

## Note

# Reaction of dwarf cashew clones to *Colletotrichum gloeosporioides* isolates in controlled environment

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**ABSTRACT:** The cashew (*Anacardium occidentale* L.) crop is an important source of income for the population of the Brazilian Northeast, and anthracnose disease caused by *Colletotrichum gloeosporioides* leads to significant production loss. However, there is little information on either the host resistance or the variation in the aggressiveness of the pathogen under controlled environment. The reaction of commercial (CCP-06, CCP-09, CCP-76 and CCP-1001) and one non-commercial (CAP-14) dwarf cashew clones was assessed against 36 isolates of this fungus controlled environmental conditions. All the isolates, including those from hosts other than cashew, were able to cause lesions on leaves and stems of most clones, albeit to different degrees. Clone CCP-06 was the most susceptible, while clone CCP-1001 showed a level of resistance to a number of the isolates, including isolate 905, while isolates 910 and 912 were aggressive to all clones. Injury increased the susceptibility of the clones to all isolates, indicating that resistance also might be associated with structural barriers that hinder penetration. Ripened cashew apples (8-week-old), of commercial clones were susceptible to isolates 905 and 910. Immature pseudo-fruits (2-week-old), with exception of clone CCP-76 which was susceptible to both isolates showed resistance against these isolates, suggesting the presence of structural and chemical barriers. Developed nuts (8-week-old), however, were more resistant than immature nuts (2-week-old) to both isolates, probably due to their thicker exocarp cuticle and reduced number of stomatal pores.

**Key words:** *Anacardium occidentale*, anthracnose, resistance, aggressiveness

## Reação de clones de cajueiro-anão a isolados de *Colletotrichum gloeosporioides* em ambiente controlado

**RESUMO:** A cultura de caju (*Anacardium occidentale* L.) é uma fonte de renda importante para a população do Nordeste brasileiro, sendo a antracnose causada por *Colletotrichum gloeosporioides* a doença que mais conduz a perdas de sua produção. Entretanto, há pouca informação para a resistência desse hospedeiro ou para a variação na agressividade do patógeno em ambiente controlado. A reação de clones comerciais (CCP-06, CCP-09, CCP-76 e CCP-1001) e um não comercial (CAP-14) de cajueiros anão-precoce, contra 36 isolados desse fungo, foi avaliada sob condições controladas. Todos os isolados, incluindo os provenientes de diferentes hospedeiros, foram capazes de causar lesões sobre folhas e hastes da maioria dos clones, ainda que em graus diferenciados. O clone CCP-06 foi o mais suscetível, enquanto o clone CCP-1001 foi resistente a mais isolados, incluindo o 905, enquanto os isolados 910 e 912 foram agressivos a todos os clones testados. A injúria mecânica das folhas aumentou a suscetibilidade dos clones a todos os isolados, indicando que a resistência pode também estar associada a barreiras estruturais que impedem a penetração. Os pseudofrutos amadurecidos (8 semanas) dos clones comerciais avaliados, foram suscetíveis a ambos os isolados testados (905 e 910), mas os jovens (2 semanas), com exceção daqueles do clone CCP-76, suscetíveis aos dois isolados, apresentaram resistência a ambos, sugerindo a ação de obstáculos estruturais e químicos diferenciados. As castanhas desenvolvidas (8 semanas), no entanto, foram mais resistentes a ambos os isolados do que as jovens (2 semanas), talvez devido à espessa cutícula do exocarpo e baixo número de estômatos.

**Palavras-chave:** *Anacardium occidentale*, antracnose, resistência, agressividade

### Introduction

Until three decades ago, the production of cashew (*Anacardium occidentale* L.) in Brazil was based on seed propagated trees of the “Common” or “Giant” type, which lack uniformity for commercial characteristics. Such trees were grown widely-spaced in a semi-wild state

and this, combined with the high level of heterozygosity in the crop, restricted the amount of disease present. With the introduction of vegetatively-propagated dwarf clones of cashew, which are more uniform for agronomic characteristics (Barros and Crisóstomo, 1995), outbreaks of several diseases have become more prevalent in commercial orchards (Cardoso et al., 1999).

Anthracnose disease, caused by the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. [the anamorphic stage of *Glomerella cingulata* (Stoneman) Sp. and Schrenk], is widely distributed throughout the cashew-growing areas of the world, and has caused economic losses also in Brazil (Freire et al., 2002). The only practical management strategy for this pathogen involves general sanitation practices and chemical control (Cardoso et al., 1999), but the preferred option should be the use of clones with improved resistance to the pathogen. However, any breeding programme must take into account the variation in the pathogen population.

There is little information on either host resistance or pathogenic variation in the cashew anthracnose pathosystem under controlled environment. Therefore, tests under controlled conditions were carried out on injured and not injured leaves, stems and fruits of different dwarf cashew clones, with the purpose to verify: i) their reaction against 36 distinct *Colletotrichum* isolates, from cashew and associated plants from different regions of Brazil; and ii) the variability between the tested isolates.

