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Communications in Plant Sciences (January-June 2016), Volume 6, Issues 1-2, Pages 19-25
Commun. Plant Sci. (ISSN 2237-4027)
www.complantsci.wordpress.com
Manuscript received on June 08, 2016, and published on July 05, 2016

Sources, survival and transmission of *Cryptosporiopsis* sp., leaf and nut blight pathogen of cashew (*Anacardium occidentale* Linn)

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ABSTRACT

The germination of conidia was studied from 2 hours to 16 hours after incubation at an interval of two hours period. The germination of macroconidia microscopically was followed to understand the timing of key events. Ten infected cashew nuts were placed each on a Petri dish containing malt extract agar and incubated at 27 °C for 10 days. *Cryptosporiopsis* sp. pathogen was detected in nut samples of cashew clones. Leaf and nut blight pathogen remained viable up to one year in plant debris stored under laboratory condition at a minimum temperature range from 4 to 5 °C. There was a rapid population decline on viable counts of *Cryptosporiopsis* sp. recovered from sterile and unsterile soil after various periods of time. In debris buried at a depth of 7 cm in sterile or unsterile soil they survived for four months with 8% and 5% of disease samples with viable pathogen. Saprophytic survival capacity of the fungus *Cryptosporiopsis* sp. in cashew field revealed that the pathogen survival was 80% up to four months of incubation but fell to 40% after 6 months. It was demonstrated that plant debris, soil and nuts could harbour sufficient inoculum to cause disease in new plants.

Keywords: Survival, Fungus, Viability, Conidia, Germination.

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INTRODUCTION

Cashew (*Anacardium occidentale* L.) is one of the most popular tree nuts on the local and world markets because of its competitive price, long shelf life, relatively low fat content and excellent flavour. However, despite the strong market demand, total production of raw nuts is going down in recent years due to fungal diseases. Leaf and nut blight caused by *Cryptosporiopsis* sp. affecting cashew nut production in Tanzania, causes 48.4% crop loss annually (ACRR 2006). Direct infection of young leaves and nuts is a major effect (Sijaona et al. 2006).

Until 2005, *Cryptosporiopsis* sp. (leaf and nut blight) was infrequent pathogen of Anacardiaceace hosts throughout East Africa. Zhu et al. (2012) reported that several *Cryptosporiopsis* species are endophytes whereas some of the species are pathogenic to woody plants causing plant diseases. Ciesla et al. (1996) found out that *C. eucalypti* spread through rain splash and wind. The fungus may be transmitted through contaminated seed or chaff. The severe outbreaks of cashew leaf and nut blight in Tanzania and more recently in Kenya may be related to lack of knowledge of the survival ability of the fungus. Fungal pathogens have been shown to overwinter by means of spores and structures like mycelium or sclerotia for many months causing infections in succeeding seasons. Govindarao and Dakshinamurti (1964) reported a fungal pathogen that overwintered as mycelium in dormant state on the branches. The pathogen was able to cause new infections on new shoots by conidia production under suitable conditions.

Suhag and Gover (1973) observed that Sphaceloma ampelinum pathogen survived in the cane as the mycelium and was able to produce conidia that caused infection. The pathogen was recorded to penetrating and causing infections in unwounded shoots, twigs, stems and tendrils. Suhag and Grover (1977) reported that plant debris infected by pathogens is the principal source of infection. Pathogens can resist harsh summer and winter conditions. Ellis and Erincik (2005) reported that a fungus pathogen overwintered as sclerotia and remained viable for 3-5 years. Peres et al. (2005) studied *Colletotrichum acutatum*, a strawberry anthracnose causal agent. He reported the pathogen was a poor saprophyte but could survive for various periods.

This present investigations identify the inoculum sources and evaluate their implication in the survival and transmission of cashew leaf and nut blight pathogen.

