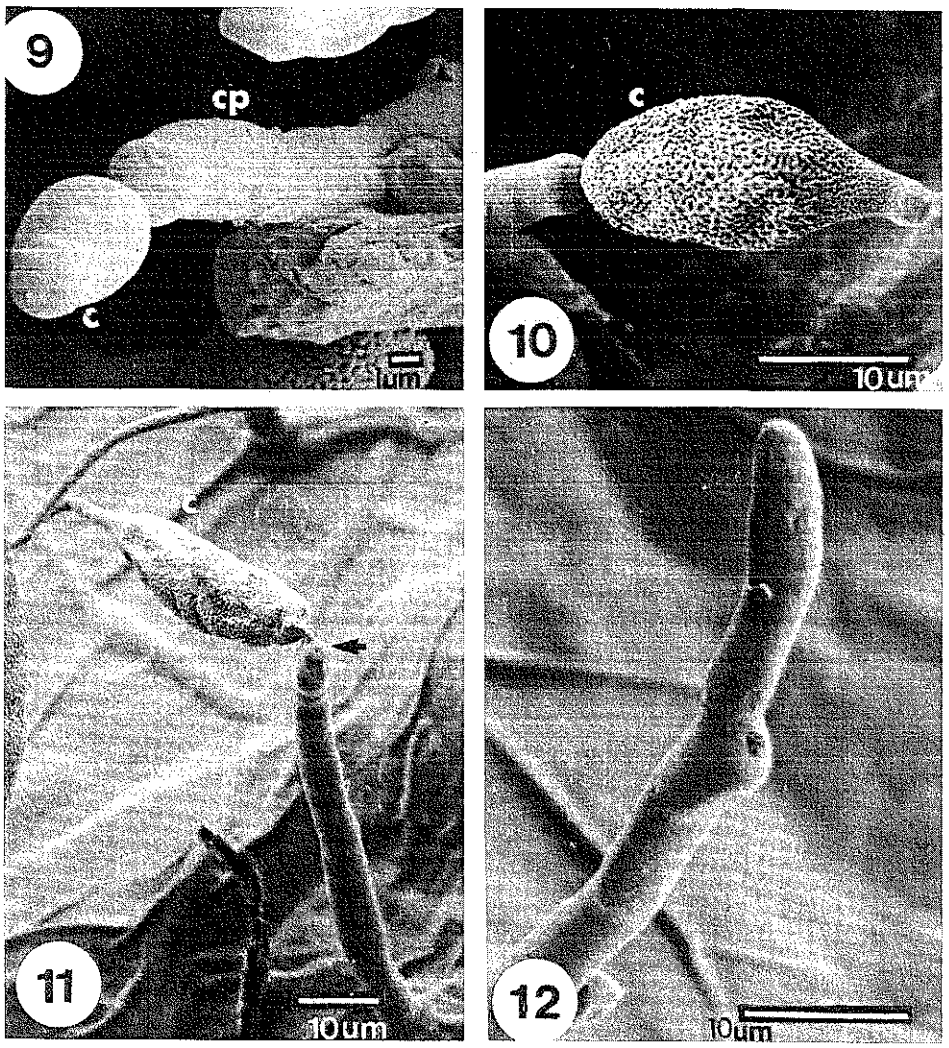
(Fig. 1), and 3) those at full size and densely verrucose, but without beaks (Fig. 10). Mature conidia had both transverse and longitudinal septae (Fig. 1). Cultures on all the media except PDA, produced more conidia with long filiform, aseptate beaks than with short, septate or no beaks (Table 1). Conidial body and beak sizes were variable on the different media and on cowpea leaves. All cultures on artificial media produced significantly longer conidia than those formed on cowpea leaves (Table 1). Conidia produced on CDA had the largest bodies (93 × 23 µm), and the shortest beaks, except for those on MEA. Conidial beaks were the longest on WA, reaching lengths of up to 330 µm (Table 1, Fig. 2), while on cowpea leaves they reached lengths of up to 238 µm. The long, filiform beaks were aseptate, whereas shorter beaks that were converted into secondary conidiophores showed septation. Branched (Fig. 3) and unbranched (Fig. 4) septate conidiophores were produced on all the culture media.



Figs 5–8. 5: Hypha (h) of *Altenaria cassiae,* growing on the surface of a cowpea leaf, differentiated into a conidiophore (cp); **6**: conidiophore (cp) of *A. cassiae* emerging through a stoma (s); **7**: single-branched conidiophore of *A. cassiae* (cp) emerging directly through the epidermis; **8**: cylindrical, unbranched conidiophores (cp) of *A. cassiae* grouped in a bundle. An enlarged apex (arrow) is visible at the site of conidium production.

Seventy-two hours hpi, profuse fungal growth was observed on the leaf surface of cowpea plants.