### Material and Methods

All the experiments using cashew leaves/stems were carried out in Bristol-UK, 51°25'N; 2°39'W; and the altitude is 27 m. Experiments using cashew fruits (nuts) and pseudo-fruits (apples) were carried out in the research station of Pacajús, state of Ceará, Brazil, 04°10'S; 38°27'W; and the altitude is 60 m.

#### Fungal isolates and inoculum production

The source of the 36 isolates of *C. gloeosporioides* studied is described in Table 1. Spores were produced by subculturing isolates in 100 mL *Colletotrichum* medium (CM) (Mathur et al., 1950) dispensed in 250 mL flasks. Spore suspensions were obtained by adding ca. 20 mL sterile distilled deionized water (SDW) to 7 days old flask cultures and shaking. All isolates were single-spored prior to experimental use.

#### Cashew clones

Seeds of the commercial cashew clones CCP-06, CCP-09, CCP-76, CCP-1001, and the experimental clone CAP-14 (derived from a mutant of CCP-76) were supplied by CNPAT/EMBRAPA, and stored in a cold room (10°C) at the MPPD/LARS. Seeds were sown in a soil-sand-peat moss mixture (1:1:1 enriched with 2.5 kg m<sup>-3</sup> of triple superphosphate and 1 kg m<sup>-3</sup> of potassium chloride, pH 5.5) in 2 L plastic pots. The pots were maintained in a controlled environment chamber (16 h light at 280 µmol PAR m<sup>-2</sup> s<sup>-1</sup>, 80% relative humidity at 35°C) and were watered daily. After 10-15 days, when the seedlings emerged, the temperature of the chamber was reduced to 25°C. Forty five-day-old seedlings were used for the inoculation experiments.

#### Fruit material

Intact cashew apples and nuts were harvested at different maturation stages (1-9 weeks) from commercial

clones in experimental field plots of Pacajús, CE, Brazil, and taken to the laboratory for immediate inoculation tests.

#### Pathogenicity Assays on Stem Segments and Leaves

Stem segments bordering the youngest leaves, and the first and second youngest fully-expanded and intact leaves from 45-day-old plants were excised and the cut ends of the excised tissue sealed with warm, molten paraffin wax. The stems segments (ca. 5 cm) were arranged on aluminium supports within plastic boxes (20 × 12 × 4 cm) lined with moist filter paper. Excised leaves were placed flat on square propylene sheets inside transparent plastic boxes (24 × 24 × 2 cm) lined with moist tissue paper. The leaves were wounded or maintained intact prior to inoculation. For the wounding treatment, ten parallel incisions, sufficient to penetrate the epidermis, were made with sterile forceps on the adaxial or abaxial surface of leaves. Drops (10 mL) of conidial suspensions (10<sup>6</sup> spores mL<sup>-1</sup>) were then placed, with the aid of a microdispenser (EDP-1, Rainin Instrument Co.) at intervals of 1 cm, on the stems or on either side of the mid-vein of the adaxial or abaxial surface of the leaf lamina. The plant material was incubated in a controlled environment cabinet [25°C, 100% relative humidity (r.h.), for 24 h in the dark, followed by a photoperiod regime with 16 h light at 280 µmol PAR m<sup>-2</sup> s<sup>-1</sup>]. The material was evaluated for both symptom type and disease severity 2-7 days after inoculation. Symptom types were scored on a 0 - 5.0 scale, where 0 = no macroscopic symptoms; 1 = few small flecks (1 mm); 2 = flecks between 2-3 mm/limited lesions; 3 = many coalescent flecks, lesions between 4-7 mm; 4 = water-soaked lesions, larger than 7 mm; 5 = spreading coalescent lesions with sporulation. For final tabulation of the data, symptom types were categorized into three broad reaction classes: R = resistant (scores 0-2); M = moderately resistant (scores 2.1-3.0) and S = susceptible (scores 3.1-5.0). Disease severity (whole leaf) was scored on a 0-5 scale, where 0 = no necrotic tissue (NT); 1 = ≤ 5% NT; 2 = 6-20% NT; 3 = 21-35% NT; 4 = 36-60% NT; 5 = 61-100% NT.

The factorial experiment consisted of a completely random design, for each clone-isolate combination, involving ten stem segments and three leaves for each position (first and second) and inoculated surface (abaxial or adaxial), and it was repeated three times. Equal number of stem segments and leaves were treated in the same way with sterile distilled water. The data were transformed to  $\sqrt{x+0.5}$  for analysis of variance and comparison between the means by the LSD test ( $p \leq 0.05$ ).

Subsequently, three isolates (905, 910, and 912) that were able to cause disease for all the clones, or that were unable to do it for at least one of them, were chosen for further study on attached leaves. Conidial suspensions (10<sup>6</sup> spores mL<sup>-1</sup>) of these isolates were sprayed on leaves of 45-day-old plants (three per clone for each isolate). Inoculated plants were enclosed in wet plastic bags and

Table 1 – *Colletotrichum gloeosporioides* isolates used in this study.