MATERIAL AND METHODS

Effect of temperature and leaf age on conidial germination. Conidia of *Cryptosporiopsis* sp. from potato dextrose agar plate were dusted onto glass microscope slides using a paint brush. Two slides were suspended on a rubber bung seated over water in a sealed plastic container (10 x 50 cm depth). Individual containers were placed in incubating rooms at a range of temperatures from 10-35 °C, with five replicate containers for each temperature treatment. After 3 days incubation, the percentage of conidial germination was determined using a microscope.

Leaf discs (1 cm diam.) were cut from 2 week- and 8 week-old leaves from seedlings after planting. Leaf discs were arranged on fine gauze with upper leaf surfaces facing upward and placed on rubber bungs in plastic containers. Conidia of *Cryptosporiopsis* sp. from potato dextrose agar plate were dusted onto these discs. Containers were incubated at 25 °C for 3 days and percentage germination of conidia on leaf discs was determined under a microscope.

The germination of conidia was studied from 2 hours to 16 hours after incubation at an interval of two hours period. Leaf and nut blight conidia were brushed off from infected cashew leaves and dropped on to PDA and incubated at 27 °C. Fifteen days old culture of *Cryptosporiopsis* sp. spore suspension (106 spores mL^{-1}) was prepared in sterilized tap water. A drop of spore suspension was placed on clean sterilized slides. Such slides were kept in moist chamber at room temperature. Observations on spore germination (x400) were recorded at 2, 4, 6, 8, 10, 12, 14 and 16 hours after incubation for spore germination. Germination was considered if the length of the germ tube was longer than the spore. Three replications were maintained for each treatment. From each slide, 100 spore counts were taken for percentage germination calculation and data were analyzed statistically.

Survival of the pathogen in cashew infected leaves and seeds under different storage conditions. Ten infected cashew nuts were placed each on a petri dish containing malt extract agar and incubated at 27 °C for 10 days. Similar samples were surface sterilized by immersing for 10 s in 70% methanol, followed by immersion in sodium hypochlorite for 1 min and rinsing in running tap water for 2 min before incubation. The samples were examined after 10 days for the presence of the pathogens.

The present investigation on viability and survival of *Cryptosporiopsis* sp. was undertaken as a part of epidemiological study during 2011-12 at Naliendele Research Institute, Mtwara to obtain information about

the perpetuation of the pathogen during the off-season. The diseased leaf material was brought to the lab and air dried in shade for 24 hours. Later it was divided into five lots and packed in paper folds and kept at different storage conditions viz., freeze (4–5 ºC), under tree shade (18–22 ºC), room temperature (20–25 ºC), greenhouse (25–28 ºC) and field condition (28–30 ºC) in separate lots. Periodical isolations at monthly interval were made on selective medium to determine its survival till the pathogen lost its viability. For each time observation, pathogen viability was confirmed by observing the growth and spore germination. Percent germination of conidia on each type of stored leaf was recorded before their preservation. The viability of conidia on leaf under different storage conditions were regularly examined by checking germination under a microscope.

Survival of leaf and nut blight pathogen in sterilized and unsterilized soils. Dry conidia of the pathogen were obtained from 20 heavily sporulating cultures on malt extract agar. Equal quantities of inoculum were added separately to 2 g of sterile/unsterile soil in small nylon bags and buried 7 cm deep in 15 cm diameter pots filled with sterile/unsterile soil, respectively. The pots were taken outside in April 2012. Samples were taken immediately at 5-day intervals for 10 weeks by removing 0.2 g of soil from each nylon bag and placing it in 10 mL of 2.5% malt extract agar containing 0.3% agar. The suspension was shaken to disperse the soil, and then 2ml aliquots were placed on selective agar plates and spread evenly with a sterile glass rod. Plates were incubated at 27 °C with a 12-h day and the mean number of germinating conidia on five plates was assessed after 3 days.

