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Chlamydospore formation by *Corynespora cassiicola*

Ricardo Ribeiro Oliveira[1], Bárbara de Melo Aguiar[1], Dauri José Tessmann[1], Valérie Pujade-Renaud[2,3] & João Batista Vida[1]

[1]Departamento de Agronomia, Universidade Estadual de Maringá, 87020-900, Maringá, PR, Brazil; [2]CIRAD, UMR AGAP, F-63000 Clermont-Ferrand, France; [3]Clermont Université, Université Blaise Pascal, UMR 547 PIAF, BP 10448, F-63000, Clermont-Ferrand, France

Author for correspondence: João Batista Vida, e-mail: jbvida@uem.br

ABSTRACT

The fungus *Corynespora cassiicola* is an important pathogen that causes necrotic lesions in several plant species. Saprophytism and parasitism habits of plants are common survival strategies for this pathogen. Few studies referred to the formation of chlamydospores by *C. cassiicola*. The objective of this study was to test the formation of chlamydospores by several *C. cassiicola* isolates from different agronomic crops and weeds. Fifteen isolates were analysed by *in vivo* and *in vitro* tests. Six isolates from four host plants (*Cucumis sativus*, *Lantana camara*, *Malpighia glabra* and *Vernonia cinerea*) were able to produce chlamydospores, both *in vitro* and *in vivo*. *In vitro*, chlamydospore production was highest for the *Malpighia glabra* isolate and one *Cucumis sativus* isolate, intermediate for two other *C. sativus* isolates, and lowest for the *Vernonia cinerea* and *Lantana camara* isolates. However, no difference in the relative number of chlamydospores produced among isolates was observed *in vivo*.
Key words: leaf spot, survival, target spot.

Chlamydospores are resting structures that are mainly produced by root pathogens (Machardy & Beckman, 1981; Meronuck & Pepper, 1968) and are an effective survival strategy in the absence of host tissue (Garrett, 1970). Furthermore, chlamydospores may be an important primary inoculum source for phytopathogenic fungi (Agrios, 2005; Amorim, 1995).

Corynespora cassiicola is a leaf pathogen from tropical and sub-tropical climates infecting more than 300 plant species (Farr & Rossman, 2011). In Brazil, this pathogen causes serious damage on soybean (*Glycine max*) (Almeida et al., 2001), parthenocarpic cucumber (*Cucumis sativus*) (Verzignassi et al., 2003) and tomato (*Lycopersicon esculentum*) (Leroy & Lourd, 1989).

In spite of the extensive research about *C. cassiicola*, little is known about its capacity to produce chlamydospores. Olive et al. (1945), Demidova (1962) and Sarma & Nayudu (1971) reported the formation of chlamydospores by isolates of *C. cassiicola* from *Vigna sinensis*, *C. sativus* and *Solanum melongena*, respectively. The primary survival strategies of *C. cassiicola* are believed to be through the parasitism of host plants and saprophytic behaviour, as reported on soybean (Almeida et al., 2001), sesame (Wulff & Pascholati, 1997), in cucurbitaceous stubble and weeds (Rêgo & Carrijo, 2000), and in parasitizing weeds (Souza & Silva, 2001; Cutrim & Silva, 2003). Thus, the aim of this research was to evaluate, *in vitro* and *in vivo*, chlamydospore formation of 15 isolates of *C. cassiicola* from several host species.

The isolates of *C. cassiicola* used in this study were obtained from single-spore cultures growing on potato dextrose agar (PDA) (Table 1).

For the evaluation of the *in vitro* chlamydospore production, each isolate was subcultured five times in Petri dishes containing PDA, as described by Olive et al. (1945), for 20 days each time, in a heated chamber at 25±2ºC under continuous fluorescent light. At the end of the fifth cultivation, the agar surface was scraped in the central region of each Petri dish quadrant, and a mycelium sample was obtained and analysed using an optical microscope equipped with an objective ocular micrometer to quantify and measure the chlamydospores.

For the *in vivo* chlamydospore production, seedlings of *C. sativus*, *L. esculentum*, *Lactuca sativa*, *Cucurbita maxima*, *G. max*, *Lantana camara*, *Malpighia glabra*, *Pipper hispidinervium*, *Coleus barbatus*, *Commelina benghalensis* and *Vernonia cinerea* were grown in plastic pots containing a sterilized commercial substrate (pine bark dust/vermiculite) which had been autoclaved for two periods of 1 hour at 120ºC at intervals of 24 hours.

Seedlings were inoculated with 1 x 10^4 spores/mL grown on PDA medium for 12 days. All leaves of the host plants were inoculated by atomisation of the conidia suspension onto the leaf surface (until the solution ran off the tissue) using the DeVilbiss n°15 atomiser. The plants were maintained in a humidity chamber for 48 hours at 25±2ºC with a 12 hour photoperiod. The plant development stage at inoculation was recorded as the number of leaves present (Table 1).