Isolates(LARS)	Origin	Infected Organ	Locality
905	<i>Rubus brasiliensis</i>	Leaf	Maceió - Alagoas <sup>1</sup>
906	<i>Spondias purpurea</i> L.	Leaf	Maceió - Alagoas <sup>1</sup>
907	<i>Carica papaya</i> L.	Leaf	Maceió - Alagoas <sup>1</sup>
908	<i>Dioscorea</i> sp.	Leaf	Maceió - Alagoas <sup>1</sup>
909	<i>Malpighia glabra</i> L.	Leaf	Maceió - Alagoas <sup>1</sup>
910	<i>Anacardium occidentale</i> L.	Leaf	Maceió - Alagoas <sup>1</sup>
911	<i>Anacardium occidentale</i> L.	pseudo-fruit	Maceió - Alagoas <sup>1</sup>
912	<i>Anacardium occidentale</i> L.	nut	Cruz das Almas - Bahia <sup>1</sup>
913	<i>Anacardium occidentale</i> L.	pseudo-fruit	Cruz das Almas - Bahia <sup>1</sup>
914	<i>Anacardium occidentale</i> L.	Leaf	Conceição do Almeida-Bahia <sup>1</sup>
915	<i>Anacardium occidentale</i> L.	flower	Praia do Forte - Bahia <sup>1</sup>
916	<i>Anacardium occidentale</i> L.	Leaf	Indiaroba - Sergipe <sup>1</sup>
917	<i>Anacardium occidentale</i> L.	Leaf	Penedo - Alagoas <sup>1</sup>
918	<i>Anacardium occidentale</i> L.	Leaf	Maceió - Alagoas <sup>1</sup>
919	<i>Anacardium occidentale</i> L.	Leaf	Marechal Deodoro - Alagoas <sup>1</sup>
920	<i>Anacardium occidentale</i> L.	Leaf	Marechal Deodoro - Alagoas <sup>1</sup>
921	<i>Anacardium occidentale</i> L.	Leaf	Recife - Pernambuco <sup>2</sup>
922	<i>Anacardium occidentale</i> L.	pseudo-fruit	Belém - Pará <sup>3</sup>
923	<i>Anacardium occidentale</i> L.	Leaf	Fortaleza - Ceará <sup>3</sup>
924	<i>Anacardium occidentale</i> L.	Leaf	Pacajus - Ceará <sup>3</sup>
925	<i>Anacardium occidentale</i> L.	pseudo-fruit	Piracicaba - São Paulo <sup>3</sup>
926	<i>Anacardium occidentale</i> L.	Leaf	Cruz das Almas - Bahia <sup>3</sup>
927	<i>Anacardium occidentale</i> L.	pseudo-fruit	Fortaleza - Ceará <sup>3</sup>
928	<i>Anacardium occidentale</i> L.	pseudo-fruit	Brasília (DF) - Goiás <sup>3</sup>
929	<i>Anacardium occidentale</i> L.	pseudo-fruit	Pacajus - Ceará <sup>4</sup>
930	<i>Anacardium occidentale</i> L.	Leaf	Goiana - Pernambuco <sup>4</sup>
931	<i>Anacardium occidentale</i> L.	Leaf	Recife - Pernambuco <sup>4</sup>
932	<i>Anacardium occidentale</i> L.	Leaf	Fortaleza - Ceará <sup>4</sup>
933	<i>Anacardium occidentale</i> L.	Leaf	Areia - Pernambuco <sup>4</sup>
934	<i>Anacardium occidentale</i> L.	Leaf	Natal - Rio Grande do Norte <sup>4</sup>
935	<i>Anacardium occidentale</i> L.	Leaf	Recife - Pernambuco <sup>4</sup>
936	<i>Anacardium occidentale</i> L.	Leaf	Maceió - Alagoas <sup>4</sup>
937	<i>Anacardium occidentale</i> L.	Leaf	Olinda - Pernambuco <sup>4</sup>
938	<i>Anacardium occidentale</i> L.	Leaf	João Pessoa - Paraíba <sup>4</sup>
939	<i>Anacardium occidentale</i> L.	Leaf	Manaus - Amazonas <sup>5</sup>
940	<i>Anacardium occidentale</i> L.	Leaf	Pacajus - Ceará <sup>5</sup>

Isolates were provided in 1996 by : <sup>1</sup>Prof. Dr. Eurico Lemos, “Centro de Ciências Agrárias - UFAL”, Maceió/AL; <sup>2</sup>Dr. M.B. Figueiredo, “Seção de Micologia - Instituto Biológico de São Paulo”, SP; <sup>3</sup>Dr. E.E. Bach, “Seção de Bioquímica Vegetal - Instituto Biológico de São Paulo”, SP; <sup>4</sup>Prof. Dr. M. Menezes, “Departamento de Fitossanidade - UFRPe”, Recife/Pe; <sup>5</sup>Dr. F.P. Freire, “Centro Nacional de Pesquisa da Agroindústria Tropical - EMBRAPA”, Fortaleza/CE.

incubated in a controlled environment cabinet as described above. Control plants were sprayed with sterilized deionised water. After 48 hours the plastic bags were removed and the plants observed daily for 10 days. The symptoms were scored as described above. The experiment consisted of a completely random design, with three repetitions for each assay. The data were also trans-

formed to  $\sqrt{x+0.5}$  for analysis of variance and comparison of the average scores by the LSD test ( $p \leq 0.05$ ).