RESULTS

Effect of temperature and leaf age on conidial germination. Conidial germination of *Cryptosporiopsis* species infecting cashew was significantly affected by temperature ($P<0.0001$). Greatest germination (up to 80%) was recorded at 30 °C, although germination was generally low after 3 days of incubation (Figure 1). Some conidia germinated at 20 and 30°C but no germination occurred at 10 or 35 °C. The optimal temperature for germination was between 25 and 30 °C, revealing more than 70% of conidia germinated. Germination of the *Cryptosporiopsis* sp. pathogen occurred over a wide temperature range (10-35 °C). From these studies it is evident that infection will not take place below 10 °C.

Only a small proportion (1.6%) of the conidia germinated when placed on leaf discs from old leaves (8 weeks-old), but many conidia germinated (94%) on leaf discs from 2 weeks old leaves. Overall, conidial germination increased significantly with longer incubation times, especially from 4 to 12 h. Macroconidia of cashew leaf and nut blight are typical of *Cryptosporiopsis* having ellipsoidal, rounded at the apex, tapering into a scar at the base. Within the 2 h of incubation, 60% of swollen macroconidia were observed. However, germination was not revealed at 2 hours after incubation. Appearance of Germ tubes occurred in spores by 4 h (Figure 2). By 10 h, over 40% of macroconidia had at least one germ tube.

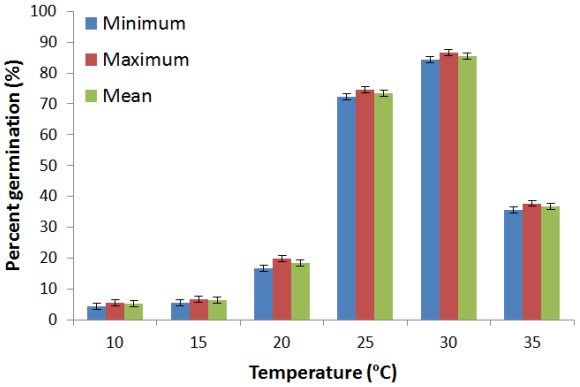

Figure 1. Percentage germination of *Cryptosporiopsis* sp. conidia on glass slides at different temperatures in saturated humidity.

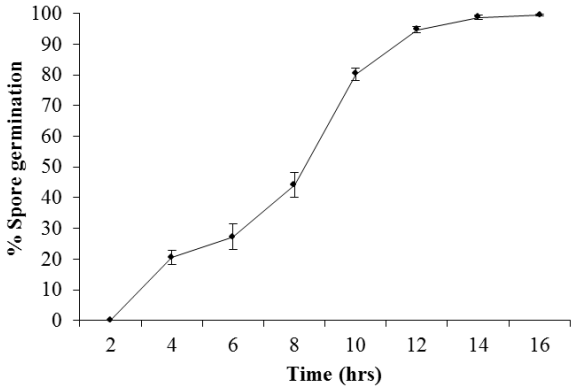

Figure 2. Conidial germination of *Cryptosporiopsis* sp. During the time.

The spore germination at various incubation periods was found to differ significantly. Maximum germination (99%) was observed after incubating the conidia for 16 h. A majority of spores had a single germ tube. Germ tubes developed hyphal branching after 12h of incubation. More than 94% of macroconidia had germinated after 12 h of incubation. Spore germination after 16h of incubation was over 99%. Germination tubes developed branched hyphae after 24h of incubation. At this time developing conidiophores started to appear (Figure 3). Terminal end germination was preferred by leaf and nut blight macroconidia.