Hyphae growing on the plant surface differentiated into conidiophores (Fig. 5) by 24 hpi, whereas conidiophores emerged through stomata (Fig. 6) or directly through the epidermis (Figs 7, 8) by 96 hpi. By 168 hpi conidiophores were abundant and young developing conidia were present. Conidiophores formed on cowpea leaves were straight or curved and borne singly (Fig. 7) or in bundles (Fig. 8). The conidiophores were septate, branched (Fig. 7) or unbranched (Fig. 8) and 30–120 × 4–6 µm in size. Conidiophores were cylindrical in shape, but enlarged apically at the site of conidium production (Fig. 8). In most instances a single conidium was produced at the apex of a conidiophore (Fig. 9). Initially the conidium was round, bud-like and smooth (Fig. 9). Mature conidia were elliptical to obvate and densely verrucose (Figs 10, 11), with sizes ranging from 50–85 × 13–25 µm, excluding the length of the beak. Once the mature conidium seceded, a small pore (±0.5 µm) representing the conidio-



Figs 9–12. 9: Young smooth, bud-like conidium (c) of *Altenaria cassiae* developing at the apex of a conidiophore (cp). **10**: mature and densely verrucose beakless conidium (c) of *A. cassiae* with base broader than the apex; **11**: mature, verrucose conidium (c) of *A. cassiae* with a pore (arrow) visible at the apex of the conidiophore; **12**: proliferating primary conidiophore of *A. cassiae* after the mature conidium has been detached.

genous locus, was visible at the apex of the conidiophore (Fig. 11). Occasionally a proliferating primary conidiophore was observed (Fig. 12).

Discussion

As reported for *A. cassiae* isolated from *Cassia* spp. (Simmons 1982; David 1991), *A. cassiae* isolated from cowpea also produced a mixed conidium population of three conidial types both in vitro and in vivo. Typical long, filiform, aseptate, beaked conidia and conidia with shorter beaks, converted into secondary conidiophores were more frequently observed than mature, beakless conidia. However, on PDA short, septate, beaked and beakless conidia were more prevalent. This may occasionally result in the incorrect identification of the fungus as a different *Alternaria* spp. It is therefore necessary to define conidial sizes and types on specific media, to avoid confusion. The occurrence of several conidial types within one population, may possibly be linked to a mode of survival and dissemination.

A mean body size on all the different media of 75 × 18 µm was calculated for *A. cassiae*. This is

slightly smaller than the 90 × 20 µm and 80–85 × 20–25 µm reported for *A. cassiae* on artificial media by Simmons (1982) and Mims et al. (1997), respectively. However these dimensions fall within the range of 65–90 × 20 µm reported by David (1991) for conidia of *A. cassiae* on potato carrot agar (PCA).

Conidial beak lengths of up to 330 µm were observed in culture, and mean beak length on all the media was approximately 105 µm. According to Simmons (1982) and David (1991), beak lengths of 100–125 µm are more usual, the longest beak length reported by both these authors being 200 µm. Results obtained in the present study indicated that *A. cassiae* produced conidia with conidial beaks longer than 105 µm more frequently on WA, V8-agar and CMA.

Conidial formation and detachment were similar to that described for other *Alternaria* spp. The emergence of conidiophores of *A. cassiae* through the epidermis and stomata as well as the differentiation of hyphae growing on the leaf surface into conidiophores, indicated the initiation of sporulation. As was found in this study with *A. cassiae*, Allen et al. (1983) observed two ways of conidiophore development in *Alternaria helianthi* (Hansf.) Tubaki & Nish. on sunflower (*Helianthus annuus* L.). *A. helianthi* produced conidiophores that protruded through stomata as well as other sites on the leaf, but conidiophores were also formed from mycelium growing across the leaf surface. Conidiophores were more darkly pigmented and morphologically different from vegetative hyphae, the latter being thinner. The length of conidiophores of *A. cassiae* in the present study varied from 30–100 µm, corresponding well with the conidiophore lengths of 36–112 µm reported by David (1991).

Tips of conidiophores of *A. cassiae* were slightly swollen where the conidia protruded, similar to those of *A. porri* on onion (*Allium cepa* L.) leaves (Aveling et al. 1991). The conidial initial of *A. cassiae* was bud-like, also similar to that of *A. porri* (Aveling et al. 1991) and appeared smooth to slightly verrucose. David (1991) described the young conidia of *A. cassiae* as smooth-walled and slightly verrucose. Results of the present study, however, indicated that the conidia became densely verrucose as they matured. Transverse and longitudinal septa were distinct in mature conidia of *A. cassiae* on cowpea leaves. Simmons (1982) reported that mature conidia of *A. cassiae* had transverse and intercalated longitudinal septa, giving the conidium a surface view of 12–20 compartments. Characteristic conidiophore proliferation, as described by Simmons (1982), was observed occasionally, but only after a mature conidium was dislodged.

Acknowledgement

We thank the National Research Foundation for funding.

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Accepted 29 July 2002
Associate Editor was F C Wehner