Differences in virulence among isolates were examined by comparing the number of clones on which isolates induced symptoms. Aggressiveness among the isolates was measured by comparing the variation in the degree of symptoms (mean disease severity) on the

cashew clones which were susceptible to all pathogen isolates. The LSD test ( $p \leq 0.05$ ) was used to compare every possible pair of treatment means and rank the relative aggressiveness among the isolates.

### Pathogenicity Assays on Pseudo-fruits and Fruits

Three whole apples and attached nuts of each clone, in different stages of maturation (three per stage), were surface disinfected by consecutive immersion for 5 min in 75% (v:v) EtOH, 10 min in 65% NaOCl (10% available chlorine) and 1 min in 75% EtOH. The samples of each clone were rinsed in sterilized distilled water, dried with sterile tissue paper, placed inside plastic boxes (50 × 30 × 20 cm) lined with moist tissue paper, and axially marked with black permanent ink using a lumocolor pen. Drops (10 mL) of conidial suspension ( $10^6 \text{ mL}^{-1}$ ) of LARS-isolates 905 and 910 were placed at 1 cm intervals onto an area of 1.5 cm of the axial line of the surface of apples and nuts. Each side of the axial line was inoculated with one different isolate. The boxes were then closed and incubated in a controlled environment room (25°C, 100% r.h., for 24 h in the dark followed by a photoperiod with 16 h light at  $280 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ ). Both symptom type and disease severity were evaluated as described before, 96 hours after inoculation. The experiment (completely random design) was repeated three times and the data were transformed to for analysis of variance and comparison of the average scores by the LSD test ( $p \leq 0.05$ ).

## Results and Discussion

The reaction of intact leaves of all clones varied, suggesting differences in host susceptibility, irrespective of the *C. gloeosporioides* isolate (Table 2). Clone CCP-1001 was resistant to 22% of the isolates and moderately-resistant to 22% of them, while clone CCP-06 was susceptible to 97.2% of the isolates. The non commercial clone CAP-14 was resistant and moderately-resistant to 30.5% and 38.8% of the isolates, respectively. The clones CCP-09 and CCP-76 were respectively susceptible to ca. 81 and 67% of the isolates. The same results were seen on the stem segments (data not shown). This suggests that under field conditions, none of the clones would remain free of anthracnose indefinitely.

Virulence of the pathogen isolates was determined by comparing the number of host genotypes on that they produced symptoms of an interaction considered as compatible (susceptibility). Isolates 910, 912, 913, 919, 932 and 939 induced symptoms in all the clones, while their aggressiveness (disease severity) varied slightly (Table 2). The remaining 30 isolates were pathogenic to some of the different clones, while isolates 926, 927, 930 and 936 were only pathogenic on CCP-06. All clones were moderately-resistant to isolate 922. Analysis of the transformed data (Table 3) indicated differences ( $p \leq 0.05$ ) in the relative susceptibility of the different hosts, between isolates and in the host-isolate interaction. After seven days, the disease reaction type of the first and second young leaves of all the clones to each isolate was gener-

ally only slightly influenced by the surface inoculated (data not shown).

The younger first leaves of cashew seedlings were more susceptible than the older second leaves (Figure 1). This result is similar to observations on other host-*Colletotrichum* combinations (Bentes and Matsuoka, 2002; Waller, 1992). Disease was also less severe in attached than in detached leaves, although host reaction among the clones was not modified (Figure 1). Wounding invariably gave rise to necrotic lesions, even in clone-isolate combinations that were resistant when leaves were intact (Figure 2). Such resistance to *C. gloeosporioides* may therefore be associated with structural barriers that hinder penetration at the epidermal level.