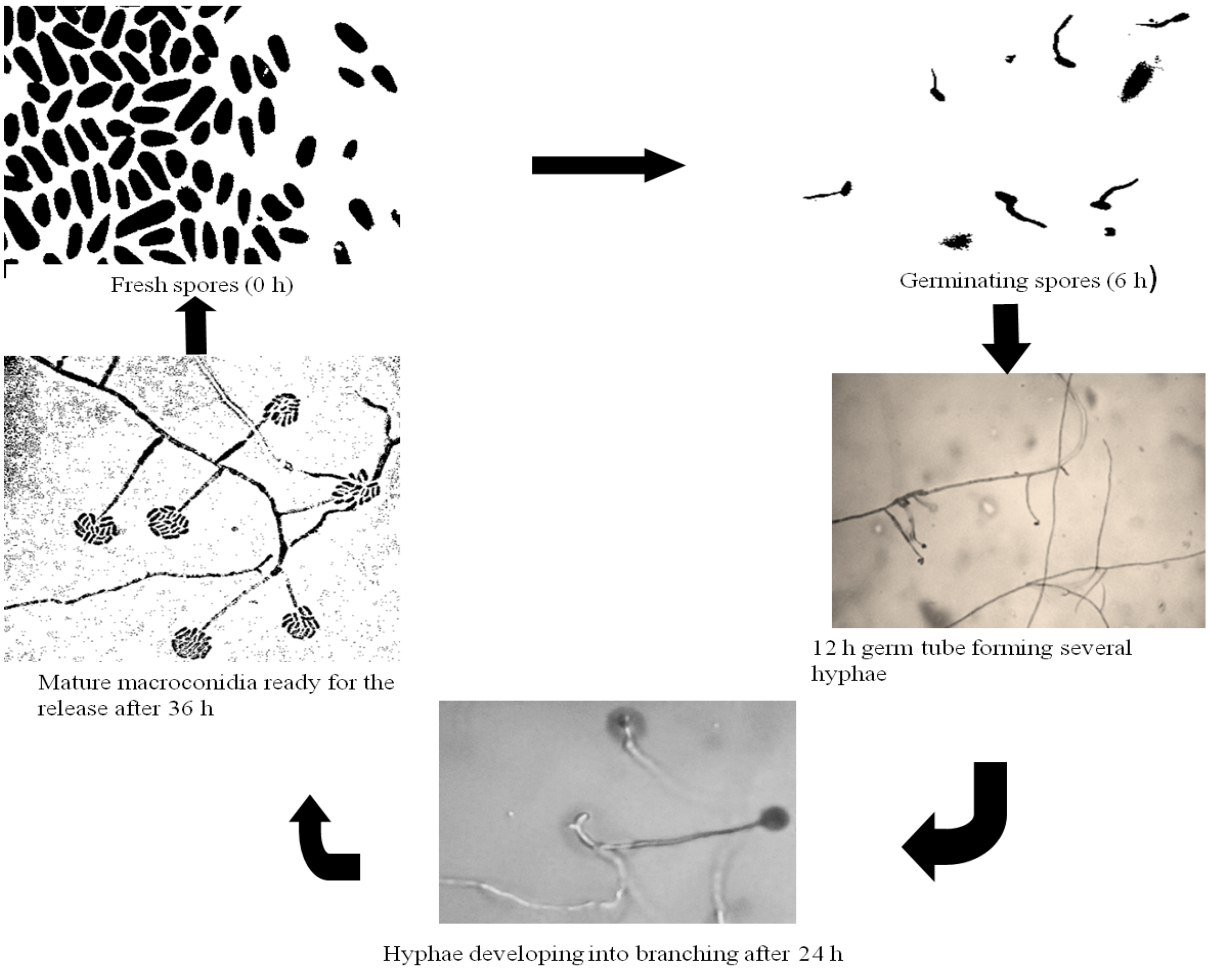

Figure 3. Light micrographs of *Cryptosporiopsis* sp, macroconidia germination causing cashew blight (X100).

Survival of the pathogen in cashew infected leaves and seeds under different storage conditions. Studies on survival of *Cryptosporiopsis* sp. and germination percentage in cashew seeds was undertaken for a period of 360 days at an interval of one month and the results are presented in Table 1. Data revealed that there was a sharp decline in survivability of the fungus over the period of storage, however the germination percentage increased with time, further it was observed that initially *Cryptosporiopsis* sp. fungus recorded 25.54 per cent survival in seed at 30 days. Later there was a gradual decrease in the survivability of fungus.