TABLE 1 - Chlamydospore formation by *Corynespora cassiicola* isolates from different host plants on PDA medium and on inoculated leaves of different host plants

Isolate	Host plant	Plant development stage (number of leavesfully expanded)	Formation of chlamydospores[1]		Average dimension (length x width [µm])[2]	
			In vitro	*In vivo*	*In vitro*	*In vivo*
IA	*Cucumis sativus*	4	++	+	14.38 x 12.50	14.78 x 14.22
PB	*Cucumis sativus*	4	++	+	15.00 x 14.38	14.65 x 14.17
JQ	*Cucumis sativus*	4	+++	+	13.13 x 13.75	13.56 x 13.46
LP05	*Cucurbita maxima*	4	-		-	-
493AA	*Glycine max*	4	-		-	-
777AA	*Glycine max*	4	-		-	-
LP02	*Lycopersicon esculentum*	5	-		-	-
GS01	*Carica papaya*	6	-		-	-
JMP220	*Lantana camara*	8	+	+	13.75 x 12.50	13.64 x 12.53
LP04	*Malpighia glabra*	14	+++	+	14.38 x 13.10	13.08 x 12.67
LP01	*Lactuca sativa*	10	-		-	-
LP07	*Piper hispidinervium*	12	-		-	-
RWB321	*Coleus barbatus*	7	-		-	-
CP03	*Commelina benghalensis*	12	-		-	-
GS02	*Vernonia cinerea*	6	+	+	12.50 x 12.50	13.12 x 12.79

[1]Relative amount of chlamydospores: (-) chlamydospores absent, (+) up to 2 chlamydospores per field of view at 400x magnification, (++) 3 to 5 chlamydospores and (+++) 6 to 22 chlamydospores;
[2]Average of 40 chlamydospores from each replication.

When large necrotic lesions had formed on inoculated leaves, approximately 10 to 20 mm in diameter, the leaves were detached and placed in a laminar flow cabinet where they were disinfected for 1 min in 0.5% sodium hypochlorite. After disinfection, the leaves were placed in acrylic boxes containing two sterilised sheets of filter paper moistened with sterilised distilled water. The acrylic boxes were maintained at 24°C with a 12 hours photoperiod. When the fungal growth was observed on the leaves, mycelial fragments were examined under an optical microscope for the presence of chlamydospores. The relative quantity of chlamydospores and the dimensions of 40 chlamydospores from each repetition were collected for each chlamydospores producing isolate. The experiment was completely randomised, with five repetitions for each isolate. Each Petri dish (*in vitro*) or leaf (*in vivo*) was considered an experimental unit. The experiment was repeated twice.

From the fifteen isolates analysed, only six produced chlamydospores both *in vivo* and *in vitro* (Table 1). These were the three isolates from *C. sativus* and the isolates from *L. camara*, *M. glabra*, and *V. cinerea*.

Differences were observed among the six isolates in the amount of chlamydospores formed *in vitro*. One isolate from *C. sativus* (JQ) produced a high quantity of chlamydospores, while the other two (IA and PB) had a medium production, under the same conditions. The isolates from *L. camara* and *V. cinerea* produced a low amount of chlamydospores. The isolate from *M. glabra* produced a high quantity of chlamydospores, comparable to that of the *C. sativus* isolate JQ (Table 1).

In vivo, mycelium growth occurred on the surface of the leaf lesions of all the host plants inoculated with *C. cassiicola* after five days of incubation in the humidity chamber. However, only the six isolates mentioned above developed chlamydospores. By comparing the *in vivo* and *in vitro* conditions, it appeared that the PDA growth medium was more favourable for chlamydospore production than the host leaf tissue. The relative abundance of chlamydospores produced *in vivo* was low and similar for all six isolates (Table 1).

The average dimensions of the chlamydospores observed *in vitro* were 13.86 µm in length and 13.12 µm in width. The largest chlamydospore size was found for the PB (*C. sativus*) isolate (15.00 µm x 14.38 µm), and the smallest was observed for the GS02 (*V. cinerea*) isolate (12.50 µm x 12.50 µm) (Table 1). We found that chlamydospore formation *in vitro* occurred in three possible forms: terminal, intercalary or chains of up to five chlamydospores (Figure 1), regardless of the isolate. Chlamydospores produced *in vivo* exhibited similar dimensions; however, in contrast to the results obtained under *in vitro*, only terminal and intercalary chlamydospores were observed (Figure 1 A, B and C).