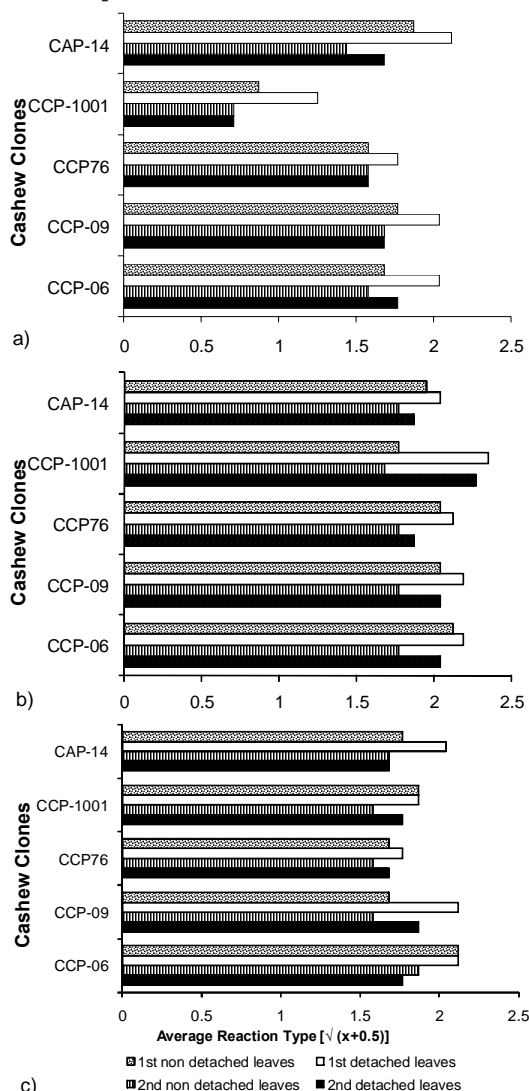


Figure 1 – Disease severity on the first (1st) and second (2nd) intact (detached and attached) leaves of *Anacardium occidentale* clones, 120 h after inoculation (abaxial surface) with LARS isolates of *Colletotrichum gloeosporioides*: a) 905; b) 910; c) 912. Averages (three experiments in triplicates) of the transformed data  $\leq 1.58$  (type 2) were scored as resistant; from 1.58 to 1.87 (types 2 and 3) as moderately resistant, and  $> 1.87$  (type 3) as susceptible

Table 2 – Reaction classes (RC)<sup>1</sup> and disease severity scores (DS)<sup>2</sup> on young detached first leaves of different clones of *Anacardium occidentale*, 120 h after inoculation (abaxial surface) with different isolates of *Colletotrichum gloeosporioides*, in a controlled environment.

Isolates (LARS)	CCP-06		CCP-09		CCP-76		CCP-1001		CAP-14	
	RC	DS	RC	DS	RC	DS	RC	DS	RC	DS
905	S	3.7 ± 0.6	S	3.7 ± 0.6	S	3.3 ± 0.6	R	1.0 ± 0	S	4.3 ± 0.6
906	S	4.0 ± 0	S	4.0 ± 0	S	5.0 ± 0	M	3.0 ± 0	S	4.0 ± 0
907	S	4.0 ± 0	R	1.0 ± 0	S	4.3 ± 0.6	R	0.3 ± 0.6	S	3.7 ± 0.6
908	S	4.7 ± 0.6	S	5.0 ± 0	S	4.7 ± 0.6	R	1.7 ± 0.6	M	3.0 ± 0
909	S	3.7 ± 0.6	S	4.0 ± 0	S	3.7 ± 0.6	R	1.0 ± 0	R	1.7 ± 0.6
910	S	4.3 ± 0.6	S	4.3 ± 0.6	S	4.0 ± 0	S	5.0 ± 0	S	4.0 ± 0
911	S	4.7 ± 0.6	S	4.7 ± 0.6	M	3.0 ± 0	R	1.0 ± 0	M	3.0 ± 0
912	S	5.0 ± 0	S	4.0 ± 0	S	4.3 ± 0.6	S	4.3 ± 0.6	S	3.3 ± 0.6
913	S	4.0 ± 0	S	4.0 ± 0	S	4.7 ± 0.6	S	4.0 ± 0	S	3.7 ± 0.6
914	S	5.0 ± 0	S	5.0 ± 0	M	3.0 ± 0	R	2.0 ± 0	M	3.0 ± 0
915	S	5.0 ± 0	S	4.0 ± 0	S	4.7 ± 0.6	M	2.7 ± 0.6	M	3.0 ± 0
916	S	5.0 ± 0	S	5.0 ± 0	S	5.0 ± 0	R	1.0 ± 0	R	2.0 ± 0
917	S	5.0 ± 0	S	4.3 ± 0.6	S	5.0 ± 0	S	4.7 ± 0.6	M	3.0 ± 0
918	S	5.0 ± 0	S	5.0 ± 0	M	3.0 ± 0	R	0	R	1.7 ± 0.6
919	S	5.0 ± 0	S	4.7 ± 0.6	S	4.3 ± 0.6	S	4.3 ± 0.6	S	3.7 ± 0.6
920	S	4.7 ± 0.6	S	4.3 ± 0.6	R	2.0 ± 0	R	2.0 ± 0	M	3.0 ± 0
921	S	5.0 ± 0	S	4.7 ± 0.6	R	2.0 ± 0	R	0	R	0
922	M	3.0 ± 0	M	3.0 ± 0	M	2.3 ± 0.6	M	3.0 ± 0	M	3.0 ± 0
923	S	4.0 ± 0	S	3.7 ± 0.6	S	4.0 ± 0	M	3.0 ± 0	M	3.0 ± 0
924	S	5.0 ± 0	S	4.3 ± 0.6	S	4.0 ± 0	R	2.0 ± 0	M	2.7 ± 0.6
925	S	3.7 ± 0.6	S	3.7 ± 0.6	S	4.0 ± 0	R	2.0 ± 0	S	3.7 ± 0.6
926	S	5.0 ± 0	R	1.0 ± 0	R	1.0 ± 0	R	1.0 ± 0	R	1.3 ± 0.6
927	S	4.0 ± 0	M	3.0 ± 0	M	3.0 ± 0	M	3.0 ± 0	M	2.3 ± 0.6
928	S	5.0 ± 0	S	5.0 ± 0	S	4.7 ± 0.6	R	1.0 ± 0	R	2.0 ± 0
929	S	4.0 ± 0	R	1.0 ± 0	S	4.7 ± 0.6	R	1.0 ± 0	M	3.0 ± 0
930	S	4.0 ± 0	R	1.0 ± 0	M	3.0 ± 0	R	0	R	1.0 ± 0
931	S	5.0 ± 0	S	4.7 ± 0.6	S	4.7 ± 0.6	R	1.0 ± 0	R	2.0 ± 0
932	S	5.0 ± 0	S	4.0 ± 0	S	5.0 ± 0	S	4.0 ± 0	S	4.0 ± 0
933	S	5.0 ± 0	S	5.0 ± 0	S	5.0 ± 0	R	2.0 ± 0	M	3.0 ± 0
934	S	5.0 ± 0	S	4.7 ± 0.6	S	5.0 ± 0	R	2.0 ± 0	S	4.7 ± 0.6
935	S	4.3 ± 0.6	S	3.7 ± 0.6	M	3.0 ± 0	M	3.0 ± 0	R	2.0 ± 0
936	S	4.0 ± 0	R	1.7 ± 0.6	M	3.0 ± 0	R	0	M	3.0 ± 0
937	S	5.0 ± 0	S	5.0 ± 0	R	0	R	0	R	2.0 ± 0
938	S	5.0 ± 0	S	4.3 ± 0.6	S	4.7 ± 0.6	M	3.0 ± 0	R	1.0 ± 0
939	S	5.0 ± 0	S	5.0 ± 0	S	4.0 ± 0	S	3.7 ± 0.6	S	3.7 ± 0.6
940	S	4.7 ± 0.6	S	4.0 ± 0	S	4.0 ± 0	M	3.0 ± 0	M	3.0 ± 0