The fungus remained viable at low percentage (5.45) up to 360 days. The germination percentage gradually increased with increase in the storage period and reached up to 85 per cent after 360 days of storage.

Cashew leaf blight pathogen *Cryptosporiopsis* sp. remained viable up to one year in plant debris stored under laboratory condition at a minimum temperature range from 4 to 5 °C (Table 2). The present investigation showed that leaf and nut blight pathogen remained viable for a maximum time of 12 months under 4-5 °C in the refrigerator, 8 months under tree Shade (18-22 °C), 8 months under laboratory conditions (20-25 °C), 4.5 months under greenhouse conditions (25-28 °C) and 3.5 months under natural/field conditions.

Table 1: Studies on survival of *Cryptosporiopsis* sp in cashew seeds and its effect on germination.

Sl no.	Storage period (days)	Percent Survival of fungus	Percent germination of seed
1	30	25.54	45
2	60	24.34	55
3	90	23.23	58
4	120	20.12	62
5	150	19.22	64
6	180	18.56	65
7	210	16.4	70
8	240	15.45	73
9	270	14.35	75
10	300	14.22	79
11	330	10.33	80
12	360	5.45	85

Survival of the pathogen in sterilized and unsterilized soils. *Cryptosporiopsis* sp. causing leaf blight of cashew was conducted in the sterilized and unsterilized soil under in vivo condition and the results

obtained are given in Tables 3 and 4. Pathogen survived for 28 weeks and 16 weeks under sterilized and unsterilized soils, respectively. *Cryptosporiopsis* sp. survived in 45% and 30% of the diseased samples on both surfaces of sterile and unsterile soils respectively (Table 3). However, when buried in sterilized and unsterilized soil, the pathogen could survive on 8% and 5% of the diseased samples, respectively.

Saprophytic survival capacity of the fungus *Cryptosporiopsis* sp. in cashew field revealed that the pathogen survival was 80 per cent up to four months of incubation but fell to 40 per cent after 6 months (Table 4). The fungus *Cryptosporiopsis* sp. overwintered on cashew plant debris. Spores produced on infected leaves stored at 8 °C and 25 °C were highly pathogenic to cashew plants.

Table 2. Survival of conidia of *Cryptosporiopsis* sp .causing blight of cashew under different temperatures.

SI. No	Percent viable conidia at different storage conditions					
	Storage period (Days)	Freeze (4-5°C)	Tree Shade (18-22 °C)	Room/Lab (20-25 °C)	Green house (25-28 °C)	Field (28-30 °C)
1	15	90.31	82.15	80.18	76.73	66.17
2	30	88.25	76.52	77.26	68.15	57.23
3	45	87.10	70.23	73.10	61.24	42.30
4	60	85.75	67.88	69.55	55.18	30.75
5	75	83.23	63.40	65.33	46.29	15.20
6	90	80.41	54.73	56.81	23.11	9.23
7	105	77.50	48.25	50.42	10.35	4.56
8	120	73.62	40.08	42.36	8.56	0
9	135	70.78	35.66	36.91	3.67	0
10	150	67.45	30.19	29.24	0	0
11	165	63.81	25.43	21.66	0	0
12	180	60.11	20.78	15.38	0	0
13	195	57.75	16.76	14.21	0	0
15	210	54.75	15.65	13.05	0	0
16	225	53.72	10.03	10.65	0	0
17	240	48.98	6.34	8.89	0	0
18	255	45.05	0	0	0	0
19	270	38.15	0	0	0	0
20	285	35.78	0	0	0	0
21	300	30.15	0	0	0	0
22	315	29.01	0	0	0	0
23	330	25.22	0	0	0	0
24	345	20.19	0	0	0	0
25	360	10.21	0	0	0	0

Table 3. Percentage of diseased leaf samples with viable inoculum of *Cryptosporiopsis* sp .after incubation in soil or on the soil surface.