In the literature, there are only two reports regarding chlamydospores formation by *C. cassiicola in vivo* (Demidova, 1962; Sarma & Nayudu, 1971). The authors reported the importance of this structure to survival of the fungus in plant debris. Chlamydospores were also observed by Olive et al. (1945) in old cultures from successive transfers. Here we present evidence of chlamydospore formation by *C. cassiicola* in *in vivo* and *in vitro* tests, using several isolates and plant hosts. We suggest that the

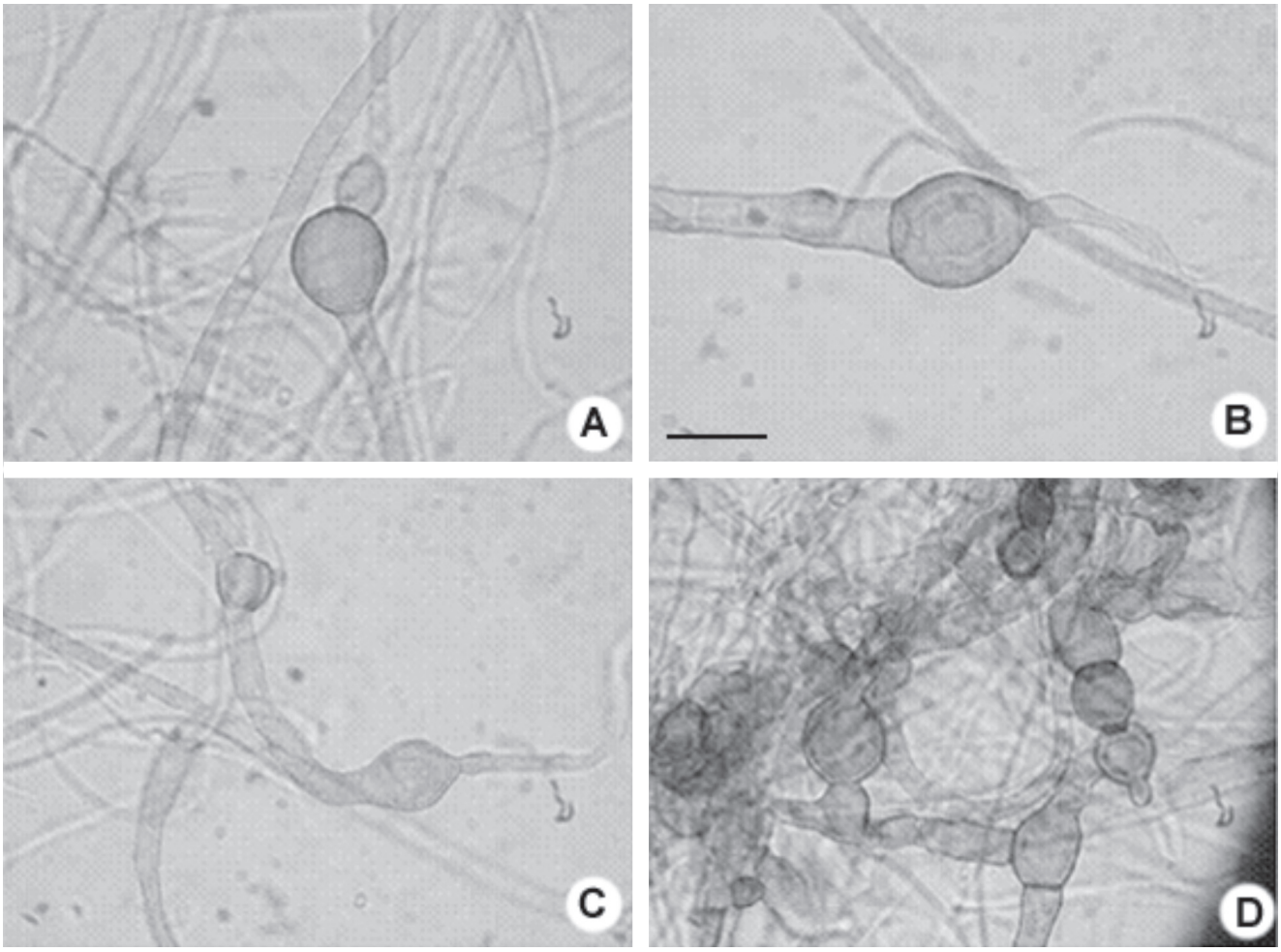

FIGURE 1 - Chlamydospores formed *in vitro* and *in vivo*: **A.** terminal formation; **B** and **C**. intercalary formations; **D.** chain formations. Scale bar for B = 10 µm.

chlamydospores of *C. cassiicola* produced *in vivo* may contribute as an important source of primary inoculum.

Chlamydospores allow the fungi to survive in soil or plant debris. Meronuck & Peper (1968) reported the formation of chlamydospores among *Bipolaris sorokiniana* conidia. Chinn (1976) showed that these structures may be the survival forms of this pathogen in the soil. Although *C. cassiicola* is mostly known as a pathogen infesting the aerial portions of the plant, the observation of chlamydospores suggests that this fungus may also have the capacity to survive in the absence of a living host, or under unfavourable conditions. The importance of chlamydospores as a primary inoculum source for *C. cassiicola* will have to be further investigated.

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