<sup>1</sup>Scale of symptoms types: 0 = no symptoms; 1 = few small flecks (1 mm); 2 = flecks 2-3 mm, limited lesions; 3 = coalescent flecks, lesions 4-7 mm; 4 = water-soaked lesions, higher than 7 mm; 5 = spreading coalescent lesions with sporulation. Averages (three repetitions, three replicates each) of the non-transformed scores, were categorized into three broad reaction classes: Resistant (R: ≤ 2), Moderately Resistant (M: 2.1-3.0) and Susceptible (S: > 3.0). <sup>2</sup>Scale of disease severity: 0 = no necrotic tissue (NT); 1 = ≤ 5% NT; 2 = 6–20% NT; 3 = 21-35% NT; 4 = 36-60% NT; 5 = 61-100% NT. Averages (three repetitions, three replicates each) of the non-transformed data ≤ 2.0 suggested low aggressiveness, and > 3.0 suggested high aggressiveness.

Table 3 – Analysis of variance for the average disease severity scores (transformed data  $\sqrt{x+0.5}$ ), of three repetitions with three replicates each, on young detached first leaves (abaxial surface) of different clones of *Anacardium occidentale*, 120 h after inoculation with different isolates of *Colletotrichum gloeosporioides* in controlled environment.

Source	DF	SS	MS	VR (F)	Tab. F (1%)
Clones	4	33.67942	8.41986	946.2330 **	3.3715
Isolate	35	25.20646	0.72018	80.0798 **	1.6938
Clones x Isolates	140	37.32995	0.26664	29.6664 **	1.3746
Treatments	179	96.21583	0.53752	59.7686 **	1.3434
Error	360	3.23760	0.00899		
Total	539	99.45343	CV=4.91784%	LSD for clones = 0.21	LSD for isolates = 0.30