Treatment	Time (months)									
	1	2	3	4	5	6	7	8	9	10
Cryptosporiopsis sp										
On surface of unsterile soil	100	95	95	80	50	40	30	0	0	0
On surface of sterile soil	100	100	100	90	55	48	45	0	0	0
Buried in unsterile soil	45	57	22	5	-a	-	-	-	-	-
Buried in sterile soil	75	71	26	8	-	-	-	-	-	-

a-, No leaf debris recovered.

Table 4. Percentage of viable counts of *Cryptosporiopsis* sp. recovered from sterile and unsterile soil after various periods of time.

Pathogen/soil	Mean no. germinating conidia initially	Time(days)									
		0	5	10	15	20	25	30	35	40	45
Cryptosporiopsis sp											
Unsterile soil	3872	100	85	62	27	23	4	3	0.1	0	0
Sterile soil	506	100	90	70	48	14	9	8	0.3	0.1	0

DISCUSSION

The information temperature is useful for the prediction of infection periods. The results obtained with the in vitro germination of *Cryptosporiopsis* sp. clearly show how temperature affects spore germination. Various incubation periods affects the leaf and nut spore germination. The life cycle of leaf and nut blight conidia starts with the germination of infectious propagules. This determines the infection and host penetration. Maximum germination reached over 99% after 16h of incubation. However, after 12 hours of incubation there was 90 percent spore germination. Earlier studies by Ullstrup (1966) reported conidial germination taking place 6 to 18 hours after incubation. In this study, an incubation period of four hours was needed for germination of *Cryptosporiopsis* sp. This present research showed that conidial germination has a role in disease epidemiology.

The availability of young tender cashew leaves showed to trigger an infection in all cashew genotypes. Mature leaves appeared to be resistant from *Cryptosporiopsis* sp. infection causing cashew blight. Therefore the control measures of *Cryptosporiopsis* sp. causing cashew blight can be scheduled to coincide with specific host phenological observations like the availability of young tender leaves.

Leaf and nut blight pathogen remained viable for 8, 3.5 and 12 months in plant materials stored at under laboratory (20-25 °C), natural field (28-30 °C) and refrigerated conditions (4-5 °C), respectively. Accumulation of plant debris from successive seasons, owing to reduced cultivations, was probably responsible for increases in leaf and nut blight (*Cryptosporiopsis* sp.) in East Africa. The disease has also become more common and severe in Tanzania in recent years, probably as a result of the increasing popularity of minimum cultivation techniques. Infected cashew debris can be effectively reduced by stubble burning, which in many cases reduces rather than eradicate inoculum. Infected stem and leaf debris act as a potent source of inoculum for many diseases. *Cryptosporiopsis* sp. (cause of leaf and nut blight) can survive on intact cashew leaves, stem and nuts. The short generation time and prolific sporulation of the *Cryptosporiopsis* fungus ensures such rapid build-up of blight. The results from present investigation indicate clearly that cashew leaf and nut blight pathogen overwinters in plant debris. The overwintering is likely to serve as a secondary inoculum source for succeeding season. Ellis and Erincik (2005) reported that anthracnose pathogen in grapes was able to survive up to 941 days.

The fungus Cryptosporiopsis sp overwintered on infected cashew leaves and during the following flushing season spores were formed on the mycelium located in and on old lesions. The survival of *Cryptosporiopsis* sp. from infested cashew residue showed that local epidemics of leaf and nut blight caused by *Cryptosporiopsis* sp. mostly originated from conidia on infested cashew residues.

The germination percentage gradually increased with increase in the storage period. The results indicate the potentiality of cashew seed as a carrier of primary inoculum. This study indicates that since *Cryptosporiopsis* sp. can survive for a longer period, care must be taken while exchanging the cashew seeds for sowing purpose. Leaf and nut blight pathogen can be inactivated by soaking seeds for long periods in a systemic or protectant fungicide.