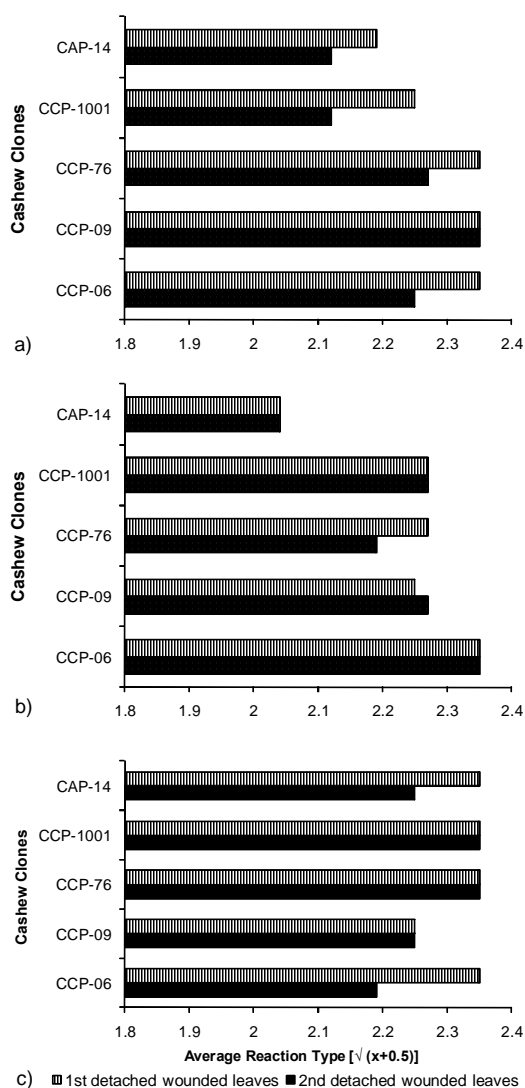


Figure 2 – Disease severity on the first (1st) and second (2nd) detached and injured leaves of *Anacardium occidentale* clones, 120 h after inoculation (abaxial surface) with LARS isolates of *Colletotrichum gloeosporioides*: a) 905; b) 910; c) 912. Averages (three experiments in triplicates) of the transformed data  $\leq 1.58$  (type 2) were scored as resistant; from 1.58 to 1.87 (types 2 and 3) as moderately resistant, and  $> 1.87$  (type 3) as susceptible.

Although the abaxial surface of young cashew leaves has a higher number of stomata (ca. 60,000-100,000  $\text{cm}^{-2}$ ) and trichomes (ca. 3,000  $\text{cm}^{-2}$ ) than the adaxial surface (no stomata and ca. 1,000  $\text{cm}^{-2}$  trichomes) (Ohler, 1979), which might suggest easier fungal penetration and infection in the former, inoculation of the later gave similar disease severity. Then, albeit penetration via stomata and cells beneath trichomes in young leaves occurs in cashew, it does not play the special role as it does in some other host-*C. gloeosporioides* interactions, such as tea, rubber and northern jointvetch (Ando and Hamaya, 1986; Senéchal et al., 1987; Tebeest et al., 1978).

Furthermore, as older cashew leaves have a tougher texture and higher number of trichomes on the adaxial surface than its younger leaves (Shaper and Chacko, 1991), and they are less susceptible to anthracnose, the few sites of infection may be due to invasion through injuries and stomatal openings. Perennial evergreen plants of resource-poor environments tend to have high levels of immobile defences, such as waxy cuticle, thick epidermis, tannins, flavonoids and lignins in the outer epidermal cell wall of leaves and in the outer cortical tissues of stems, without the metabolic costs of turnover. There is a correlation between structural barriers/tannin levels and the relative vulnerability of such plants to certain pathogens (Edwards, 1992).

Penetration through wounds have also been identified as essential for *C. musae* to cause crown and finger stalk rot in banana, for *C. gloeosporioides* to cause dieback in cassava, or for *G. cingulata* f. sp. *camelliae* to cause leaf blotch, canker and dieback in *Camellia* species (Van der Bruggen and Maraite, 1987; Waller, 1992). Van der Bruggen et al. (1990) found that *C. gloeosporioides* can penetrate tissues of a resistant cultivar of cassava through a puncture made either by a hot needle or an insect (*Pseudotheraptus devastans*). Infection of cashew by *C. gloeosporioides* in the field is often preceded by infestation with the insect *Helopeltis antonii* Müller (Varma and Balasundaran, 1990).

The five dwarf cashew clones used in this work were included in the field trials reported by Cardoso et al. (1999), when these authors evaluated disease levels on dwarf cashew clones over two seasons in Brazil, but their performance differed slightly, once that CAP-14 was the most resistant and CCP-09 the most susceptible in the

field. This probably happened because of the phenology of the crop, and the timing of leaf emergence (flushing) and blossoming. With CAP-14, for instance, the flushing and blossom phase preceded the main rainfall season, which is favourable for infection and epidemic spread. Hence, disease escape may play a critical role in addition to the genetic resistance of leaves to the pathogen.

The fact that isolates from host species other than cashew (LARS 905-909) were able to cause anthracnose in this plant is not a new observation for *C. gloeosporioides*. Several authors have reported cross-infections in controlled environmental conditions with *Colletotrichum* isolates from many fruit crops (Abang et al., 2006; Serra et al., 2008; Xiao et al., 2004). In some instances, isolates from different hosts had identical rDNA and similar mtDNA banding patterns (Alahakoon et al., 1994), while heterogeneity was observed within populations of *C. gloeosporioides* on almond, apple, avocado, mango and strawberry, all of which possess a teleomorphic stage (Freeman et al., 1998). Furthermore, a heterothallic isolate from pecan, able to infect apple, was found to mate with isolates of *C. gloeosporioides* f. sp. *aeschynomene*, a mycoherbicide of northern jointvetch weed (Cisar et al., 1994).

Polymorphism of the D2 sequences of the 28S rDNAs of the 36 isolates studied in the present work (data not shown) had high homology between themselves and with *C. gloeosporioides* from distantly-related hosts, being not enough to clearly distinguish inside the species [the four sub-groups of isolates have origins as distant as *Rubus brasiliensis* (LARS 905), *Dioscorea* sp.

(LARS 908), *Malpighia glabra* (LARS 909), *Spondias purpurea* L. (LARS 906), *Carica papaya* (LARS 907) and *A. occidentale* (LARS 910)]. But they were all able to infect different clones of cashew, providing evidences for the anthracnose cross-infection in cashew.