The present study suggests that there was a potential secondary inoculum existing that can cause infection. Leaf and nut blight saprophytic ability reduced considerably in unsterilized soils when compared to sterilized soils. Biological antagonism and competition could be attributed to the poor saprophytic ability of leaf and nut blight pathogen. Shree and Luke (1983) reported similar findings in sorghum field where *E. turcicum* survival ability reduced with time. The reduction in saprophytic ability of the fungus in unsterilized soils suggests that the soil borne inoculum is not playing an important role in inoculum sources in the following seasons. The poor saprophytic ability of the fungus might be attributed to the antagonists encounter and low oxygen in soil. Similar studies by Peres et al. (2005) reported that *C. acutatum*, a strawberry anthracnose causal agent was a poor saprophytic pathogen but was able to survive in soil for various periods.

The present study provided evidence that there existed a potential secondary inoculum in the cashew field that can cause new infections. It was revealed that pathogen survival ability in soil was achieved by the leaf and nut blight pathogen ability to colonize on diseased cashew debris from the previous season. It is revealed that the cashew debris in the field upper layer should be considered as the possible infection sources. The cashew debris may be assisting in disease perpetuation. Suhag and Grover (1973) reported that *C. acutatum* overwintered as and its mycelium was able to produce conidia under suitable weather conditions.

Integrating infected cashew debris inside the soil will accomplish inoculum reduction. This is because soil saprophytes microbial activity will be favored in optimal moisture and temperature conditions thus hastening decomposition of cashew debris. This will reduce the ability of the fungus structures to survive to the next season therefore providing inoculum. The leaf and nut blight mycelium growing in the soil might be

subjected to lysis therefore chances of forming survival structures are reduced.

CONCLUSION

Leaf and nut blight conidial germination has a role in disease epidemiology. Availability of young cashew leaves triggered an infection in all cashew genotypes. *Cryptosporiopsis* sp. can survive on intact cashew leaves, stem and nuts. The short generation time and prolific sporulation of the *Cryptosporiopsis* fungus ensures such rapid build-up of blight. The study indicates the potentiality of cashew seed as a carrier of primary inoculum. *Cryptosporiopsis* sp. can survive for a longer period and therefore care must be taken while exchanging cashew seeds for sowing purpose. That pathogen is able to survive in soil by colonizing on diseased cashew debris from the previous season.

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RESUMO

Fontes, sobrevivência e transmissão de *Cryptosporiopsis* sp., patógeno de folha e castanha de caju (*Anacardium occidentale* Linn). A germinação de conídios foi estudada entre 2 horas e 16 horas após a incubação, com um intervalo de duas horas por período. A germinação de macroconídios foi analisada microscopicamente para entender o calendário de eventos-chave. Dez castanhas de caju infectadas foram colocadas em placa de Petri contendo ágar de extrato de malte, incubadas a 27 ° C durante 10 dias. O patógeno *Cryptosporiopsis* sp. foi detectado em amostras de castanha de clones de cajueiro. O patógeno de folha e castanha permaneceu viável até um ano em restos vegetais armazenados sob condições de laboratório em diferentes temperaturas mínima de 4 para 5 °C. Houve rápido declínio da população viável nas contagens de *Cryptosporiopsis* sp. extraído a partir do solo esterilizado e não esterilizado depois de vários períodos de tempo. Em restos enterrados a uma profundidade de 7 cm no solo estéril ou não estéril, eles sobreviveram por quatro meses com 8% e 5% dos casos de doença com patógeno viável. A capacidade de sobrevivência saprofítica do fungo *Cryptosporiopsis* sp. no caju em campo revelou que a sobrevivência do patógeno foi de 80% até quatro meses de incubação, mas reduziu para 40% após 6 meses. Foi demonstrado que restos vegetais, solo e castanhas podem abrigar inóculo suficiente para causar doenças em plantas novas.

Palavras-chave: Sobrevivência, Fungo, Viabilidade, Conídio, Germinação.

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