#### Pathogenicity assays with fruits and pseudofruits

The detached ripened pseudo-fruits (apples) of the commercial clones were all susceptible to isolates 905 and 910. A quiescent infection was verified on immature apples (except for the clone CCP-76), while immature nuts were more susceptible than mature ones (Table 4). In most tropical unripened fruits, *C. gloeosporioides* initially gains entry through the cuticle, but it is further restricted within the epidermal layer in a latent or dormant form. It is only able to colonise the tissues after fruit ripening, when host-physiological changes stimulate further pathogen development (Prusky and Plumbley, 1992).

The high content of tannins in cashew pseudo-fruit peel decreases with maturity (Wardowski and Ahrens, 1990). Tannins and their oxidation products are effective inhibitors of fungal enzymes, and epicatechin (tannin) from the pericarp of unripe resistant avocado fruits inhibits endopolygalacturonase, pectate-lyase and lipoxygenase (LOX) produced by *C. gloeosporioides*. Decay by *C. gloeosporioides* in post-harvest avocado is related to the degradation of an antifungal diene, which is catalyzed by LOX (Prusky and Plumbley, 1992; Wattad et al., 1994). Therefore, the concentration of tannins also plays a role in the restriction of anthracnose of young cashew pseudo-fruits.

Table 4 – Reaction class (RC)<sup>1</sup> scores on pseudo-fruits and attached nuts of different clones of *Anacardium occidentale* at different stages of development, 96 h after inoculation with isolates 905 and 910 of *Colletotrichum gloeosporioides* in a controlled environment.

Isolates	Growth Stage (weeks)	Clones							
		CCP-06		CCP-09		CCP-76		CCP-1001	
		nut	pseudo-fruit	nut	pseudo-fruit	nut	pseudo-fruit	nut	pseudo-fruit
905	1	3.0 ± 0*	2.0 ± 0	3.0 ± 0*	0	3.0 ± 0*	2.0 ± 0	3.0 ± 0*	0
	2	3.0 ± 0*	2.0 ± 0	3.0 ± 0*	0	3.0 ± 0*	3.3 ± 0.6	3.0 ± 0*	0
	3-4	3.0 ± 0*	3.0 ± 0	3.0 ± 0*	2.0 ± 0	3.0 ± 0*	4.0 ± 0	3.0 ± 0*	2.0 ± 0
	5-6	1.0 ± 0*	3.0 ± 0	1.0 ± 0*	3.0 ± 0	1.0 ± 0	4.0 ± 0	0	3.0 ± 0
	7-8	0	4.0 ± 0	0	4.0 ± 0	1.0 ± 0	4.0 ± 0	0	4.0 ± 0
	9	0	5.0 ± 0	0	5.0 ± 0	0	5.0 ± 0	0	5.0 ± 0
910	1	3.0 ± 0*	0.7 ± 0.30	3.0 ± 0*	0	3.0 ± 0*	2.0 ± 0	3.0 ± 0*	0
	2	3.0 ± 0*	1.0 ± 0	3.0 ± 0*	0	3.0 ± 0*	3.7 ± 0.6	2.7 ± 0.6*	0
	3-4	2.0 ± 0*	1.6 ± 0.6	3.0 ± 0*	0.3 ± 0.6	3.0 ± 0*	4.0 ± 0	3.0 ± 0*	1.6 ± 0.6
	5-6	0	3.0 ± 0	1.0 ± 0	2.0 ± 0	1.0 ± 0	4.0 ± 0	0	2.7 ± 0.6
	7-8	0	3.0 ± 0	0	3.0 ± 0	1.0 ± 0	4.0 ± 0	0	3.0 ± 0
	9	0	4.0 ± 0	0	4.0 ± 0	0	5.0 ± 0	0	4.7 ± 0.6

<sup>1</sup>Average (three repetitions, three replicates each) of the non-transformed data (symptom type) based on a 0-5 scale [0 = no symptoms; 1 = few small flecks (1 mm); 2 = flecks between 2-3 mm/limited lesions; 3 = coalescent flecks (lesions 4-7 mm); 4 = water-soaked lesions (>7 mm); 5 = spreading lesions with sporulation]. Data were categorized into three broad reaction classes: Resistant (R: ≤ 2.0), Moderately Resistant (M: 2.1-3.0) and Susceptible (S: >3). \*Green Exocarp.

## Conclusions

Although under a standardised set of conditions only a narrow range of dwarf cashew clones (five) was tested, with a small sample of isolates (36) from cashew and intercropped plants from some Brazilian regions, the clone CCP-06 was the most susceptible, CCP-1001 was the most resistant and none of the clones were significantly resistant to all of the tested isolates. These isolates also varied in their virulence/ aggressiveness towards cashew, and it is unlikely that based on the differential reactions found the isolates can be grouped into subpopulations. The genetic basis of the quantitative control of the differential interactions identified is not currently known. More work analyzing field populations of the pathogen by genetic markers is required.

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